INVESTIGATION OF THE ROLE OF THE SEROTONIN 2C RECEPTOR POLYMORPHISMS IN ANTIDEPRESSANT-ASSOCIATED WEIGHT CHANGE IN TURKISH POPULATION

A THESIS SUBMITTED TO
THE GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
OF
MIDDLE EAST TECHNICAL UNIVERSITY

BY

UĞUR ÜZEL

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR
THE DEGREE OF MASTER OF SCIENCE
IN
BIOLOGY

NOVEMBER 2024

Approval of the thesis:

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ABSTRACT

INVESTIGATION OF THE ROLE OF THE SEROTONIN 2C RECEPTOR POLYMORPHISMS IN ANTIDEPRESSANT-ASSOCIATED WEIGHT CHANGE IN TURKISH POPULATION

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November 2024, 99 pages

Major depressive disorder (MDD) and anxiety disorders have become highly prevalent in recent years, especially following the COVID-19 pandemic. Although selective serotonin reuptake inhibitors (SSRIs) are the first-line treatment for these disorders, weight change is a significant side effect that may interfere with patient adherence to treatment and could also lead to additional health problems. A serotonin receptor subtype, serotonin 2C receptor (5-HT2CR), regulates feeding behavior through hypothalamic pathways. This project aimed to investigate the role of three 5-HT2CR polymorphisms: -759C/T, -697G/C, and 68G/C (Cys23Ser), previously linked to weight or feeding abnormalities, in SSRI-associated weight change in a small patient group in Türkiye. Due to its clinical convenience, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) technique was used to determine polymorphic alleles. Weight changes were analyzed in relation to the alleles and the administered SSRIs after 4 weeks of SSRI use. The findings indicated that baseline body mass index (BMI) was higher in carriers of the -697C allele, who were more common in our study population.

Keywords: SSRI, Antidepressant, Serotonin 2C Receptor, Weight Change, SNP

ÖZ

TÜRK POPÜLASYONUNDA ANTİDEPRESAN İLİŞKİLİ KİLO DEĞİŞİMİNDE SEROTONİN 2C RESEPTÖR POLİMORFİZMLERİNİN ROLÜNÜN ARAŞTIRILMASI

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Kasım 2024, 99 sayfa

Majör depresif bozukluk (MDB) ve anksiyete bozuklukları son yıllarda, özellikle COVID-19 pandemisinden sonra oldukça yaygın hale gelmiştir. Seçici serotonin geri alım inhibitörleri (SSRI'ler), bu psikiyatrik bozuklukların tedavisinde ilk tercih edilen ilaçlar olmasına rağmen, en önemli yan etkilerinden biri olan kilo değişikliği ek sağlık sorunlarına sebep olabilir veya hastanın tedaviye devamını engelleyebilir. Bir serotonin reseptör alt tipi olan serotonin 2C reseptörü (5-HT2CR) hipotalamik yolaklar aracılığıyla beslenme davranışını düzenlemektedir. Bu proje, daha önce kilo veya beslenme anormallikleri ile ilişkilendirilen üç 5-HT2CR polimorfizmi olan -759C/T, -697G/C, ve 68G/C (Cys23Ser)'nin SSRI'lerle ilişkili kilo değişimindeki rolünü Türkiye'den küçük bir hasta grubunda araştırmaktayı amaçlamıştır. Klinik uygunluğu nedeniyle polimorfik allelleri belirlemek için polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi (PCR-RFLP) tekniği kullanılmıştır. Kilo değişimi 4 haftalık SSRI kullanımı sonunda aleller ve kullanılan ilaçlar ile karşılaştırılarak analiz edilmiştir. Bulgular, -697C alel taşıyıcılarının daha yüksek başlangıç vücut kitle indeksi (VKİ) değerlerine sahip olduğunu ve bu varyant alelin deney dahilindeki populasyonda daha yaygın olduğunu göstermiştir.

Anahtar Kelimeler: SSRI, Antidepresan, Serotonin 2C Reseptörü, Kilo Değişimi, SNP

To the misfits for not letting me feel alone

ACKNOWLEDGMENTS

I would like to express my gratitude to my supervisor Assoc. Prof. Dr. Tülin Yanık for guiding and supporting me in the challenges I faced throughout my graduate education, and for providing the environment I needed to work freely and pursue my ideas. I am deeply grateful to my lab mate and mentor Seyda Tuğçe Durhan for helping me with the experimental applications and interpretations. I am also thankful to my lab mates Ceren Akçasoy and Zehra Koyuncu for putting up with my quirks, gifting me Hello Kitty products and pink items to boost my morale.

I wish to thank Prof. Dr. Mehmet Ak for his guidance in project formation, and Dr. Zeynep Karaman for her assistance with the collection of human samples. I also extend my gratitude to all contributing medical personnel at the Psychiatric Service of Necmettin Erbakan University, Faculty of Medicine, Konya, Türkiye.

I am profoundly thankful to my flatmate Ebru Özkök for all the enjoyable times and her generosity in sharing her home. And of course, no words can truly reflect my gratitude to my family members and Ekin Sayın for their selfless and endless support, kindness, and understanding. In addition, I wish to express my heartfelt thanks to my cat Mantar for making these last months bearable. This achievement would not have been possible without you.

Lastly, I sincerely appreciate the examining committee members Prof. Dr. Çağdaş Devrim Son, Assoc. Prof. Dr. Tülin Yanık and Prof. Dr. Mehmet Ak for their valuable insights and feedback.

I am grateful to the Scientific and Technological Research Council of Türkiye for awarding me the TÜBİTAK 2211 National Graduate Scholarship under the 2210-A MSc program. This work was supported by the Support Program for Research Universities (ADEP), Project number: ADEP-108-2023-11242, the Council of Higher Education (YÖK), Ankara, Türkiye.

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ABBREVIATIONS

5-HT	5-hydroxytryptamine
5-HT2CR	Serotonin 2C Receptor
α-MSH	Alpha-Melanocyte Stimulating Hormone
AgRP	Agouti-Related Peptide
ARC	Arcuate Nucleus
bp	Base Pair
BMI	Body Mass Index
C	Cystosine
CART	Cocaine- and Amphetamine-Regulated Transcript
CI	Confidence Interval
CNS	Central Nervous System
Cys	Cysteine
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide Triphosphate
EDTA	Ethylenediaminetetraacetic Acid
EtBr	Ethidium Bromide
G	Guanine
GI	Gastrointestinal
GPCR	G Protein-Coupled Receptor

IQR	Interquartile Range
MC4R	Melanocortin 4 Receptor
MDD	Major Depressive Disorder
mRNA	Messenger Ribonucleic Acid
NFW	Nuclease-free Water
NPY	Neuropeptide Y
OCD	Obsessive-Compulsive Disorder
OECD	Organization for Economic Cooperation
OR	Odds Ratio
PCR	Polymerase Chain Reaction
PCR-RFLP	Polymerase Chain Reaction–Restriction Fragment Length Polymorphism
POMC	Pro-Opiomelanocortin
SD	Standard Deviation
Ser	Serine
SNP	Single-Nucleotide Polymorphism
SSRI	Selective Serotonin Reuptake Inhibitor
WHO	World Health Organization

CHAPTER 1

INTRODUCTION

1.1 The Serotonergic System

Serotonin, also known as 5-hydroxytryptamine or 5-HT, is a monoamine neurotransmitter synthesized from the amino acid tryptophan. It was first discovered in 1937 by Erspamer and Vialli, who originally named the molecule enteramine. Eleven years later, it was established in the literature with its current name as a serum vasoconstrictor (Rapport et al., 1948). The serotonergic system is a network of pathways in the central nervous system (CNS) comprising serotonin, serotonergic neurons, serotonin receptors, serotonin transporters, and serotonin-metabolizing enzymes. Even though nearly a century has passed since its discovery, studies on the serotonergic system continue to prove its relevance and value in life sciences. The system is implicated in numerous physiological and cognitive functions, covering almost every fundamental process such as appetite, mood, sleep, temperature regulation; cardiovascular, gastrointestinal (GI), reproductive function, and pain (Berger et al., 2009). Accordingly, the serotonergic system is associated with the pathophysiology of a multitude of neuropsychiatric and neurological disorders, particularly depression and anxiety.

Although almost all neurological processes underlying any behavior or psychological state are somehow regulated by the serotonergic system, curiously enough, by a wide margin, most of the serotonin is found outside of the CNS. Approximately 95% of the serotonin is secreted from enterochromaffin cells in the GI tract for the regulation of smooth muscle contractions, while some are concentrated in the platelets for their function in the vascular system (Gershon & Tack, 2007). Only a small amount of serotonin in the body is located in the CNS, produced and secreted from a region called the raphe nuclei in the midline of the

brain stem (Bakshi & Tadi, 2022). These serotonergic neurons have projections encircling all brain regions and the spinal cord to release 5-HT to the synapses.

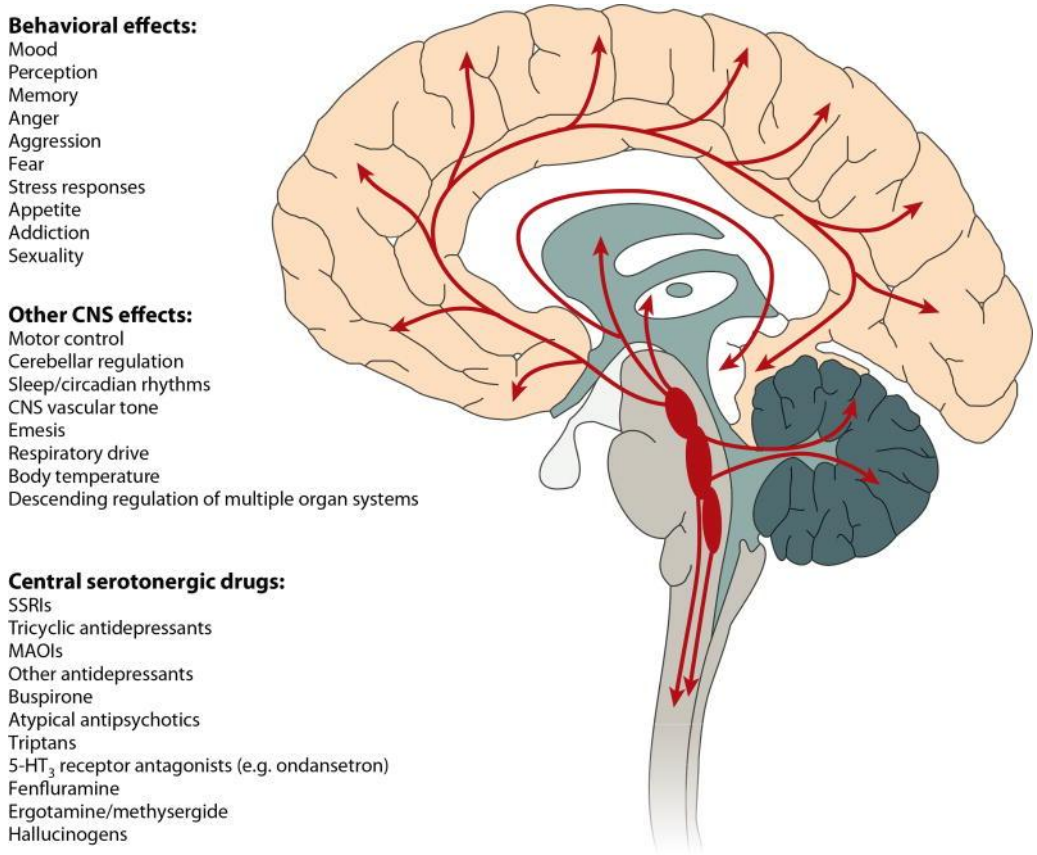

Figure 1.1. Serotonergic pathways in the CNS, their effects, and the drugs that target them. Red lines with arrows indicate serotonergic projections from the raphe nuclei. 5-HT, serotonin; MAOI, monoamine oxidase inhibitor; SSRI, selective serotonin reuptake inhibitor (Berger et al., 2009).

In Figure 1.1, the neurological pathways of the serotonergic projections, their modulatory effects over the CNS, as well as pharmacological agents affecting serotonergic neurotransmission are illustrated. Serotonin mediates almost every type of behavioral, psychological, and perceptual process an animal has. For the organism's physiological balance and survival, the serotonin system functions to communicate basic needs such as food, sleep, and reproduction with high-level cognitive functions such as learning, memory, fear, anger, happiness, and reward. For this reason, modulation of the serotonergic system as a research target in psychiatric disorders has been highly relevant in clinical research (Lin et al., 2014).

1.2 Depression and Anxiety

Mental health has been on the decline in the global population over the past decades (Wu et al., 2023). According to the World Health Organization (WHO), 1 in 8 people were suffering from a psychiatric illness in 2019, with anxiety and depressive disorders positioned at the top of the list. The same report draws attention to a considerable increase in 2020 due to the COVID-19 pandemic, with approximately 26% and 28% increases for these two groups of disorders, respectively (WHO, 2022). Moreover, a recent cross-national study reaching over 29 countries and more than 150,000 participants estimated that about 50% of the population will develop at least one of the considered mental disorders by the age of 75 (McGrath et al., 2023). The study revealed that major depressive disorder (MDD), and anxiety disorders are among the most prevalent mental disorders. These conditions negatively influence the patients' health, as well as create a significant burden on society and the economy (Sartorius, 2001).

The American Psychiatric Association's (2013) *Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition* (DSM-5) defines depressive disorders as a group of mood disorders characterized by depressed mood, feelings of guilt, reduced pleasure and energy, changes in appetite and weight, difficulties in concentrating, sleep disturbances and suicidal ideation. Clinical depression or major depressive disorder is one of the foremost disabilities that make patients suffer from unemployment and disconnection from society. Furthermore, patients' condition can be negatively impacted by MDD, leading to medical complications such as type 2 diabetes, hypertension, pulmonary and coronary diseases (Bains & Abdijadid, 2023). On top of that, research conducted in Denmark, Finland, Sweden, and Italy populations showed substance abuse and suicide attempts greatly reduced the life expectancy of antidepressant-treated depression inpatients and outpatients by 6.3 to 21.0 years, highlighting the importance of comorbid conditions and risk factors (Korhonen et al., 2021).

Anxiety disorders originate from a pathologically exaggerated or misplaced form of the natural emotion of fear. They haunt society and individuals as one of the most prevalent psychiatric disorders, though the exact rates are unknown due to the lack of official diagnosis for many cases. DSM-5 groups different forms of anxiety disorders, such as specific phobia, social anxiety disorder, panic disorder, and generalized anxiety disorder, under the umbrella term "anxiety disorders". These are characterized by cognitive symptoms causing intense fear and poor mental performance; physiological symptoms such as increased heart rate, dizziness, and nausea; behavioral symptoms such as restlessness, isolation, and avoidance; and emotional symptoms such as irritability and fearfulness (American Psychiatric Association, 2013). Similar to MDD, anxiety disorders can significantly increase patients' morbidity due to aggravation of life-threatening conditions, risk of substance, abuse and suicide (Chand & Marwaha, 2023).

Table 1.1. Examples of antidepressants according to generations and groups.

Antidepressants				
First Generation		**Second Generation**		
TCAs	***MAO Inhibitors***	***SSRIs***	***SNRIs***	***Atypicals***
Imipramine Amitriptyline Clomipramine	Phenelzine Selegiline patch	Fluoxetine Paroxetine Citalopram Escitalopram Sertraline	Venlafaxine Desvenlafaxine Duloxetine	Trazodone Bupropion Mirtazapine

MAO, monoamine oxidase; SSRI, selective serotonin reuptake inhibitor; SNRI, serotonin-noradrenaline reuptake inhibitor; TCA, tricyclic antidepressant (Kumral & Bayam, 2020).

In 1967, Alec Coppen proposed serotonin deficiency as the primary neurochemical pathogenesis for depression and anxiety. The serotonin hypothesis of depression continued to be relevant in the following research and still holds its influence despite contradictory results (Moncrieff et al., 2022). Aligning with this hypothesis, various antidepressant medications with differing types of pharmacological profiles

have been developed, and some of the most commonly prescribed examples are shown in Table 1.1. The first-generation antidepressants, monoamine oxidase inhibitors (MAOIs) and tricyclic antidepressants (TCAs), worked on regulating naturally occurring 5-HT levels. MAOIs increase the serotonin concentration by inhibiting its oxidation by blocking the monoamine oxidase enzyme; nonetheless causing systemic-level side effects due to their pharmacological activity outside of the brain (Laban & Saadabadi, 2023). TCAs help relieve depression and anxiety symptoms by inhibiting serotonin and norepinephrine reuptake, however, their affinity to postsynaptic cholinergic, muscarinic, and histamine receptors brings along numerous unwanted adverse effects (Moraczewski et al., 2023).

In the late 1980s, a new chapter began with the introduction of selective serotonin reuptake inhibitor (SSRI) antidepressant fluoxetine (Wong et al., 2005). Second-generation antidepressants, namely SSRIs, serotonin-norepinephrine reuptake inhibitors, and atypicals, with their markedly better selectivity, tolerability, efficacy, safety, and substantially fewer side effects have surpassed first-generation antidepressants and have been dominating the market ever since (Ferguson, 2001).

1.3 Selective Serotonin Reuptake Inhibitors (SSRIs)

SSRI antidepressants are the first-line pharmacological treatment option and the most commonly prescribed medication for the management of major depressive disorder and anxiety disorders (Chu & Wadhwa, 2023). The growing mental health crisis brought along increased use of psychiatric medication, particularly antidepressants. The Organization for Economic Co-operation and Development's (OECD) data collection from 18 European countries between the years of 2000 and 2020 pointed out a dramatic increase in antidepressant use, more than doubling the initial values (OECD, n.d.). The situation is no different in Türkiye. According to the Republic of Türkiye Ministry of Health's Health Statistics Yearbook 2021, daily defined dose (DDD) consumption per 1,000 people increased from 33 to 52 from 2010 to 2020 (Figure 1.2). Clearly, the sudden surge in SSRI consumption

brings about additional issues due to the circumstances discussed above. In this case, they are the side effects of these medications.

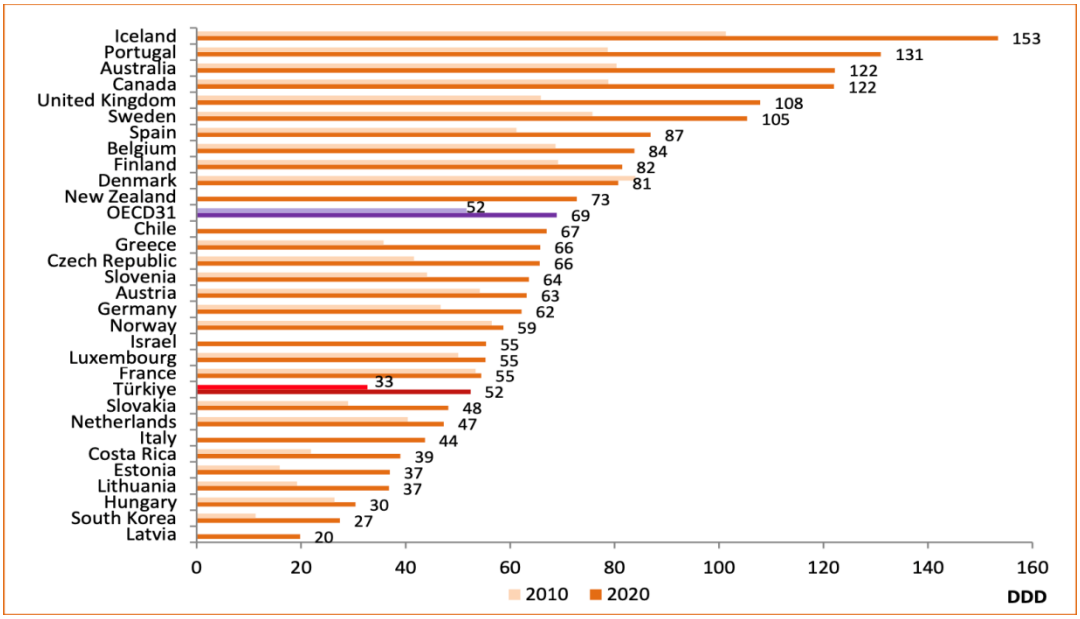

Figure 1.2. International antidepressant consumption comparison per 1,000 population in 2010-2020. DDD, defined daily dose; OECD, the Organization for Economic Co-operation and Development (T.C. Sağlık Bakanlığı, n.d.).

Serotonin transporter or 5-HT reuptake transporter (SERT or 5-HTT), a highly selective protein for serotonin, transports serotonin from the synaptic cleft into the presynaptic serotonergic neurons. SSRI antidepressants selectively bind and block SERT, therefore inhibiting their reuptake function (Figure 1.3). By this action, 5-HT molecules spend more time in the synapse, binding 5-HT receptors on the postsynaptic membrane. Evidently, this chain of molecular activity relieves the pathophysiological basis of depressive symptoms in the patients (Chu & Wadhwa, 2023). Although side effects start to emerge in the acute term, the therapeutic effects of SSRI antidepressants may take up to 6 weeks to manifest, during which neurochemical adaptations in the CNS take place, such as receptor desensitization and down-regulation (Richelson, 2001). Another hypothesis suggests that these interactions initiate a cascade of structural changes that trigger neuroplasticity in the long term (Sharp & Collins, 2023). This hypothesis implies that with the prolonged use of SSRIs, rewiring of the neuronal networks results in improvements

in the patient's depressive symptoms by behavioral means such as reflecting on emotional experiences. Consequently, besides the complications surfacing due to previously mentioned psychiatric conditions, a new dimension emerges with the detrimental side of discontinuing SSRI treatment.

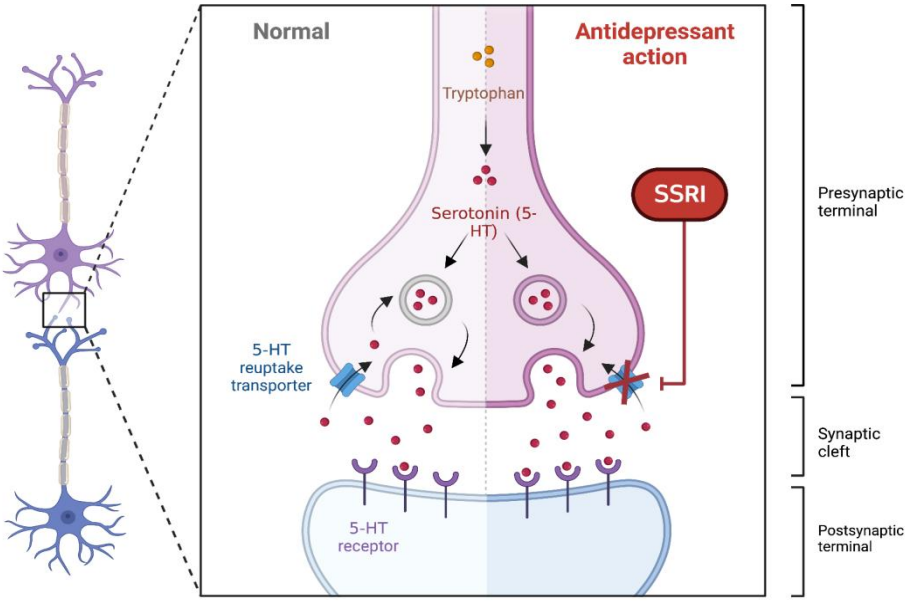

Figure 1.3. SSRI mechanism of action. 5-HT, serotonin; SSRI, selective serotonin reuptake inhibitor (BioRender Science Templates, n.d.).

1.3.1 Side Effects of SSRIs

Comparable to many other types of medication or antidepressants, SSRIs may cause side effects. These adverse effects manifest themselves in various ways, ranging in their severity in the sense of complications they create and how much they bother the patient. Common side effects can be listed as agitation, appetite, and weight changes, blurred vision, dizziness, drowsiness, dry mouth, headaches, insomnia, sexual dysfunction, etc. (NHS, n.d.). As seen in Figure 1.4, a survey of roughly 700 patients ranks the side effects according to the instance being mentioned by patients. In a study conducted by Hu et al. in 2004 on 401 depressed patients, 86% of whom were treated with SSRIs reported at least 1 side effect and 52% of them reported more than 3 side effects. In addition, 219 patients (55%)

reported to the physicians that at least one of the side effects was substantially bothersome. Inherently, these unwanted adverse effects give rise to discontinuation of the treatment. Up to 25% of patients quit their SSRI treatment due to side effects that are too hard to endure, the most frequent ones being sexual dysfunction and weight change (Kelly et al., 2008; Cascade et al., 2009).

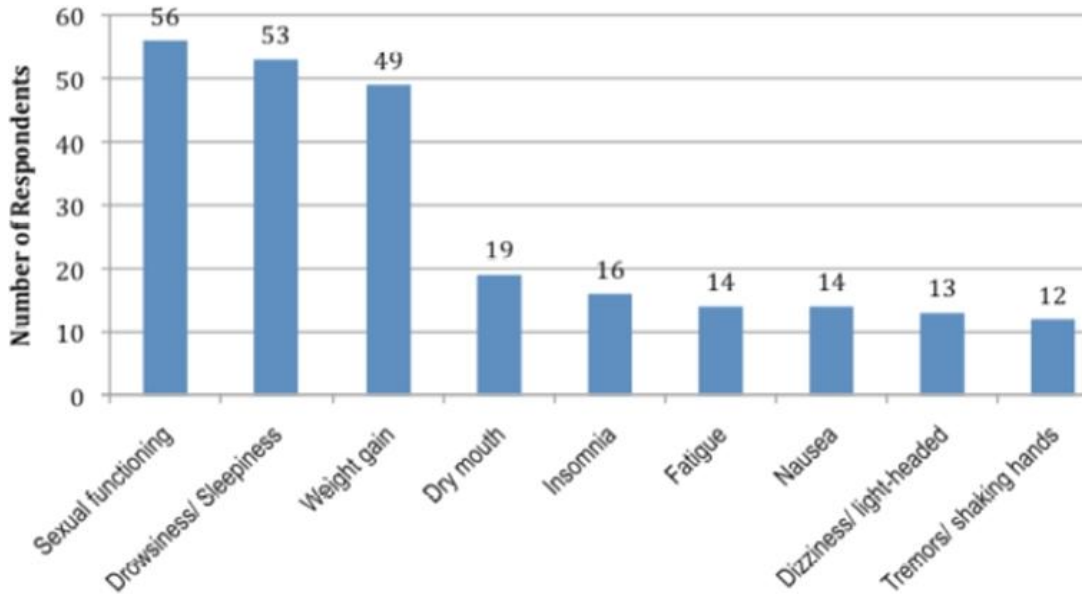

Figure 1.4. Commonly reported SSRI side effects by patients (Cascade et al., 2009).

In a cross-sectional study from Türkiye, Uguz et al. (2015) demonstrated statistically significant weight gain caused by non-SSRI antidepressants mirtazapine, venlafaxine, and duloxetine, as well as by the SSRI antidepressants sertraline, paroxetine, citalopram, escitalopram, with the exception of fluoxetine. A meta-analysis on weight change caused by antidepressants evaluated SSRI-associated weight gain findings and categorized them into three risk levels: high, medium, and low (Gill et al., 2020). Drugs that are considered high-risk for inducing weight gain were defined to cause >7% weight change and ≥1.5 kg weight gain. Medium-risk ones caused >7% weight change and 0.4-1.4 kg weight gain. And, the low-risk group caused no weight change or loss.

In 2010, Mastronardi et al. argued that long-term antidepressant use can cause a persistent increase in obesity risk. In parallel to the rise in mental health incidences,

the rate of obesity is also increasing both globally and locally in Türkiye. According to OECD (n.d.) data sets from 2021, approximately 18% of the adult population were obese. The numbers go as high as 20.2% according to the Turkish Statistical Institute's (TÜİK Kurumsal, n.d.) 2023 report. Obesity is a disease that causes complications in patients' lives and increases their mortality risk. It is a risk factor for a complex spectrum of symptoms, ranging from cardiovascular diseases to neurodegenerative ones.

Citalopram and paroxetine are SSRIs in the high-risk group, escitalopram is in the middle-risk group, and fluoxetine is categorized as low-risk. Fluoxetine has been repeatedly mentioned in the literature for causing body weight loss with acute use in human and similarly, significant body weight loss and calorie intake reduction in rats (Serralde- Zuñiga et al., 2022; Heisler, 1997; Aggarwal et al., 2016). Although the mechanism of action is similar among different SSRIs, there are differences in receptor binding and their downstream signaling properties. One of the differential properties of fluoxetine from other SSRIs is its antagonistic affinity to the serotonin 2C receptors (5-HT2CR) (Ni & Miledi, 1997).

1.4 The Neurobiology of Feeding

The hypothalamic region of the brain is central to regulating satiety, feeding behavior, and energy metabolism. There are groups of clustered neurons that have similar functions located in different nuclei of the hypothalamus. In the arcuate nucleus (ARC), agouti-related peptide (AgRP) and neuropeptide Y (NPY) neurons are orexigenic and stimulate appetite, whereas cocaine- and amphetamine-regulated transcript (CART) and pro-opiomelanocortin (POMC) neurons are anorexigenic and inhibit appetite (Ahima & Antwi, 2008). The state of hunger stimulates AgRP/NPY neurons to release AgRP and NPY neurohormones which stimulate appetite and decrease energy metabolism. Conversely, activation of POMC/CART neurons trigger the release of alpha-melanocyte stimulating hormone (α-MSH) and CART, causing appetite suppression and increase in energy metabolism

(Millington, 2007). These hypothalamic pathways are closely interconnected with the serotonergic system (Figure 1.5).

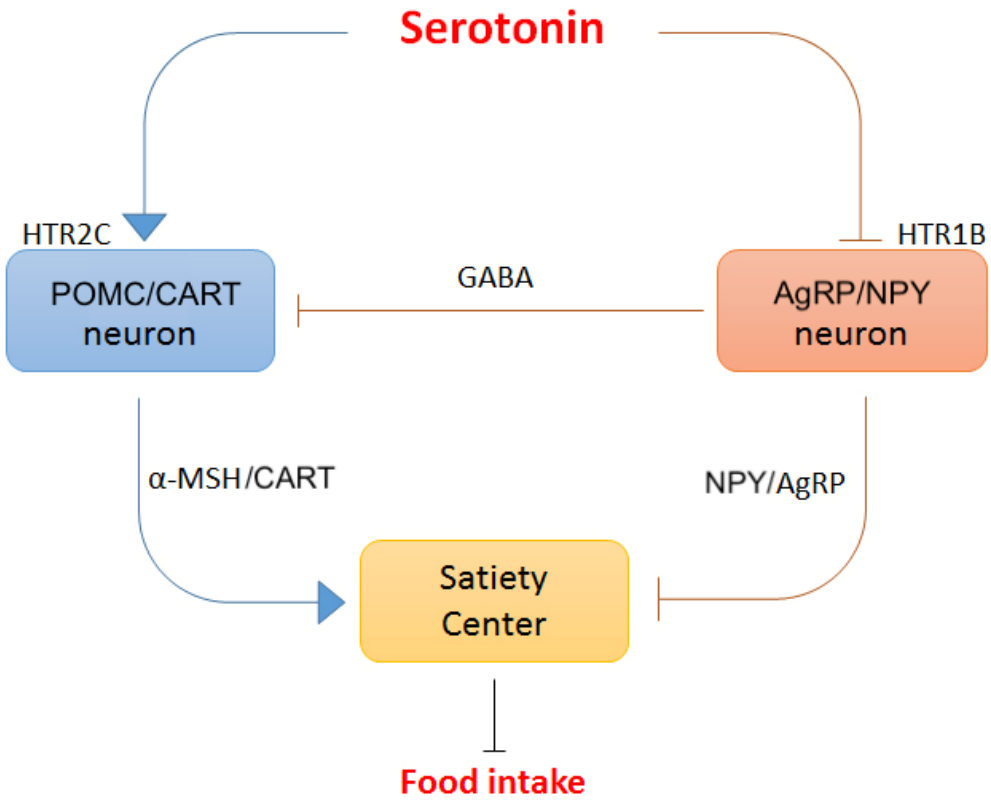

Figure 1.5. Hypothalamic appetite circuit regulation by the serotonergic system. α-MSH, alpha-melanocyte stimulating hormone; AgRP, agouti-related peptide; CART, cocaine- and amphetamine-regulated transcript; HTR1B, serotonin 1B receptor; HTR2C, serotonin 2C receptor; GABA, γ-aminobutyric acid; NPY, neuropeptide Y; POMC, pro-opiomelanocortin (Modified from Namkung et al., 2015).

The serotonergic system regulates feeding behavior and weight change. Blundell (1977) demonstrated that increased 5-HT concentration in the synaptic cleft or direct activation of serotonin receptors decreased food intake, whereas decreased 5-HT concentration or receptor blockade increased feeding. Recent updates present 5-HT1B, 5-HT2C, and 5-HT6 receptor subtypes as relevant in regulating satiety via serotonergic pathways (Voigt & Fink, 2014). Appetite and weight changes by the hypothalamic circuit (Figure 1.5) where the ARC 5-HT2CR activity modulates POMC secretion and α-MSH production from POMC neurons modulate

melanocortin 4 receptors (MC4R) neurons in the paraventricular nucleus have been shown with the use of 5-HT2CR agonists (Heisler et al., 2002; Lam et al., 2007).

1.5 Serotonin Receptors

There are at least 15 subtypes of serotonin receptors cloned and classified under seven families according to their structural and functional properties (Kroeze et al., 2002). They are named numerically to indicate the family and alphabetically for subtype, as in 5-HT1AR, 5-HT2BR, etc., and belong to the G protein-coupled receptor (GPCR) superfamily with the exception of ligand-gated ion channel 5-HT3 receptors. 5-HT1 and 5-HT5 receptors inhibit cyclic AMP production through G-protein coupling, while 5-HT4, 5-HT6, and 5-HT7 activate adenylyl cyclase and increase cyclic AMP; on the other hand, 5-HT2 receptors activate phospholipase C affecting the inositol phosphate system, while ligand-gated 5-HT3 receptors transport Na+, K+ and Ca2+ ions (Bakshi & Tadi, 2022). Yohn et al. (2017) examine that evidence suggests each type of serotonin receptor contributes to the pathophysiology of depression and anxiety to varying degrees (Figure 1.6). Presynaptic serotonin receptors function as autoreceptors, binding 5-HT and regulating serotonergic transmission tone, whereas postsynaptic serotonergic receptors bind 5-HT to trigger signal transduction, in turn regulating multiple neuronal networks (Lin et al., 2023).

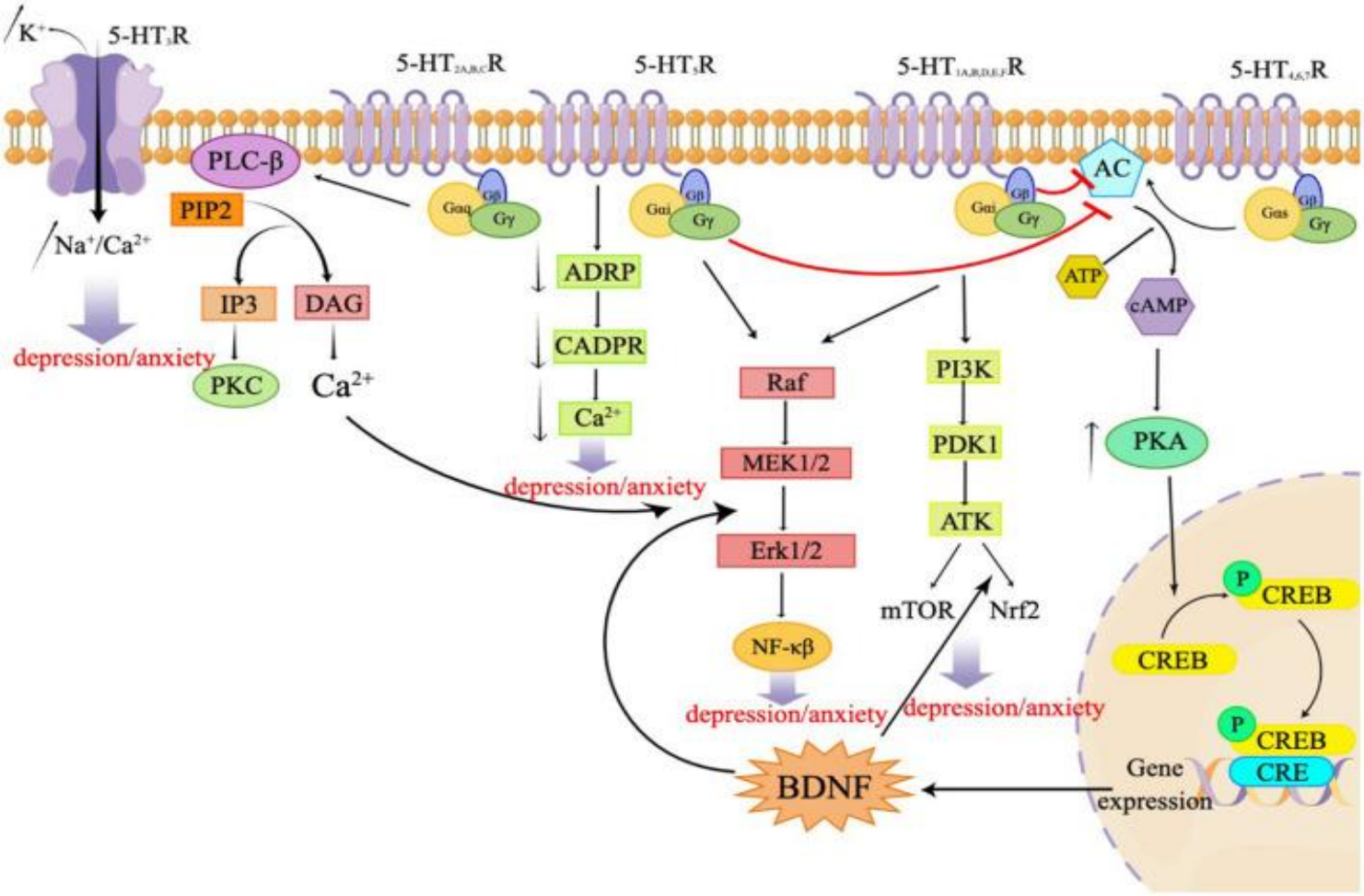

Figure 1.6. Serotonergic signal transduction pathways implicated in depression and anxiety(Lin et al., 2023).

1.5.1 The Serotonin 2C Receptor (5-HT2CR)

The G-protein coupled receptor 5-HT2CR is encoded on the X chromosome (Xq23) with the notation *HTR2C* (NCBI, n.d.). 5HT2CRs are prevalent in the CNS and are primarily located in the choroid plexus, as well as various brain regions associated with neurological, neuropsychiatric, and neuropharmacological research, including the amygdala, hypothalamus, cerebral cortex, striatum, and substantia nigra (Abramowski et al., 1995; Sharma et al., 1997; Clemett et al., 2000). Additionally, the activity of 5-HT2CR is associated with medical problems, such as anxiety, depression, substance use disorders, psychotic disorders, and obesity (Kimura et al., 2009; Millan, 2005; Kasper et al., 2013; Canal et al., 2014; Higgins et al., 2013).

Numerous studies conducted on the subject presented a clear and close association between the activity of the receptor, feeding, and weight gain. Tecott et al. (1995) observed the development of obesity and abnormal feeding behavior following genetic deletion of the *HTR2C*. Accordingly, 5-HT2CR agonists decreased feeding in mice and rats when acutely administered, while chronic administration decreased body weight in obese animals (Bickerdike, 2003). Similarly, 5-HT2CR antagonists, which include many of the commonly prescribed antipsychotic medications, reversed 5-HT2CR agonist-induced appetite suppression (Reynolds et al., 2006). Administration of olanzapine, one of the aforementioned pharmaceuticals, in male Wistar rats caused a reduction in hypothalamic POMC gene expression and plasma α-MSH levels, leading researchers to consider the involvement of the 5-HT2CR (Sezlev-Bilecen et al., 2016). Recently, He et al. (2022) identified rare loss-of-function variants of *HTR2C* by sequencing severely obese individuals. Their knock-in studies in mice showed these variants the resulted in obesity, disrupted social behavior, and impairment in POMC neurons, which they suggested may be the underlying cause.

Serotonin 2C receptor messenger RNA's (mRNA) nucleotides can be edited by double-stranded RNA adenosine deaminase enzyme to produce different isoforms

of the receptor (Fitzgerald et al., 1999). Curiously, 5-HT2CR is the only known GPCR to go through this type of mRNA editing process. Unedited natural isoform carries out its constitutive activity independent from ligand binding at a maximal level, while this activity declines proportionally to the accumulated edits of the isoforms (Figure 1.7) (Marion et al., 2004). Similarly, 24 different isoforms of the receptor produced by mRNA editing show decreased sensitivity in their response to G-protein activation triggered by agonist binding up to 15-25 times, proportional to the editing accumulation (Gurevich et al., 2002). In essence, the mRNA editing process of 5-HT2CR serves as an agonist-induced desensitization mechanism of the receptor that occurs corresponding to the chemical structure of the agonist and the downstream effector pathway triggered (Stout et al., 2002; Felsing et al., 2019). In speculation, these findings indicate SSRIs might cause both body weight gain and loss through 5-HT2C receptor binding.

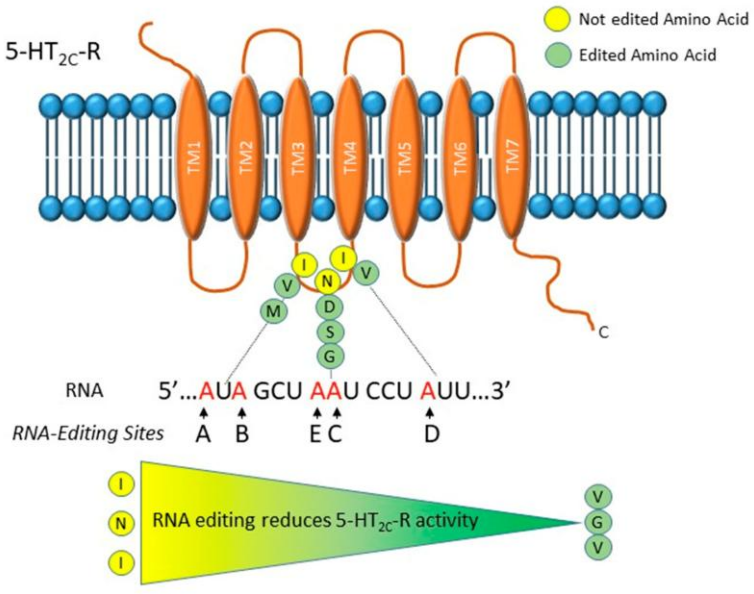

Figure 1.7. Representation of 5-HT2CR editing sites, different isoforms, and their activity levels (Barbon & Magri, 2020).

It has been demonstrated that chronic fluoxetine administration in rats causes region-specific changes in the 5-HT2CR mRNA expression and mRNA editing patterns (Barbon et al., 2011). Several behavioral and cellular research suggested

findings on the therapeutic effects of SSRIs are associated with the desensitization of the 5-HT2CR (Bristow et al., 2000; Yamauchi et al., 2004; Martin et al., 2015). It was hypothesized that serotonergic drugs modulate satiety through the 5-HT2CR and downstream melanocortin pathways. However, neither the SSRI fluvoxamine nor the 5-HT2CR neutral antagonist SB-242084 produced an anorexigenic effect when administered alone. Instead, appetite suppression was observed only when these drugs were combined. Also, it was demonstrated that a selective 5-HT1BR agonist that acts on NPY/AgRP neurons of the ARC to inhibit 5-HT2CR activity, reduced the observed effects and increased hypothalamic POMC expression (Nonogaki et al., 2006). Consistently, another study has shown long-term fluoxetine use decreased 5-HT2CR signaling and production of POMC/α-MSH in hypothalamic neurons, consecutively increasing feeding. In the former study, researchers were able to reverse SSRI-induced changes in feeding behavior through external MC4R stimulation (Ortuño, et al., 2021). In summary, current findings suggest the serotonin 2C receptors and peptide secretions from POMC neurons in the hypothalamus may contribute to SSRI-associated weight gain.

1.6 Genetic Polymorphisms

Genetic mutations refer to changes in the nucleotide sequence of the genome. They can result from internal factors such as DNA replication errors or external factors such as mutagenic chemicals or ionizing radiation (Brown, 2002). When these mutations occur in the germline and are non-lethal, they can be inherited by offspring and may spread throughout a population. If a genetic variant is present in more than 1% of a population, it is classified as a genetic polymorphism; otherwise, it is referred to as a rare mutation (Karki et al., 2015). These variations are called alleles, and individuals can be homozygous, heterozygous or hemizygous based on the number of copies of a specific allele they carry. Homozygous individuals have two identical copies of the same allele, while heterozygous individuals carry two different alleles. When the polymorphic region is located on

the X chromosome, males are considered hemizygous, as they carry only one copy of the allele.

Most genetic polymorphisms are silent, meaning they do not affect the gene's expression or functionality, though some may be involved in disease pathogenesis (Kikutake & Suyama, 2023). Functional changes can occur when mutations alter promoter or enhancer regions, influencing transcriptional levels, mRNA stability, and translation (Bull, 2013). Additionally, mutations may impact protein stability, folding, localization signals or amino acid sequence, potentially disrupting normal protein function. The most common type of genetic polymorphism, single-nucleotide polymorphisms (SNPs), refers to base changes at a point in the genome caused by factors such as insertions, deletions, or substitutions (Kirk et al., 2002). However, other forms of genetic variation involving multiple base pairs, such as repetitive sequences or duplications, also exist (Gummadi & Guddati, 2021).

1.6.1 Single-Nucleotide Polymorphisms (SNPs)

As the most common type of genetic polymorphism, SNPs make up approximately 1 in every 1000 nucleotides of the human genome (Wu et al., 2023). It is estimated that an individual carries around 14 million SNPs in their genome (Gummadi & Guddati, 2021). These mutations can occur both within and outside gene coding sequences. SNPs occurring in coding sequence regions are categorized as either synonymous or nonsynonymous mutations. Synonymous mutations do not alter the amino acid sequence of the translated protein, while nonsynonymous mutations include missense mutations, which change the amino acid sequence, and nonsense mutations, which introduce a premature stop codon (Oelschlaeger, 2024).

SNPs play a crucial role in pharmacogenetics, where they can influence the absorption, distribution, excretion, metabolism, and effects of the drugs, as well as the body's responses to them (Gray, 2000). SNP frequencies vary among populations, which explain why certain populations have higher susceptibility to

particular diseases or different responses to the same drug treatment (Myles et al., 2008). Linkage disequilibrium often leads to the inheritance of SNPs in haplotypes, which are groups of alleles that are inherited together (Bull, 2013). They impact the function of key enzymes responsible for drug metabolism, transport, and efficacy (Gummadi & Guddati, 2021). According to Ahmet et al. (2016), 20-95% of variation in drug response among individuals stems from genetic components. Identifying these variations is essential for understanding the genetic basis of complex diseases such as cardiovascular diseases and psychiatric disorders.

SNPs also have a wide range of applications in genetics. Genome-wide association studies and candidate gene approaches are commonly used to identify SNPs associated with specific traits or conditions. In pharmacogenetics, SNPs serve as biomarkers that help determine individual responses to medications, offering the potential for personalized therapies that take patients' genetic profile into account (Lailing et al., 2011). As research into genetic variations continues, the potential for SNPs to play a role in personalized medicine and customized therapeutic approaches increases, making them central to advancements in modern healthcare.

1.7 The Serotonin 2C Receptor Polymorphisms

There have been thousands of SNP entries on the *HTR2C* gene listed in the National Center for Biotechnology Information's database (HTR2C – SNP, n.d.). As anticipated, a few of them have been the subject of interest in pharmacogenetic research. Figure 1.8 shows the location of prominent polymorphisms, RNA editing sites, and the structure of the 5-HT2C receptor gene. In 1995, Lappalainen et al. identified a SNP (68G/C, Cys23Ser, rs6318) at the 68th nucleotide of the coding region (exon) of 5-HT2CR where guanine (G) is replaced by cytosine (C), resulting in the substitution of cysteine (Cys) with serine (Ser) at the 23rd amino acid position. Yuan et al. (2000) identified additional SNPs of interest in the promoter region of the gene, specifically -995G/A (rs3813928), -759C/T (rs3813929), and -697G/C (rs518147) (SNP position are numbered relative to the transcriptional start

site). Out of the mentioned, -759C/T, -697G/C, and 68G/C (Cys23Ser) had been studied the most in the context of neuropsychiatric and pharmacological research regarding the topic of weight gain and response to medication.

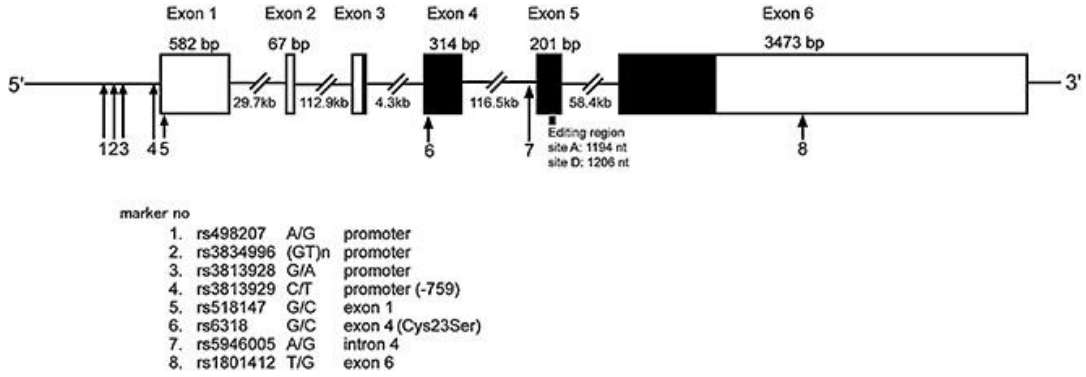

Figure 1.8. Representation of *HTR2C* genomic structure. Boxes indicate exon regions. Untranslated regions are colored in white; translated regions are colored in black. Black bar indicates RNA editing sites. Arrows indicate SNPs on the 5-HT2CR gene. SNPs are given in common/variant allele notation. bp, base pair; kb, kilobase; nt, nucleotide (Bundo et al., 2010).

1.7.1 *HTR2C*: -759C/T (rs3813929) and -697G/C (rs518147)

Variant alleles of the -759C/T and -697G/C promoter polymorphisms of the 5-HT2CR have been reported to decrease the promoter activity of the gene (McCarthy et al., 2005; Hill & Reynolds, 2007). Hill and Reynold's follow-up study in 2011 concluded the variant allele -759T was a functional polymorphism, leading to impairment in DNA-protein interactions. In genetic polymorphism studies conducted on first-episode schizophrenia patients, the -759T allele has been found to significantly decrease weight gain caused by 5-HT2CR antagonist antipsychotic drug olanzapine (Reynolds et al., 2002; Templeman et al., 2005). In a similar research with first-episode patients, both -759T and -697C variant alleles have been shown to protect against olanzapine-associated weight gain at the end of 6 weeks; and the -697C allele was suggested to be the major effector (Godlewska et al., 2009). A review article by Wallace et al. (2011) pointed out that much of the research concluding on the connection between -759C/T polymorphism's

protective effects over conditions such as antipsychotic-associated weight gain, including risperidone, clozapine, and olanzapine, obesity, and metabolic syndrome was carried out on drug-naive patients in their first and second months of treatment.

1.7.2 *HTR2C*: 68G/C (Cys23Ser, rs6318)

It has been suggested that the variant allele 68C (Ser23) did not differ in functionality compared to the wild type in past studies (Lappalainen et al., 1995; Fentress et al., 2005). However, following research associated the Ser23 allele with increased constitutive activity, faster resensitization after exposure to the 5-HT2CR inverse agonist SB-206553, and more abundant surface expression of the serotonin 2C receptor (Okada et al., 2005; Walstab et al., 2011). In a recent study, Ser23 allele variant receptors were observed to affect their pharmacological properties due to functional and intracellular localization differences (Land et al., 2019).

There has been no statistically significant association between the Cys23Ser SNP and weight gain induced by antipsychotic use other than Ujike et al.'s (2008) olanzapine study which reported additive effects with other genetic factors. However, there is substantial research going back at least 25 years that correlates Cys23Ser polymorphism with unipolar and bipolar depression, psychotic symptoms caused by Alzheimer's, drug response to clozapine, and risk of hospitalization in schizophrenia (Chagraoui et al., 2016; Li et al., 2019). Moreover, -697C and Ser23 variant alleles have been observed more frequently than the general population in patients who experience tardive dyskinesia and extrapyramidal symptoms caused by antipsychotic use (Segman et al., 2000; Gunes et al., 2006; Gunes et al., 2008). In a study comparing the distribution of Cys23Ser alleles among underweight, normal-weight, and obese adolescents, results showed similar distribution, suggesting there were no direct effects of the SNP on obesity (Lentes et al., 1997). On the other hand, there has been research linking this polymorphism with weight change and feeding behavior.

Holmes (1998) associated Ser23 allele with hyperphagia seen in late-onset Alzheimer's patients. In another study, the same allele was associated with both susceptibility to anorexia nervosa and its intensity in women (Westberg, 2002; Hu et al., 2003). Additionally, a seasonal affective disorder study on women suggested that the mutant allele is linked to lifelong low body mass index (BMI) and strongly correlated with condition-dependent weight change (Praschak-Rieder et al., 2005). Another polymorphism research unrelated to psychiatric disorders or pharmaceutical use reported a correlation between the Ser23 allele with low BMI, and found the -759T allele to be rarer among obese subjects (Bah et al., 2010). In research aimed at clarifying the role of -759C/T, -697G/C, and Cys23Ser haplotypes in metabolic abnormalities among olanzapine and clozapine users, Gunes et al. (2009) observed that the C-C-Ser haplotype was associated with higher BMI score compared to the T-C-Cys haplotype, and the homozygous C-G-Cys haplotype was significantly more common in obese subjects within the clozapine group.

1.8 Aim of the Study

Considering the global decline in mental health and half of the population being at risk of experiencing at least one psychiatric disorder in their lifetime, it is a substantial matter that needs to be addressed. All surveys conducted on the matter point towards depressive and anxiety disorders being the majority of the aforementioned psychiatric problems. These disorders share antidepressant medication use as the first-line pharmacological intervention in common, SSRI antidepressants being the most prescribed ones by a significant margin. Though current neuropsychiatric research has an understanding of the fundamentals of the pathophysiology of these disorders and SSRI antidepressants' mechanism of action, there is a notable knowledge gap in predicting their side effects. As a consequence of the variety in the pharmacological profiles of SSRIs and the

variability in patients' physiological response to them due to their genetic makeup, makes prescribing the appropriate SSRI antidepressant highly challenging.

The side effects of the selective serotonin reuptake inhibitors should be taken seriously as the psychiatric disorders themselves, because they can also be life-threatening in numerous cases. One such side effect is SSRI-associated weight change. Weight gain caused by SSRI use can increase the risk of obesity, which in turn may lead to cardiovascular health decline, and neurodegenerative disorders among other debilitating health conditions. Another facet of weight change caused by SSRI use can be the patients' dissatisfaction with their body image. This often leads to discontinuation of the medication and results in relapse into depressive states. This can bring about severe and dangerous possibilities, such as suicidal ideation and substance use disorder.

One of the major neurotransmitter systems of the central nervous system is the serotonergic system. The serotonin system regulates numerous physiological and cognitive functions, such as mood and appetite. The system has been implicated in psychiatric disorders including major depressive disorder, and anxiety disorders. Also, the main pathways through which SSRI antidepressant treatment exerts its effects belong to the serotonergic system. The serotonin 2C receptor, a subtype of serotonergic receptors, has become prominent in feeding behavior-related research due to its modulatory function in the hypothalamic feeding circuit and satiety center. The receptor also has a unique mRNA editing property that alters its activity when triggered by ligands, including potentially SSRIs. Three of the 5-HT2C receptor polymorphisms, namely -759C/T, -697G/C, and 68G/C, have been studied recurrently regarding their role in protection against antipsychotic-induced weight gain and their effects on feeding in other disorders, such as anorexia nervosa. Furthermore, SSRI antidepressants vary in their affinity to the serotonin 2C receptor, just as they vary in their potential to cause weight gain. However, there is no reported research investigating the association between SSRI-induced weight changes and the three 5-HT2CR SNPs mentioned above.

In this project, we aimed to investigate the role of -759C/T (rs3813929), -697G/C (rs518147), and 68G/C (Cys23Ser, rs6318) polymorphisms of the 5-HT2C receptor in SSRI-associated weight change. First-time users were selected from the applicants of the Psychiatric Service of Necmettin Erbakan University, Faculty of Medicine as study subjects, with no constraints on the prescribed SSRI. Demographic characteristics, height, and weight were recorded and total blood samples were collected. Patients' second weight measurements were taken 4 weeks after the start of the treatment. We specifically selected an affordable, fast, and reliable method called polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to determine the patients' alleles, due to its compatibility with clinical application. By including various SSRI antidepressant medications, we aimed to explore potential interactions between specific SNPs and these drugs. Ultimately, our main goals are to provide clinicians with a starting point for personalized SSRI prescriptions and to clarify the role of the serotonin 2C receptor's role in SSRI-associated weight gain mechanisms. The main objectives of the project were to collect patient data and samples, isolate genomic DNA from blood samples, determine patient alleles for the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms of the 5-HT2C receptor, and analyze significant differences among alleles, weight change and SSRIs.

CHAPTER 2

MATERIALS AND METHODS

2.1 Materials

2.1.1 Study Groups and Clinical Measurements

The study participants (Appendix C) were selected from the applicants of the Psychiatric Service of Necmettin Erbakan University, Faculty of Medicine. Outpatients who met the following criteria were included in the study: aged between 18 and 65 years, not having received any psychiatric treatment for the past 2 months, and approved for SSRI treatment due to any psychiatric disorder(s). The exclusion criteria for the study were decided as follows: mental retardation; a diagnosis of schizophrenia, psychotic disorder, or bipolar disorder; having comorbid medical conditions such as cardiopulmonary diseases, neurological diseases, metabolic diseases, hematologic, or other carcinomas, endocrine abnormalities, and inflammatory bowel diseases; having a history of any acute medical illness, such as an infectious disease, in the past month; use of any medication that can cause weight change, such as corticosteroids, oral contraceptives, antihistamines, and antiepileptic drugs; or a history of weight change due to psychiatric disorders before starting the antidepressant treatment.

Participants were informed about the study's objectives and procedures. Detailed written and signed consent forms were obtained (Appendix A). To maintain anonymity, patients were assigned numbers according to their inclusion order. During their first application to the clinic, their age, sex, height and weight were recorded, and whole blood samples were drawn into ethylenediaminetetraacetic acid (EDTA) tubes and stored at -80 ^{o}C. Weight measurements were taken using a scale regularly inspected by the Ministry of Health. The weight measurements were

conducted twice with a 4 week interval to assess weight changes. The initial measurements were taken before the SSRI treatment started, and the second measurements were taken 4 weeks after the SSRI treatment began. The whole blood samples were transferred to the Biological Sciences Department at Middle East Technical University, and stored following cold chain protocols.

2.1.2 Chemicals and Enzymes

The preparation and isolation of human genomic DNA from whole blood samples were performed using ethanol (34852-M, Sigma-Aldrich, St. Louis, Missouri, United States), GeneJET Whole Blood Genomic DNA Purification Mini Kit (K0781, Thermo Fisher Scientific, Waltham, Massachusetts, United States), and Invitrogen UltraPure DNase/RNase-Free Distilled Water (10977035, Thermo Fisher Scientific, Waltham, Massachusetts, United States).

Polymerase chain reactions were conducted using dNTP Set, 100 mM Solutions (R0181, Thermo Fisher Scientific, Waltham, Massachusetts, United States), DreamTaq DNA Polymerase (5U/µL) with 10X DreamTaq Buffer with 20 mM $MgCl_2$ (EP0702, Thermo Fisher Scientific, Waltham, Massachusetts, United States), Invitrogen UltraPure DNase/RNase-Free Distilled Water (10977035, Thermo Fisher Scientific, Waltham, Massachusetts, United States), and Q5 High-Fidelity 2X Master Mix (M0492S, New England Biolabs, Ipswitch, Massachusetts, United States).

Restriction enzyme digestion reactions were carried out using HinfI with rCutsmartBuffer (R0155S, New England Biolabs, Ipswitch, Massachusetts, United States), Invitrogen UltraPure DNase/RNase-Free Distilled Water (10977035, Thermo Fisher Scientific, Waltham, Massachusetts, United States), and SsiI (AciI) (10U/µL) with 10X Buffer O (ER1791, Thermo Fisher Scientific, Waltham, Massachusetts, United States).

Agarose gel electrophoresis was performed using agarose (A9539, Sigma-Aldrich, St. Louis, Missouri, United States), boric acid (1001651000, Merck, Darmstadt, Germany), ethidium bromide (E7637, Sigma-Aldrich, St. Louis, Missouri, United States), EDTA disodium salt (E5134, Sigma-Aldrich, St. Louis, Missouri, United States), GeneRuler Low Range DNA Ladder with 6X TriTrack DNA Loading Dye (SM1191, Thermo Fisher Scientific, Waltham, Massachusetts, United States), and Trizma base (T1503, Sigma-Aldrich, St. Louis, Missouri, United States).

2.1.3 Primers

Primer pairs designed for amplifying the target regions in the polymerase chain reactions were obtained from Oligomer Biyoteknoloji (Ankara, Türkiye). Desiccated oligomers were mixed with nuclease-free water (NFW) to obtain stock solutions of 100 µM concentration following the instructions provided by the manufacturer. The aforementioned stock solutions were diluted into working stock solutions of 10 µM concentration for the following PCR steps (Chapter 2.2.3). All stock solutions were stored at -20 ^{o}C. The primer sequences were chosen according to the previous publications. For the -759C/T and -697G/C promoter polymorphisms, the primer pair was referenced from Yuan et al. (2000). Primer pair for the 68G/C (Cys23Ser) polymorphism in the intron sequence was referenced from Malhotra et al. (1996).

Table 2.1. Sequence information of the primer pair used for amplifying the region covering the -759C/T and -697G/C polymorphisms of the 5-HT2CR.

Direction	**Sequence (5'-3')**	**Length (bp)**	**GC (%)**
Forward	ATCTCCACCATGGGTCTCGC	20	60
Reverse	CAATCTAGCCGCTCCAAAGG	20	55

bp, base pair.

Table 2.2. Sequence information of the primer pair used for amplifying the region covering the 68G/C (Cys23Ser) polymorphism of the 5-HT2CR.

Direction	**Sequence (5'-3')**	**Length (bp)**	**GC (%)**
Forward	TTGGCCTATTGGTTTGG**G*****AAT	21	43
Reverse	GTCTGGGAATTTGAAGCGTCCAC	23	52

*G at the 18th position of the forward primer was changed from C to introduce a restriction site for HinfI. bp, base pair.

2.2 Methods

2.2.1 Preparation and Isolation of Human Genomic DNA from Whole Blood Samples

Genomic DNA isolation from whole blood samples was the first stage of a series of technical procedures that aim to determine the polymorphic alleles of the 5-HT2C receptor gene. The following molecular biology operations administered on the specimens exclude non-DNA components of the blood. Therefore, they make the template DNA available for the PCR-RFLP. Patients' whole blood samples stored in appropriate conditions (Chapter 2.1.1) were utilized for genomic DNA isolation by using GeneJet Whole Blood Genomic DNA Purification Mini Kit according to the instructions provided by the manufacturer (Pub. No. MAN0012667 Rev. D, Thermo Fisher Scientific, 2024).

Two hundred µL of whole blood sample and 20 µL of Proteinase K were transferred into a 1.5 mL microcentrifuge tube. The tube was vortexed with a WiseMix VM-10 vortex mixer (DAIHAN Scientific, Wonju, Kangwon-do, South Korea). Then, 400 µL Lysis solution was added and incubated at 56 ^{o}C on a SHT100D Block Heater (Stuart Scientific, Staffordshire, England, United Kingdom) for 10 min. The tubes were intermittently vortexed during incubation. Later, 200 µL of 96% ethanol was added to the tube and mixed by pipetting. The mixture was transferred into a spin column and centrifuged at 6,000 X *g* for 1 min.

using a Sigma 1-15 benchtop microfuge (Sigma Laborzentrifugen GmbH, Osterode, Germany). The collection tube was discarded and the column was placed on a new 2 mL collection tube. Five hundred µL of Wash Buffer I (ethanol added) from the kit was added into the tube and centrifuged at 8,000 X *g* for 1 min. Flow-through solution was discarded and the column was placed back into the tube. This time, 500 µL Wash Buffer II (ethanol added) from the kit was added inside the tube and centrifuged at ≥20,000 x g for 3 min. with a Sigma 2-16KL refrigerated centrifuge (Sigma Laborzentrifugen GmbH, Osterode, Germany). The collection tube was emptied, then placed back into the tube, and centrifuged at 20,000 X *g* for 1 min. The column of the tube was placed into a sterile 1.5 mL microcentrifuge tube. Two hundred µL of Elution buffer was added into the center of the tube and incubated for 2 minutes at room temperature. The tube was centrifuged at 8,000 X *g* for 1 min. Finally, the column was discarded and the isolate was quantified. Purified genomic DNA solution was either used directly for succeeding experimental steps or stored at -20 ^{o}C.

2.2.2 Quantification of Genomic DNA Samples

Spectrophotometry is a reliable technique for quantifying the purity and concentration of nucleic acid-containing samples. Spectrophotometers can inform the experimenter about the DNA concentration with sensitivities up to micrograms in a unit volume (usually µg/mL). The device calculates absorbances of the sample at 230, 260, and 280 nm wavelength light. DNA and RNA absorbances peak at 260 nm, while protein absorbance peak at 280 nm. Thus, the ratio of absorbances at 260 nm and 280 nm (A_{260}/A_{280}) is used as the primary indicator of DNA isolate purity. A value between 1.7 and 1.9 for A_{260}/A_{280} is considered pure DNA. In addition to this, ratio of absorbances at 260 nm and 230 nm (A_{260}/A_{230}) is used as a secondary indicator of purity, because contaminants such as EDTA, carbohydrates, and phenol have peak absorbances near 230 nm wavelength. A_{260}/A_{230} value between 2.0-2.2 is considered pure nucleic acid.

To quantify the purity and concentration of the DNA isolates, a BioDrop µLite+ spectrophotometer (BioDrop Ltd., Cambridge, United Kingdom) was used. The nucleic acids and DNA options were selected from the device's software. DNA molecules in the isolates were suspended in the Elution Buffer of the GeneJet Whole Blood Genomic DNA Purification Mini Kit. Therefore, the same buffer was used as the blank solution for calibration. Quantification was conducted by dropping 1 µL of blank or DNA-containing solution onto the port, closing the lid of the port, and using the touch screen of the device for measurement. The port was cleaned with distilled water between every measurement and at the end. The ratios were recorded to ensure they met the required purity range for further steps.

2.2.3 Polymerase Chain Reaction (PCR)

The polymerase chain reaction is a groundbreaking technology that revolutionized the field of molecular biology. It was pioneered by Kary Mullis et al. in the 1980s, earning them the Nobel Prize in Chemistry in 1993 (Mullis et al., 1986). PCR is essentially a method to catalyze rapid, site-specific amplification of the template DNA in a test tube. The components are a DNA sample to be amplified (template DNA), a set of 20-25 base pair (bp) long forward and reverse primers to complement the target region on the template DNA, deoxyribonucleotide triphosphates (dNTPs) for building new DNA strands, buffer solution to provide optimal enzymatic conditions, and DNA polymerase to duplicate the template. The ingenuity of the method that replicates in vivo phenomenon in a tube relies on the polymerase enzyme, *Taq* DNA polymerase, acquired from a thermophilic bacteria *Thermus aquaticus. Taq* polymerase is a heat-stable enzyme that can endure high enough temperatures (95 ^{o}C) to withstand the denaturation phase of the PCR (Khehra et al., 2023). After PCR components are prepared, the tube is placed inside an electronic device called a thermal cycler. The thermal cycler repeatedly goes through three phases: denaturation for separating DNA into single strands, annealing for primer binding, and extension of single strands by the polymerase

with dNTPs (Figure 2.1). Each cycle, usually between 30-40, doubles the amount of DNA, resulting in exponential amplification. Applications of PCR in forensics, genetics, and clinical research are numerous, including detecting mutations and sequences for allelic analysis, identifying viral infections, diagnosing genetic disorders, analyzing genetic diversity, performing genetic fingerprinting, and RNA research through variants such as reverse transcriptase polymerase chain reaction (RT-PCR) and quantitative real-time polymerase chain reaction (qRT-PCR) (Zhu et al., 2020).

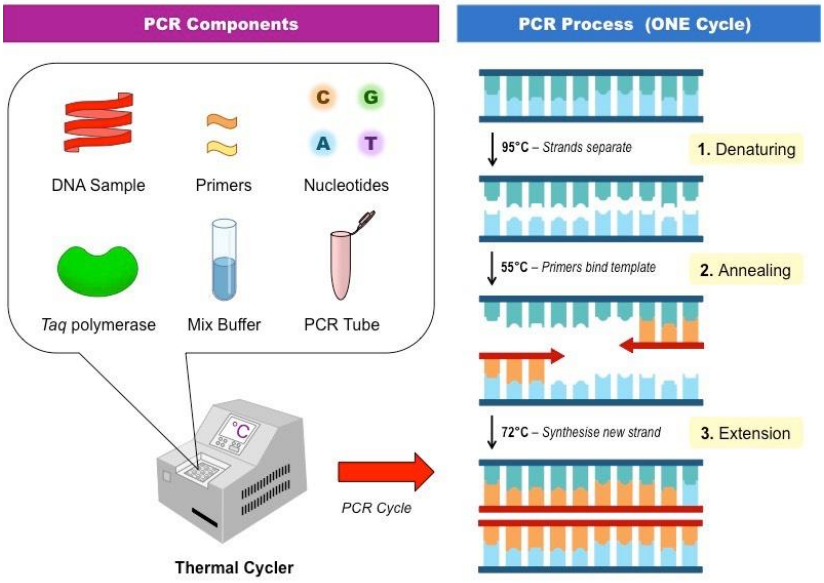

Figure 2.1. Representation of PCR components and stages. DNA, deoxyribonucleic acid; PCR, polymerase chain reaction; *Taq*, *Thermus aquaticus* (faCellitate, 2023).

To amplify only the region of interest from the whole genome is a necessity for genotyping the patients' samples. Firstly, the ingredients of the PCR mixtures were thawed on ice. Bio-Rad T100 thermal cycler (Bio-Rad Laboratories, Inc., Hercules, California, United States) was set to incubate at the appropriate initial denaturation temperatures. The components of the PCR reaction were added into the PCR tubes via Thermo Scientific Finnpipette F1 micropipettes (Thermo Fisher Scientific, Waltham, Massachusetts, United States) in order from largest to smallest volume in a sterile environment. The contents of the tubes were mixed gently by pipetting. Primers listed in Table 2.1 and Table 2.2 were used accordingly. To check

contamination in each batch of reaction preparation, a tube was prepared by replacing the template DNA with an equal amount of NFW. Due to high GC content in the primers of the -759C/T and -697G/C promoter polymorphisms, DreamTaq DNA Polymerase proved inefficient in preventing secondary structure and primer dimer formation. Therefore, Q5 High-Fidelity DNA Polymerase was preferred. Ratios of the PCR components in the mixture (Table 2.3) and reaction conditions (Table 2.5) were added as the official protocol suggested (Biolabs, n.d.). The reaction conditions for the 68G/C (Cys23Ser) polymorphism (Table 2.6) were sourced from Segman et al.'s (1997) study. To amplify the fragment covering the 68G/C site, the PCR reaction mixture ratios were replicated according to the official protocol provided with DreamTaq DNA Polymerase in the initial experiments. In later trials, it was optimized to its final form (Table 2.4) for the best results. At the end of the reactions, the contents were either used for subsequent experimental steps or refrigerated at -20 ^{o}C for storage.

Table 2.3. PCR mixture components and ratios for the -759C/T and -697G/C polymorphisms of the 5-HT2CR.

Ingredient	**Volume (µL)**	**Final Concentration**
Q5 High-Fidelity Master Mix	10	1X
Forward primer (10 µM)	1	0.5 µM
Reverse primer (10 µM)	1	0.5 µM
Template DNA (~15 ng/µL)	2.0	30 ng
NFW	6	
Total volume	**20 µL**	

Table 2.4. PCR mixture components and ratios for the 68G/C (Cys23Ser) polymorphism of the 5-HT2CR.

Ingredient	Volume (µL)	Final Concentration
10X DreamTaq Buffer*	2.0	1X
dNTP mix (10 µM each)	0.4	0.2 µM each
Forward primer (10 µM)	0.2	0.1 µM
Reverse primer (10 µM)	0.2	0.1 µM
Template DNA (~15 ng/µL)	2.0	30 ng
DreamTaq DNA Polymerase (5 U/µL)	0.2	1 U
NFW	15	
Total volume	**20 µL**	

*includes 20 mM $MgCl_2$

Table 2.5. PCR conditions for the -759C/T and -697G/C polymorphisms of the 5-HT2CR.

Stage	Temperature (^oC)	Duration	
Initial Denaturation	98	30 sec.	
Denaturation	98	10 sec.	x 35 cycles
Annealing	67	10 sec.	
Extension	72	10 sec.	
Final Extension	72	2 min.	

Denaturation, annealing and extension stages are cycled 35 times.

Table 2.6. PCR conditions for the 68G/C (Cys23Ser) polymorphism of the 5-HT2CR.

Stage	Temperature (^oC)	Duration	
Initial Denaturation	94	5 min.	
Denaturation	94	30 sec.	x 35 cycles
Annealing	57	30 sec.	
Extension	72	30 sec.	
Final Extension	72	4 min.	

Denaturation, annealing and extension stages are cycled 35 times.

2.2.4 Restriction Enzyme Digestion

Restriction enzymes or restriction endonucleases were integrated into the pool of scientific knowledge through the studies of Arber's, Meselson's, and Smith's laboratories investigating *Escherichia coli* and their defense mechanism against bacteriophages to cleave viral DNA in the 1970s (Smith & Welcox, 1970). These so-called molecular scissors cut double-stranded DNA at or near a specific sequence called recognition sites. The use of restriction endonucleases paved the road for recombinant DNA technology such as DNA cloning, genomic mapping, and epigenetic modification identification. In 1978, Arber, Nathans, and Smith were awarded the Nobel Prize in Physiology or Medicine "for the discovery of restriction enzymes and their application to problems of molecular genetics". The restriction endonucleases fall into four categories according to their structure, cleavage site properties, and cofactors requirements, namely type I, II, III, and IV (Table 2.7).

Table 2.7. Types of restriction endonucleases and their features.

	Type I	Type II	Type III	Type IV
Nuclease structure	Multimer; heterotrimer	Homodimer	Homodimer	
Recognition site pattern	Two sites, in any orientation	Small (4–8 bp); usually palindromic	Two sites, in head-to-head orientation; non-palindromic	Weak specificity
Cleavage site	Variable distance from recognition site; non-specific cleavage	Cleavage within (Type IIP) or outside (Type IIS) the recognition site	Cleavage of one strand (nicking activity) 24–25 bp from recognition site	Methylated only
Cofactor	ATP, Mg2+, SAM	Mg2+	ATP, Mg2+, SAM	ATP, GTP

ATP, adenosine triphosphate; GTP, guanosine triphosphate; Mg, magnesium; SAM, s-adenyl methionine (Snapgene, n.d.).

Exemplary restriction enzymes, their restriction sites, and cleavage patterns are shown in Table 2.8. Currently, there are more than 5,000 characterized restriction endonucleases listed on The Restriction Enzyme Database (REBASE) (Roberts et al., 2022). The nomenclature of the endonuclease is made up of the organism and the strain it is isolated from, and the Roman numeral indicates the order of

discovery. For example: EcoRI stands for **E**scherichia **co**li, **R**Y13 strain, discovery order **I.** Some restriction endonucleases, such as BamHI and EcoRI, leave “sticky ends” after restriction, which means overhanging 3' and 5’ ends. The restriction product resulting from the reaction requires matching strands, however they are more efficient in further ligation reactions. On the other hand, some restriction endonucleases, such as HaeIII, leave “blunt ends” that require no complementary strands, but the ligation reaction of products would be slower.

Table 2.8. Examples of restriction enzymes, their recognition sites, and cutting patterns.

restriction enzyme	restriction site
BamH I	5' G|GATCC 3' 3' CCTAG|G 5'
EcoR I	5' G|AATTC 3' 3' CTTAA|G 5'
Hae III	5' GG|CC 3' 3' CC|GG 5'
Hha I	5' G|CGC 3' 3' CGC|G 5'
Hind III	5' A|AGCTT 3' 3' TTCGA|A 5'

Green lines indicate cleavage pattern (Isaac Physics, n.d.)

In this study, two restriction enzymes were selected to differentiate the polymorphic allele variants. To identify polymorphic alleles of the promoter SNPs -759C/T and -697G/C, SsiI (AciI) was selected. For the coding region polymorphism 68G/C (Cys23Ser), restriction endonuclease HinfI was selected. Components of the restriction enzyme mixtures (Table 2.9 and Table 2.10) were thawed on ice and added into PCR tubes in order from largest to smallest volume in a sterile environment. The contents of the tubes were mixed gently by pipetting. A tube was prepared by replacing the PCR product with an equal amount of NFW in each batch of reaction preparation to check contamination. In addition, another tube was prepared with a known DNA sample containing cleavage site(s) for the enzyme, which produces a known restriction pattern to verify the integrity of the restriction enzyme. The tubes were incubated at 37 ^{o}C for 1 hour in a dry block

heater (Ota et al., 2007; Malhotra et al., 1996; Iordanidou et al., 2008). At the end of the procedure, the contents were either loaded onto agarose gel for electrophoresis or refrigerated at -20 ^{o}C for storage.

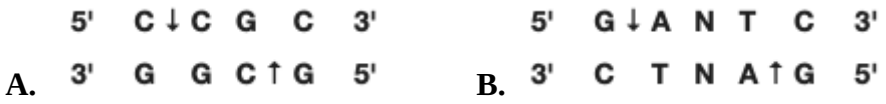

Figure 2.2. Recognition and cleavage sites of (A) SsiI (AciI) and (B) HinfI. Arrows indicate locations of the cuts.

Table 2.9. Restriction enzyme digestion reaction for the -759C/T and -697G/C polymorphisms of the 5-HT2CR.

Ingredient	**Volume (µL)**	**Final Concentration**
10X Buffer O	1	1X
SsiI (AciI) (10 U/µL)	1	1 U
PCR product	6	
NFW	2	
Total volume	**10 µL**	

Table 2.10. Restriction enzyme digestion reaction for the 68G/C (Cys23Ser) polymorphism of the 5-HT2CR.

Ingredient	**Volume (µL)**	**Final Concentration**
10X CutSmart Buffer	1	1X
HinfI (10 U/µL)	1	1 U
PCR product	6	
NFW	2	
Total volume	**10 µL**	

2.2.5 Qualification of Genomic DNA Samples by Agarose Gel Electrophoresis

Agarose gel electrophoresis is a commonly used method to separate and visualize nucleic acid fragments based on size. This method utilizes the negative electrical charge of the phosphate backbone of nucleic acids to move them through an agarose gel. Agarose is a polymer produced by *Gelidium* and *Gracilaria* genus seaweed which is made up of repeating L-galactose and D-galactose molecules (Lee et al. 2012). The gel can be prepared in various concentrations to vary in density and pore sizes. The adjustment of the gel's density is selected depending on the size of the nucleic acid fragments, with denser gels preferred for smaller fragments to increase resolution and vice versa. To visualize the nucleic acids, a fluorescent chemical, such as ethidium bromide (EtBr), selected for its binding affinity to nucleic acids is added for visualization before the polymer solidifies. Ethidium bromide signals the presence of nucleic acids by fluorescing under the ultraviolet (UV) light. The gel is placed in a tank filled with a buffer, which maintains appropriate ionic properties and pH, and subjected to an electrical current. At the end of the electrophoresis, nucleic acids migrate toward the positive electrode and form distinct band patterns. The gel is placed under UV light to visualize migration patterns. The bands are compared with a ladder (Figure 2.3) containing fragments of known sizes to determine the sizes of the fragments in the sample.

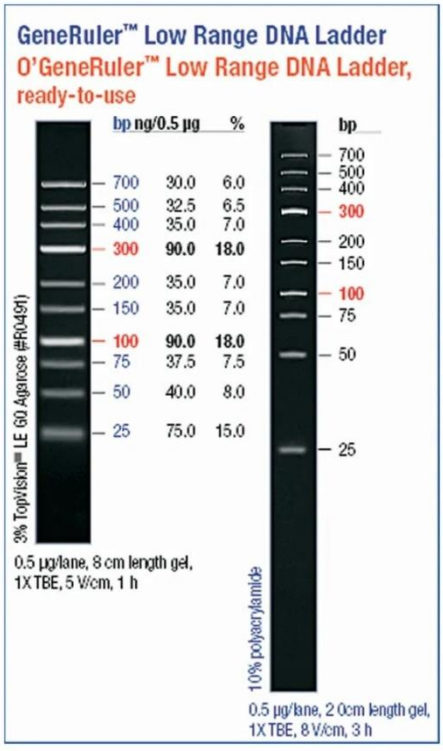

Figure 2.3. Band pattern of GeneRuler Low Range DNA Ladder on 3% agarose gel run in 1X TBE at 5V/cm for 1 hour, and on 10% polyacrylamide gel run in 1X TBE at 8 V/cm for 3 hours.

In this study, the following conditions were applied: 0.5 µL EtBr (10 mg/ml), 100 Volt (V) for 1 hour (5 V/cm), 1X TBE (Tris-Boric Acid-EDTA), 1X Thermo Scientific GeneRuler Low Range DNA Ladder, agarose gel with a 2.5% (weight/volume, g/mL) concentration. 10X TBE electrophoresis buffer stocks were prepared as shown in Table 2.11 and stored at room temperature, away from direct sunlight, for up to 6 months. 1X TBE electrophoresis buffer was prepared from aforementioned stocks by diluting them 1:10 with RNase-free water.

Table 2.11. 10X TBE electrophoresis buffer components.

Ingredients	**Amount (g per 1 L)**	**Final concentration (M)**
Boric acid	61.8	1
EDTA disodium salt	7.4	1
Trizma base	121.1	0.02

Agarose gel electrophoresis procedures were performed using Thermo Scientific Owl EasyCast B1 Mini Gel Electrophoresis Systems (Thermo Fisher Scientific, Waltham, Massachusetts, USA). Casting trays were taped along the open sides to prevent leakage of molten agarose and combs were placed before starting the following steps. To prepare 2.5% agarose gel, 2.5 g of agarose was added to an Erlenmeyer flask containing 100 mL 1X TBE buffer. The mixture was microwaved until the agarose particles were completely dissolved, which took approximately 2 min. The agarose solution was cooled to approximately 50 ^{o}C while swirling for uniform heat distribution. Five µL of EtBr working solution was added to the agarose solution and thoroughly mixed by swirling to obtain 0.5 µg/ml final concentration. The solution was poured slowly from a corner of the tray. Air bubbles were removed with a pipette tip before the gel solidified. The tray was left to sit for approximately 30 min. until the gel completely solidified.

The tank was filled with 1X TBE buffer up to the indicated line. The combs and tapes were removed from the tray, which was then placed inside the tank. Attention was paid to align migration direction, wells oriented from – (cathode) to + (anode) side. Two and a half µL of ladder was loaded into the first well. For the following

wells, 5 µL of PCR product, restriction digestion, or control samples were mixed with 1 µL 6X loading dye to reach a final concentration of 1X loading dye. Lastly, the mixtures were loaded into the gel wells.

The tank lid was closed and the electrical cords were plugged to the power supply. The power supply was set to 100 V for 1 hour (5 V/cm) and turned on. After the run, the gel was carefully taken out from the tray and placed into Peqlab Vilber Lourmat ST4 1100 Gel Documentation Imaging System (Peqlab, Erlangen, Germany). Images of the band patterns were captured and labeled. Agarose gel electrophoresis step was conducted twice for each patient sample and polymorphic site. The first was to ensure accurate amplification of the expected fragment in the PCR product, and the second was to visualize restriction products.

2.2.6 Genotyping of 5-HT2CR Polymorphic Alleles

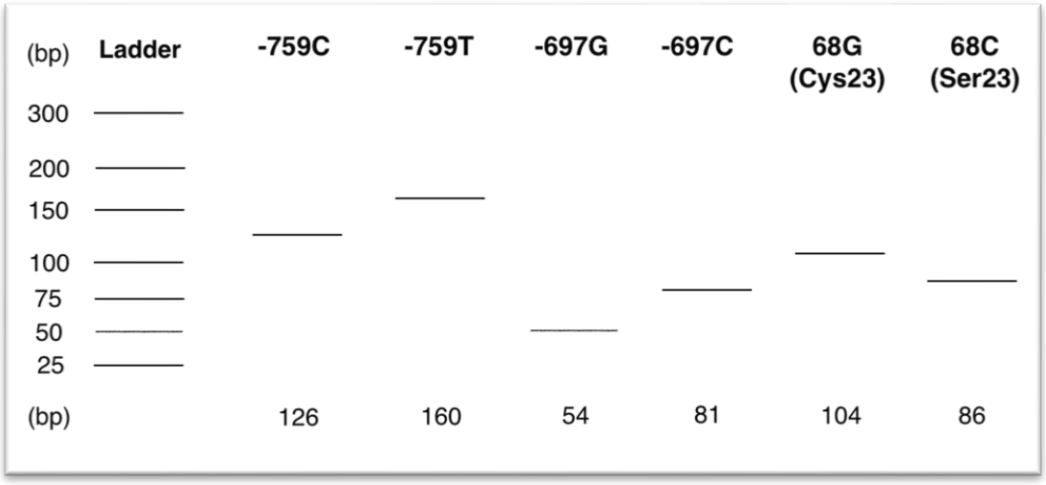

Figure 2.4. Representation of identifying band patterns expected after successful PCR-RFLP procedures for 5-HT2CR polymorphisms. The common allele -759C for the -759C/T polymorphism yields a band of 126 bp in length; the variant allele -759T yields a 160 bp band. The common allele -697G for the -697G/C polymorphism produces a band of 54 bp in length; the variant allele -697C produces an 81 bp band. The common allele 68G (Cys23) for the 68G/C (Cys23Ser) polymorphism results in a band of 104 bp in length; the variant allele 68C (Ser23) results in an 86 bp band. Bands are illustrated in comparison with the DNA ladder used in the experiments. Numbers indicate the fragment lengths in base pairs (bp).

Successful PCR amplification of the promoter region polymorphisms -759C/T and -697G/C will yield a DNA fragment of 252 bp long (Figure 2.4). Restriction enzyme AciI recognizes four regions in the aforementioned fragment as cleavage sites (Figure 2.2A and Figure 2.4). Two of those sites overlap with the -759C/T and -697G/C SNPs. For both cases, the change of sequence due to variant alleles -759T and -697C will make the sites unrecognizable by the restriction endonuclease. Therefore, samples from carriers of the variant alleles -759T and -697C will produce larger fragments after restriction digestion compared to carriers of the common variant -759C and -697G. Distinct fragment patterns expected after successful restriction digestion are shown in Figure 2.4. Due to two additional cut sites (Figure 2.5) outside of the polymorphic region, fragments of 34, 27, and 11 bp in length are produced in addition to the identifying fragments of 160, 126, 81, and 54 bp in length. However, the former set of bands was not useful for identifying genotypes and produced inconclusive bands with the agarose gel electrophoresis method due to their small size, which was below the minimum resolution.

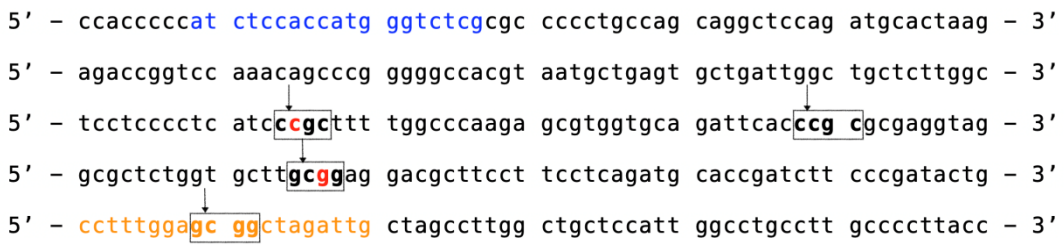

Figure 2.5. Amplified fragment sequence of the promoter polymorphisms -759C/T and -697G/C of the 5-HT2CR. The blue-colored region indicates forward primer sequence; the orange region indicates reverse. Cleavage sites for SsiI (AciI) are shown as boxes and in bold letters. Arrows indicate where the restriction endonuclease cuts the DNA. The sequence is represented with the common variants in the SNP positions (-759C and -697G, respectively, in red letters). SsiI (AciI) does not recognize transition from -759C to -759T or -697G to -697C.

Amplification of the genic polymorphic site 68G/C (Cys23Ser) yielded a DNA fragment of 104 bp in length (Figure 2.6). Although the amplified sequence does not contain any cleavage sites for the restriction endonuclease HinfI (Figure 2.2B), it was introduced into the fragment by changing C to G at the 18th position from the 5' end of the forward primer (Table 2.2 and Figure 2.6). The amplified fragment has only one cleavage site (Figure 2.6). In contrast to the previously

mentioned promoter polymorphisms -759C/T and -697G/C, the 68G/C site is only digested if the sample has variant allele 68C (Ser23) (Figure 2.2B and Figure 2.6). In summary, samples of 68G (Cys23) allele carriers produce 104 bp long fragments, while 68C (Ser23) allele produces two fragments of 86 and 18 bp in length (Figure 2.4). Obtaining an 86 bp fragment is sufficient to identify the presence of the variant allele 68C (Ser23).

Figure 2.6. Amplified fragment sequence of the coding region polymorphism 68G/C (Cys23Ser) of the 5-HT2CR. Blue-colored region indicates forward primer sequence; the orange region indicates the reverse. The cleavage site for HinfI is shown as the box and in bold letters. The arrow indicates where the restriction endonuclease cuts the DNA. The sequence is represented with the common variants in the SNP position (68G in red letter). HinfI recognizes and cuts 68G to 68C transition.

2.2.7 Statistical Analysis

Demographic variables baseline BMI (kg/m^2) and age (years) were compared with the Mann-Whitney *U* test. The distribution of the SNPs between sexes was compared with Fisher's exact test. Percent BMI change was used to compare proportional weight change from the initial measurement to 4 weeks after starting antidepressant treatment. Normality tests were conducted using the Shapiro-Wilk test. Significant weight change differences associated with allele and SSRI use were tested using the Mann-Whitney *U* test. Cut-off values of 5% and 7% BMI change were selected as the indicator of significant weight gain (Godlewska et al., 2009; Ryu et al., 2007; Uguz et al., 2015). Weight changes for each cut-off value were compared with polymorphic sites using Fisher's exact test. The numerical values were rounded to two decimal points. The mean statistics were given with standard deviation (SD) (mean±SD) and the median statistics were given with interquartile range (IQR) (median±IQR). Any value below $Q1 - 1.5 \times IQR$ or above $Q3 + 1.5 \times IQR$ was classified as an outlier, with $Q1$ being the first quartile and $Q3$ the third quartile. Statistical significance criteria was p-values less than

0.05. Power analyses were performed with the G*Power software version 3.1. All statistical calculations and plots were produced using the R software version 4.4.1.

CHAPTER 3

RESULTS

3.1 Study Population

Initially, a total of 61 patients chose to participate in the study. They were expected to revisit the clinic 4 weeks after starting treatment. The study required two weight measurements taken 4 weeks apart to analyze changes in BMI during medication use. However, only 29 (47.5%) participants, out of the 61, returned to the clinic for a second visit. Two of them returned only after 4 months, making comparison with other participants impossible. Therefore, 27 (44.3%) (n=27) patients from the initial population generated eligible data (Appendix C).

In our study population, the mean age of the participants was 35.81±10.90 (19-64) with a median of 35±15. The number of female (F) participants was 18 (67%), which was double the number of male (M) participants, who totaled 9 (33%). The mean age in the female group was 35.11±11.49 years (19-64) with a median of 33.50±16 years, while the mean was 37.22±10.10 years (19-54) with a median of 38±9 years (Figure 3.1) in the male group. The mean baseline BMI for all participants was 26.80±6.32 kg/m^2 (16.53-40.46) with a median of 26.99±9.33 kg/m^2. For females, the mean BMI was 26.16±6.03 kg/m^2 (16.53-40.27) with a median of 26.48±10.34 kg/m^2, while for males, it was 28.10±7.04 kg/m^2 (18.78-40.46) with a median of 28.09±9.71 kg/m^2 (Figure 3.2). The healthy BMI range is considered between 18.5 and 24.9 kg/m^2. Among the participants, 16 (59%; 10F, 6M) of the participants were classified as overweight, 9 (33%; 6F, 3M) who were classified as obese (BMI above 30), and 1 (4%) female participant was classified as underweight. Four different SSRIs were prescribed: 10 (37%) patients received

fluoxetine HCl, 10 (37%) received escitalopram oxalate, 4 (15%) received sertraline HCl, and 3 (11%) received paroxetine HCl tablets (Table 3.1).

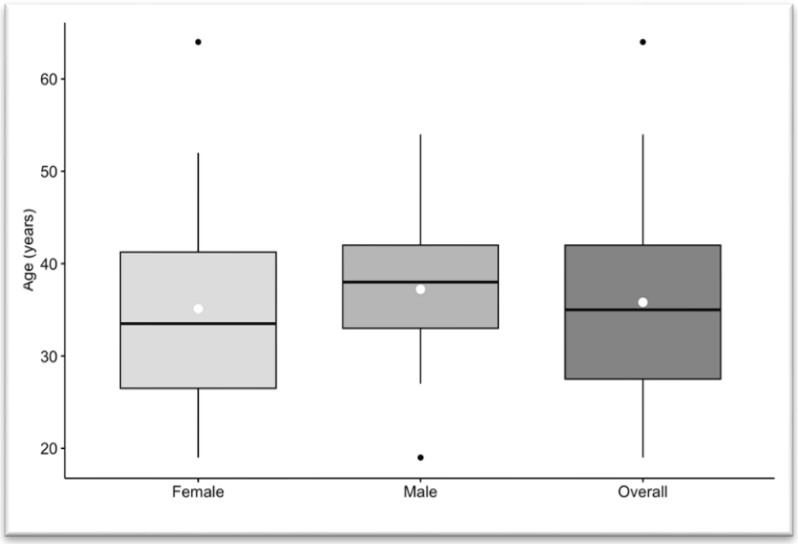

Figure 3.1. Age distribution of participants. The data shows an approximately normal distribution across all groups, centered around the age of 35 years, with males tending to be slightly older. Black dots indicate outliers; white dots indicate the mean.

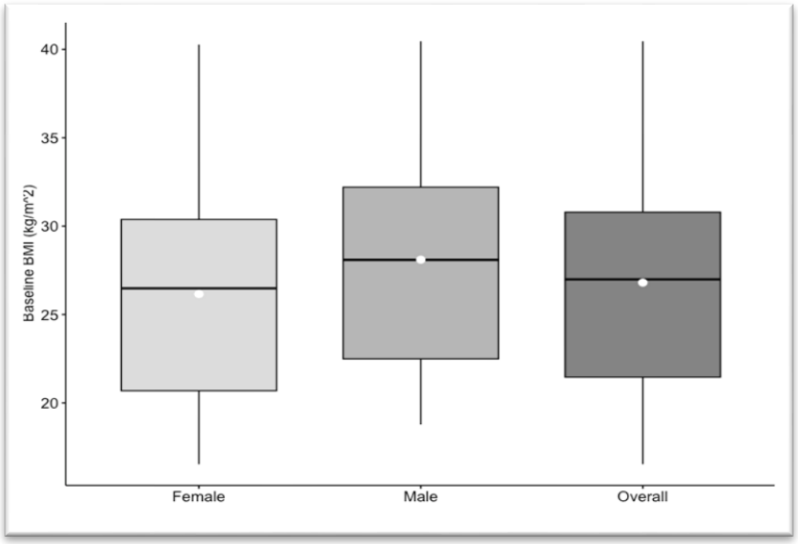

Figure 3.2. Baseline BMI (kg/m^2) distribution among participants. The majority of the study group had BMI values exceeding healthy ranges, with 59% classified as overweight and 33% as obese. The data followed an approximately normal distribution, centered around a mean of 27 kg/m^2, with males tending to have slightly higher BMI values. White dots indicate the mean.

Table 3.1. Substance and dosage information of prescribed SSRIs.

Drug	Dosage (mg/day)	Patient Count
Escitalopram	10 to 20	10
Fluoxetine	20	10
Paroxetine	10	3
Sertraline	25 to 50	4
Total		**27**

3.2 Analysis of 5-HT2CR Polymorphisms in Study Participants

To investigate the allelic and genotypic distribution of the -759C/T (rs3813929), -697G/C (rs518147), and 68G/C (Cys23Ser, rs6318) polymorphisms of the serotonin 2C receptor, PCR and subsequent restriction digestion with appropriate endonucleases were performed. The following figures demonstrate the agarose gel images corresponding to the analysis of the 5-HT2CR polymorphisms conducted on patient samples (Figure 3.3, Figure 3.4, Figure 3.5, and Figure 3.6).

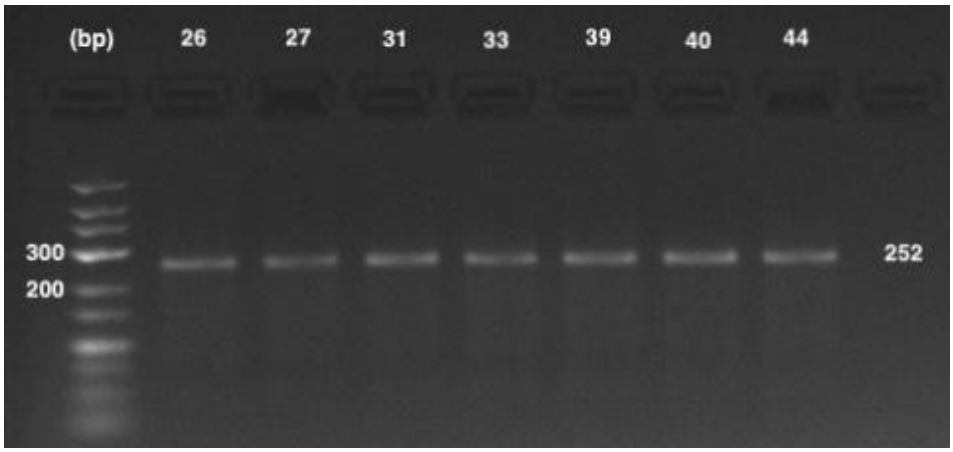

Figure 3.3. Agarose gel image of PCR products for the -759C/T and -697G/C polymorphisms of the 5-HT2CR. The numbers on the wells correspond to the patient numbers assigned to the samples. The numbers beside bands indicate fragment lengths in base pairs (bp). The target sequence, with an expected length of 252 bp, was detected in all samples.

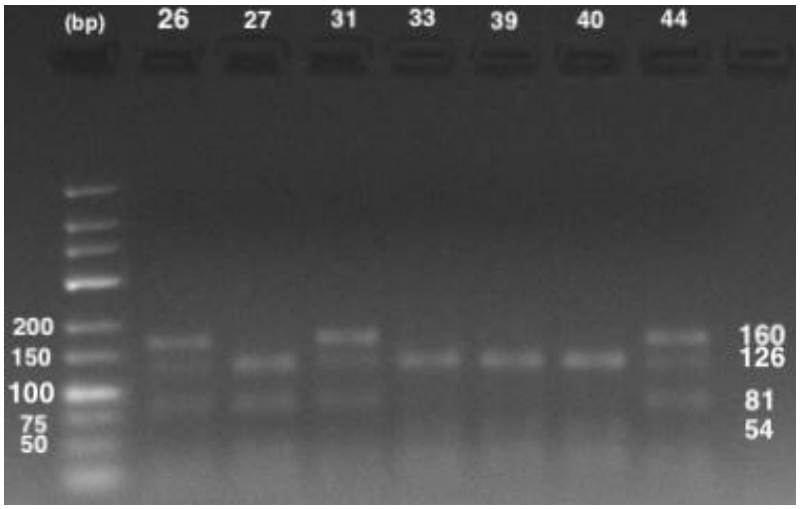

Figure 3.4. Agarose gel image of the restriction digestion products of the amplified -759C/T and -697G/C polymorphisms of the 5-HT2CR by SsiI (AciI). The numbers on the wells correspond to the patient numbers assigned to the samples. The numbers beside bands indicate fragment lengths in base pairs (bp). 160 bp bands in sample 26, 31, and 44 indicate presence of the variant allele -759T; 81 bp bands on sample 26, 27, 31, and 44 indicate presence of the variant allele -697C.

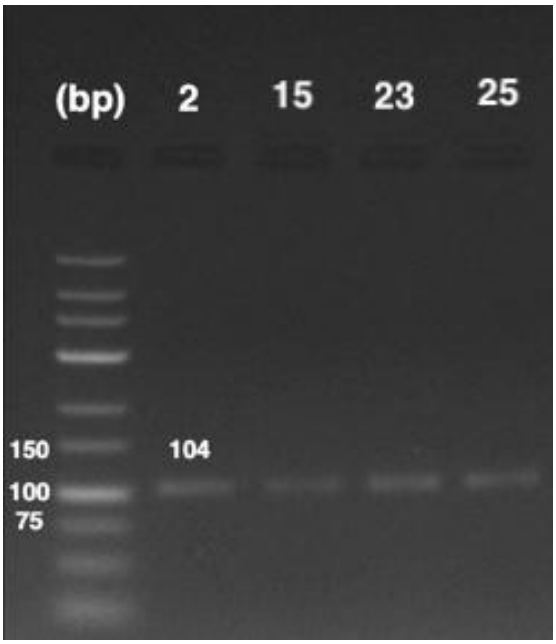

Figure 3.5. Agarose gel image of PCR product for the 68G/C (Cys23Ser) polymorphism of the 5-HT2CR. The numbers on the wells correspond to the patient numbers assigned to the samples. The numbers beside bands indicate fragment lengths in base pairs (bp). The target sequence, with an expected length of 104 bp, was detected in all samples.

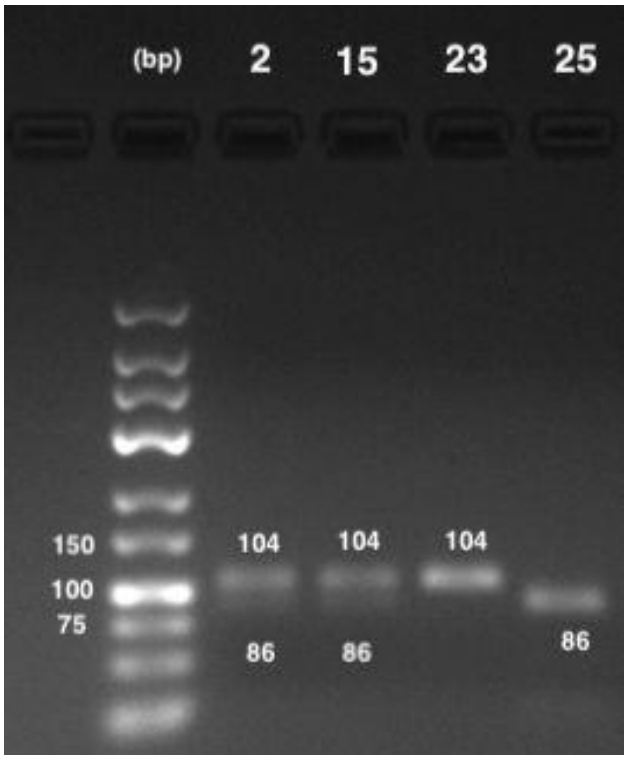

Figure 3.6. Agarose gel image of the restriction digestion products of the amplified 68G/C (Cys23Ser) polymorphism of the 5-HT2CR by HinfI. The numbers on the wells correspond to the patient numbers assigned to the samples. The numbers beside bands indicate fragment lengths in base pairs (bp). 86 bp bands in sample 2, 15, and 25 indicate presence of the variant allele 68C (Ser23).

The serotonin 2C receptor gene (*HTR2C*) is located on the X chromosome. Therefore, males are hemizygous with only one copy of the gene. Allelic distribution of the -759C/T (rs3813929), -697G/C (rs518147), and 68G/C (Cys23Ser, rs6318) polymorphisms of the 5-HT2CR is illustrated in Figure 3.7. The distribution of the -759C/T genotypes are shown in Table 3.2. Of the 27 participants, 7 (26%) carried the variant allele T, while 20 (74%) did not. Among the 9 men, 2 (22%) had the variant allele T, whereas 5 out of 18 (28%) women were carriers of the -759T. All women carrying the variant allele were heterozygous (CT). Deviation from Hardy-Weinberg equilibrium and linkage disequilibrium could not be calculated due to small sample size.

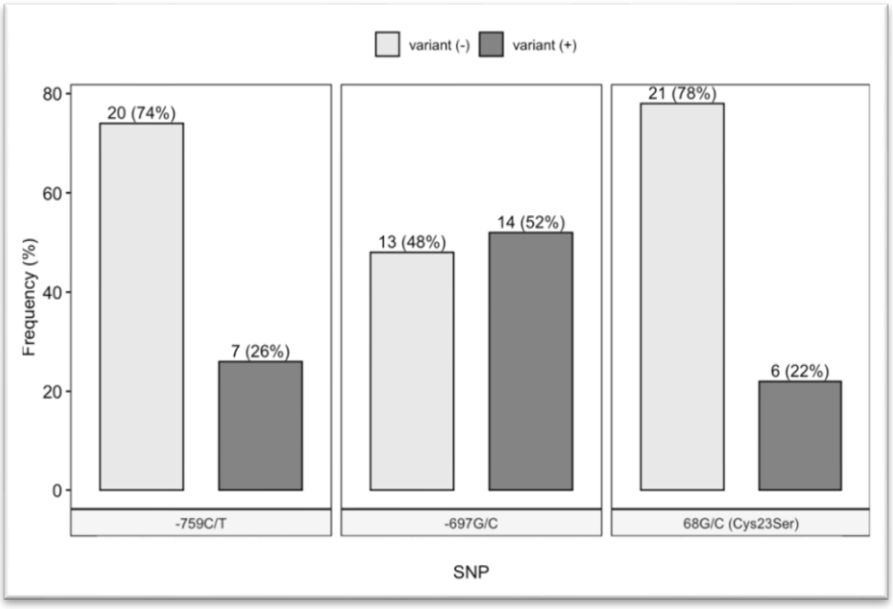

Figure 3.7. Allelic distributions of the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms of the 5-HT2CR among participants. The -759T variant allele was carried by 26% of participants, with all female carriers being heterozygous. The -697C variant allele was unexpectedly more common, found in 52% of the study population, diverging from previous literature. The 68C (Ser23) variant allele was present in 22% of participants. Overall, these statistics were similar to the only other research conducted in the Turkish population (Yücel et al., 2015). The data was obtained using PCR-RFLP from patient samples.

For the -759 polymorphic site in the promoter region, the mean age of variant allele T carriers was 31.43±12.59 years with a median of 26±20 years, while non-carriers had a mean age of 37.35±10.14 years with a median of 36±9 years. The mean baseline BMI for variant allele T carriers was 27.11±5.75 kg/m^2 with a median of 30.48±11.70 kg/m^2, while non-carriers had a mean baseline BMI of 26.70±6.64 kg/m^2 with a median of 26.48±8.61 kg/m^2 (Figure 3.8).

Table 3.2. Distribution of the 5-HT2CR -759C/T polymorphism genotypes among participants.

-759C/T	**CC/C-**	**CT**	**TT/T-**	**Total**
Male	7 (26%)	-	2 (7%)	9 (33%)
Female	13 (48%)	5 (19%)	-	18 (67%)
Total	20 (74%)	5 (19%)	2 (7%)	27 (100%)

There were no statistically significant differences in age, sex or baseline BMI values between variant carriers and non-carriers (Table 3.3). Mann-Whitney *U* tests

resulted in p=0.17 and *U*=95.5 for age, and p=0.64 and *U*=61 for baseline BMI. Fisher's exact test showed no significant difference in the distribution of the variant allele between sexes (p=1, odds ratio (OR)=0.75 and 95% confidence interval (CI)=(0.06, 6.27)). Overall, the demographic characteristics did not differ between -759T variant allele carriers and non-carriers in the study population.

Table 3.3. Demographic characteristics of participants in relation to the presence (+) or absence (-) of the variant allele -759T for the -759C/T polymorphism of the 5-HT2CR.

	-759T (+)	**-759T (-)**	**Statistics**
Mean Age (years)	31.43±12.59	37.35±10.14	p=0.17
Median Age (years)	26±20	36±9	*U*=95.5
Sex	2M, 5F	7M, 13F	p=1 OR=0.75 95% CI=(0.06, 6.27)
Mean Baseline BMI (kg/m^2)	27.11±5.75	26.70±6.64	p=0.64
Median Baseline BMI (kg/m^2)	30.48±11.70	26.48±8.61	*U*=61

Age and baseline BMI were analyzed with the Mann-Whitney *U* test; sex distribution was analyzed with Fisher's exact test. F, female; M, male; OR, odds ratio.

The distribution of the -697G/C genotypes are shown in Table 3.4. Out of 27 participants, 14 (52%) carried the variant allele C, while 13 (48%) did not. Among the 9 men, 5 (56%) had the variant allele C, while 10 out of 18 (56%) women were carriers of the -697C. Only one of the female subjects was heterozygous (GC) for the variant allele, while 9 females were homozygous for C allele. Deviation from Hardy-Weinberg equilibrium and linkage disequilibrium could not be calculated due to small sample size.

Table 3.4. Distribution of the 5-HT2CR -697G/C polymorphism genotypes among participants.

-697G/C	**GG/G-**	**GC**	**CC/C-**	**Total**
Male	5 (19%)	-	4 (15%)	9 (33%)
Female	8 (30%)	1 (4%)	9 (33%)	18 (67%)
Total	13 (48%)	1 (4%)	13 (48%)	27 (100%)

For the -697G/C polymorphic site in the promoter region, the mean age of variant allele C carriers was 34.29±13.50 years with a median of 33±18 years, while non-carriers had a mean age of 37.46±7.37 years with a median of 38±9 years. The mean baseline BMI for variant allele C carriers was 29.29±6.63 kg/m^2 with a median of 30.26±7.79 kg/m^2, while non-carriers had a mean baseline BMI of 24.13±4.88 kg/m^2 with a median of 22.49±7.96 kg/m^2 (Figure 3.8).

There were no statistically significant differences in age or sex values between variant carriers and non-carriers (Table 3.5). Mann-Whitney *U* tests resulted in p=0.21 and *U*=117 for age. Fisher's exact test showed no significant difference in the distribution of the variant allele between sexes (p=0.69, OR=0.65 and 95% CI=(0.09, 4.22)). However, baseline BMI measurements differed significantly between the groups, with -697C carriers having 5.16 kg/m^2 higher BMI values than non-carriers (p=0.04, U=49).

Table 3.5. Demographic characteristics of participants in relation to the presence (+) or absence (-) of the variant allele -697C for the -697G/C polymorphism of the 5-HT2CR.

	-697C (+)	**-697C (-)**	**Statistics**
Mean Age (years)	34.29±13.50	37.46±7.37	p=0.21
Median Age (years)	33±18	38±9	*U*=117
Sex	4M, 10F	5M, 8F	p=0.69 OR=0.65 95% CI=(0.09, 4.22)
Mean Baseline BMI (kg/m^2)	29.29±6.63	24.13±4.88	**p=0.04***
Median Baseline BMI (kg/m^2)	30.26±7.79	22.49±7.96	*U*=49

*Baseline BMI is significantly different between the groups (p<0.05). Age and baseline BMI were analyzed with the Mann-Whitney *U* test; sex distribution was analyzed with Fisher's exact test. F, female; M, male; OR, odds ratio.

The distribution of the 68G/C (Cys23Ser) genotypes are shown in Table 3.6. Of the 27 participants, 6 (22%) carried variant allele C, while 21 (78%) did not. Among the 9 men, 2 (22%) had the variant allele C, while 4 out of 18 (22%) women were carriers of the 68C (Ser23). All women carrying the variant allele were

heterozygous (GC). Deviation from Hardy-Weinberg equilibrium and linkage disequilibrium could not be calculated due to small sample size.

Table 3.6. Distribution of the 5-HT2CR 68G/C polymorphism genotypes among participants.

68G/C	GG/G-	GC	CC/C-	Total
Male	7 (26%)	-	2 (7%)	9 (33%)
Female	14 (52%)	4 (15%)	-	18 (67%)
Total	21 (78%)	4 (15%)	2 (7%)	27 (100%)

For the 68G/C polymorphic site in the coding region, the mean age of variant allele C (Ser) carriers were 37.83±16.02 years with a median value of 34.50±21 years, while non-carriers had a mean age of 35.24±9.42 years with a median value of 35±14 years. The mean baseline BMI for variant allele C (Ser) carriers was 30±6.57 kg/m^2 with a median value of 28.52±10.31 kg/m^2, while non-carriers had a mean baseline BMI of 25.89±6.09 kg/m^2 with a median value of 25.97±10.34 kg/m^2 (Figure 3.8).

Table 3.7. Demographic characteristics of participants in relation to the presence (+) or absence (-) of the variant allele 68C for the 68G/C polymorphism of the 5-HT2CR.

	68C (+)	68C (-)	Statistics
Mean Age (years)	37.83±16.02	35.24±9.42	p=0.88
Median Age (years)	34.50±21	35±14	*U*=42
Sex	2M, 4F	7M, 14F	p=1 OR=1 95% CI=(0.07, 9.20)
Mean Baseline BMI (kg/m^2)	30±6.57	25.89±6.09	p=0.24
Median Baseline BMI (kg/m^2)	28.52±10.31	25.97±10.34	*U*=42

Age and baseline BMI were analyzed with the Mann-Whitney *U* test; sex distribution was analyzed with Fisher's exact test. F, female; M, male; OR, odds ratio.

There were no statistically significant differences in age, sex or baseline BMI values between variant carriers and non-carriers (Table 3.7). Mann-Whitney *U* tests resulted in p=0.88 and U=42 for age, and p=0.24 and *U*=42 for baseline BMI.

Fisher's exact test showed no significant difference in the distribution of the variant allele between sexes (p=1, OR=0.1 and 95% CI=(0.07, 9.20)). Overall, the demographic characteristics did not differ between variant allele 68C (Ser23) cariers and non-carriers in the study population.

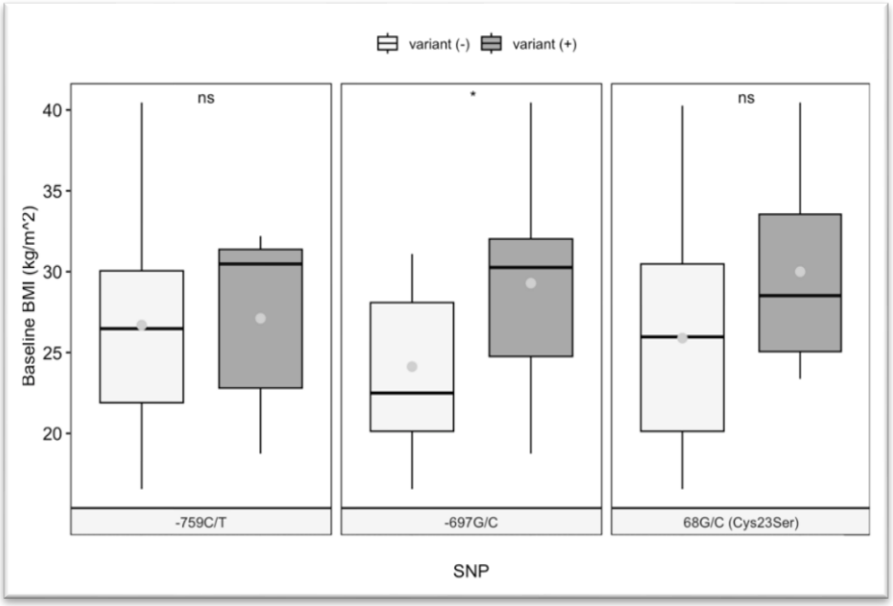

Figure 3.8. Distribution of baseline BMI (kg/m^2) among participants by presence or absence of variant alleles for the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms of the 5-HT2CR. On average, variant allele carriers for all polymorphisms had higher BMI values, with a significant difference in -697C carriers, who had a mean difference of 5.16 kg/m^2 and a median difference of 7.77 kg/m^2 compared to non-carriers (p=0.04). The data can be assumed to follow a normal distribution despite slight asymmetry. Gray dots indicate the mean. ns, non-significant; *, significant.

3.3 Analysis of Weight Change in Study Subjects

This study investigated the effects of the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms of the 5-HT2CR on SSRI-associated weight change. To quantify and compare these effects, the percent (%) BMI change among patients over the 4 weeks of SSRI treatment was analyzed. Distribution of BMI change among participants by presence or absence of the -759C/T, -697G/C and 68G/C (Cys23Ser) variant alleles is illustrated in Figure 3.10. The mean BMI change for all participants was 1.21±6.24% (-7.69-18.67), with a median value of 0±5.94%.

For females, the mean BMI change was 1.03±6.25% (-7.69-18.67) with a median of 0±5.22%, while for males it was 1.59±6.58% (-5.66-16.67) with a median of -0.35±4.69% (Figure 3.9). The difference in weight change between males and females was not statistically significant (p=0.96, *U*=82.5, 95% CI=(-5.05,3.97)).

For the -759C/T polymorphic site in the promoter region, the mean BMI change for variant allele T carriers were -0.98±3.20% with a median of -1.88±2.02%, while non-carriers had a mean BMI change of 1.98±6.90% with a median of 0±6.39% (Table 3.8). The difference in weight change between variant allele T carriers and non-carriers was not statistically significant (p=0.24, *U*=91.5). For the -697G/C polymorphic site in the promoter region, the mean BMI change for variant allele C carriers were 0.40±6.54% with a median -1.20±6.94%, while non-carriers had a mean BMI change of 2.09±6.03% with a median of 0±7.96% (Table 3.8). The difference in weight change between variant allele C carriers and non-carriers was not statistically significant (p=0.22, *U*=65.5). For the 68G/C polymorphic site in the coding region, the mean BMI change for variant allele C carriers were 2.71±9.30% with a median of 1.43±10.56%, while non-carriers had a mean BMI change of 0.79±5.30% with a median of 0±3.12% (Table 3.8). The difference in weight change between variant allele C carriers and non-carriers was not statistically significant (p=0.84, *U*=67).

Table 3.8. Analysis of weight change differences between variant allele carrier and non-carrier subjects.

SNP	*U*	p	95% CI	Mean BMI Change±SD (%)	Median BMI Change±IQR (%)
-759C/T	91.5	0.24	(-2.06, 5.94)	-0.98±3.20 (T+) 1.98±6.90 (T-)	-1.88±2.02 (T+) 0±6.39 (T-)
-697G/C	65.5	0.22	(-5.56, 2.08)	0.40±6.54 (C+) 2.09±6.03 (C-)	-1.20±6.94 (C+) 0±7.96 (C-)
68G/C	67	0.84	(-4.95, 7.24)	2.71±9.30 (C+) 0.79±5.30 (C-)	1.43±10.56 (C+) 0±3.12 (C-)

The Mann-Whitney *U* test was used for analysis. CI, confidence interval; df, degrees of freedom; SD, standard deviation; SNP, single-nucleotide polymorphism.

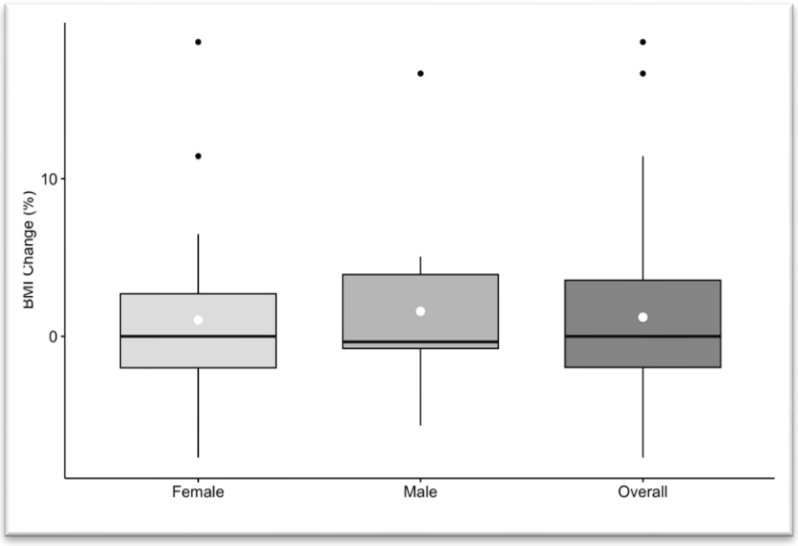

Figure 3.9. BMI change (%) distribution among participants over 4 weeks by sex. No significant BMI change was observed on average, with both mean and median values near 0. The data was skewed and demonstrated a non-normal distribution. Black dots indicate outliers; white dots indicate the mean.

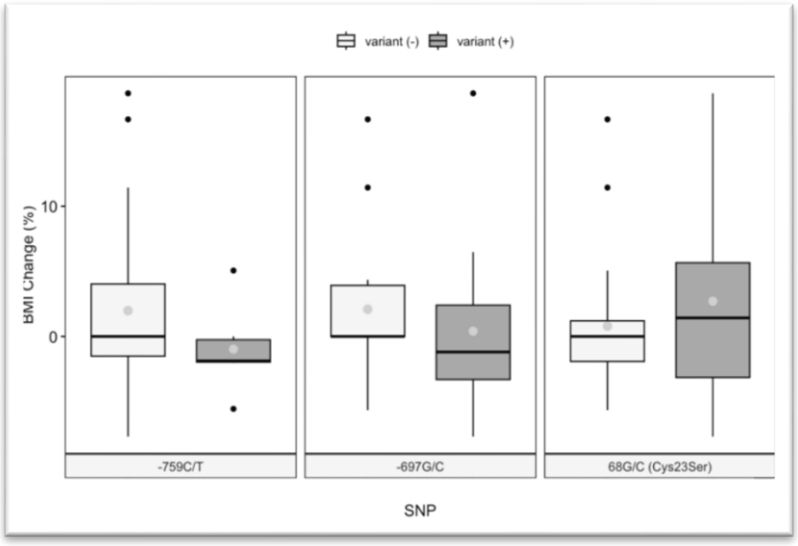

Figure 3.10. Distribution of BMI change (%) among participants by presence or absence of variant alleles for the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms of the 5-HT2CR after 4 weeks of SSRI use. No statistically significant differences were found; however, a trend demonstrated that variant allele non-carriers had a median BMI change of 0%, while variant allele carriers showed noticeable BMI changes in either direction. The data were skewed and non-normally distributed, except for -759T (p=0.32) and 68C (Ser32) (p=0.70) allele carriers. Black dots indicate outliers; gray dots indicate the mean.

3.3.1 Distribution of 5-HT2CR Polymorphisms in Relation to BMI Change Thresholds

Weight changes caused by psychiatric medications have been compared using cut-off values of 5%, 7% and 10% BMI change as significant in similar research (Ryu et al., 2007; Godlewska et al., 2009; Uguz et al., 2015). In our study, we used only 5% and 7% cut-off values, because a 10% BMI change in 4 weeks caused by SSRI treatment was highly unlikely. We also adapted these breakpoints to the negative side to quantify significant weight loss. Table 3.9 shows the distribution of study participants according to weight changes smaller than and equal to, or larger than a 5% and 7% BMI increase, as well as the presence of variant allele groups. Additionally, the table demonstrates Fisher's exact test results for comparing 5-HT2CR polymorphisms -759C/T, -697G/C and 68G/C (Cys23Ser) in these regards. None of the polymorphic sites showed a significant difference in weight gain at the 5% and 7% cut-off values. The p-values were as follows: p=1 for -759C/T, p=1 for -697G/C, and p=0.3 for 68G/C (Cys23Ser) at the 5% cut-off; and p=0.55 for -759C/T, p=0.60 for -697G/C, and p=0.55 for 68G/C (Cys23Ser) at the 7% cut-off.

Table 3.9. Distribution and comparison of variant allele carriers and non-carriers for the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms of the 5-HT2CR between BMI groups smaller than or equal to, or larger than, 5% and 7%.

Allele	≤5%	>5%	Statistics	≤7%	>7%	Statistics
-759T (+)	6	1	p=1 OR=0.68 95% CI=(0.01, 8.97)	7	0	p=0.55 OR=0 95% CI=(0, 7.20)
-759T (-)	16	4		17	3	
-697C (+)	11	3	p=1 OR=1.48 95% CI=(0.14, 21.00)	13	1	p=0.60 OR=0.44 95% CI=(0.01, 9.46)
-697C (-)	11	2		11	2	
68C (+)	4	2	p=0.30 OR=2.86 95% CI=(0.18, 35.56)	5	1	p=0.55 OR=1.85 95% CI=(0.03, 42.91)
68C (-)	18	3		19	2	

CI, confidence interval; OR, odds ratio; Inf, infinity. Fisher's exact test was used.

Table 3.10 shows the distribution of study participants according to weight changes smaller than, or larger than and equal to a -5% and -7% BMI decrease, as well as the presence of variant allele groups. Additionally, the table demonstrates Fisher's exact test results for comparing 5-HT2CR polymorphisms -759C/T, -697G/C and 68G/C (Cys23Ser) in these regards. None of the polymorphic sites showed a significant difference in weight loss at the -5% and -7% cut-off values. The p-values were as follows: p=1 for -759C/T, p=1 for -697G/C, and p=0.55 for 68G/C (Cys23Ser) at the -5% cut-off; and p=1 for -759C/T, p=1 for -697G/C, and p=0.22 for 68G/C (Cys23Ser) at the -7% cut-off.

Table 3.10. Distribution and comparison of variant allele carriers and non-carriers for the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms of the 5-HT2CR between BMI changes smaller than, or larger than or equal to, -5% and -7%.

Allele	<-5%	≥-5%	Statistics	<-7%	≥-7%	Statistics
-759T (+)	1	6	p=1 OR=0.68 95% CI=(0.03, 45.81)	0	7	p=1 OR=Inf 95% CI=(0.01, Inf)
-759T (-)	2	18		1	19	
-697C (+)	2	12	p=1 OR=0.51 95% CI=(0.01, 11.11)	1	13	p=1 OR=0 95% CI=(0, 42.00)
-697C (-)	1	12		0	13	
68C (+)	1	5	p=0.55 OR=0.54 95% CI=(0.02, 37.20)	1	5	p=0.55 OR=1.85 95% CI=(0.03, 42.91)
68C (-)	2	19		0	21	

CI, confidence interval; OR, odds ratio; Inf, infinity. Fisher's exact test was used.

3.3.2 Evaluation of Weight Change in Relation to Administered SSRI

Four different SSRIs were prescribed in this study: escitalopram, fluoxetine, paroxetine and sertraline. Due to the very small sample sizes for paroxetine (n=3) and sertraline (n=4) users, these groups were excluded from the analysis of weight change according to administered SSRI. Both the escitalopram and fluoxetine groups had a sample size of 10 participants. In the escitalopram group, out of 10 participants, 3 had the -759T variant allele, 7 had the -697C variant allele, and 3

had the 68C (Ser23) variant allele. In the fluoxetine group, out of 10 participants, 2 had the -759T variant allele, 5 had the -697C variant allele, and 3 had the 68C (Ser23) variant allele. Escitalopram group had a mean of 1.94±6.38% BMI change and a median of 0±5.12%, while fluoxetine group had a mean 0.77±5.92% BMI change and a median of -0.18±8.8% (Figure 3.11). The Mann-Whitney *U* test for BMI change between the SSRI groups showed no significant difference (p=0.82, *U*=46.5, 95% CI=(-5.67, 5.06)).

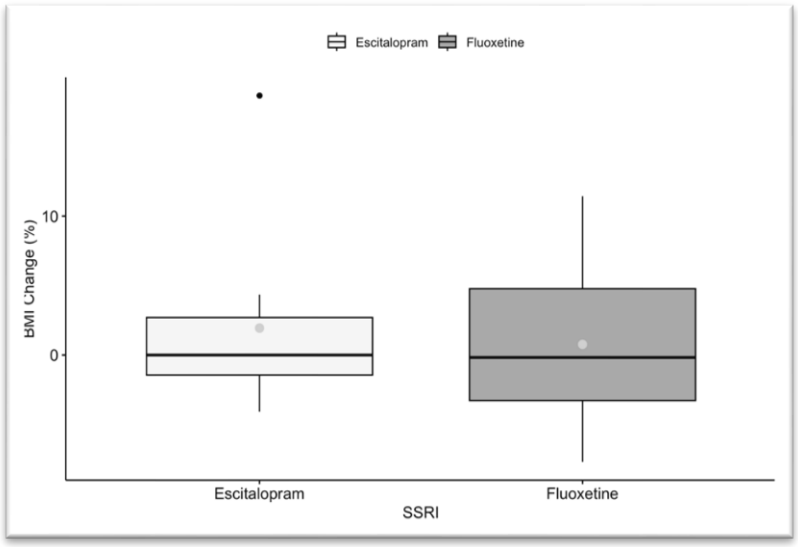

Figure 3.11. Distribution of BMI change (%) among participants by administered SSRI. No significant BMI change was observed on average in both groups, with both mean and median values near 0. The data followed an approximately normal distribution when the outlier in escitalopram groups is excluded. Black dot indicates the outlier; gray dots indicate the mean.

-759T variant allele carriers in the escitalopram group had a mean of -1.97±0.07% BMI change and a median of -1.97±0.1%, while non-carriers had a mean of 2.92±6.84% and a median of 0.6±3.78%. -759T variant allele carriers in the fluoxetine group had a mean of -0.79±5.39% BMI change and a median of -1.88±10.62%, while non-carriers had a mean of 1.44±6.41% and a median of 0±10.22%.-697C variant allele carriers in the escitalopram group had a mean of 2.77±9.29% BMI change and a median of -1.92±5.22%, while non-carriers had a mean of 1.11±1.88% and a median of 0±1.2%. -697C variant allele carriers in the fluoxetine group had a mean of -1.10±5.27% BMI change and a median of -

1.88±10.62%, while non-carriers had a mean of 5.12±5.81% and a median of 3.92±11.43%. 68C (Ser23) variant allele carriers in the escitalopram group had a mean of 5.93±11.62% BMI change and a median of 3.2±22.75%, while non-carriers had a mean of 0.23±2.15% and a median of 0±3.12%. 68C (Ser23) variant allele carriers in the fluoxetine group had a mean of -0.52±7.09% BMI change and a median of -0.35±14.17%, while non-carriers had a mean of 1.32±5.89% and a median of 0±8.8%.

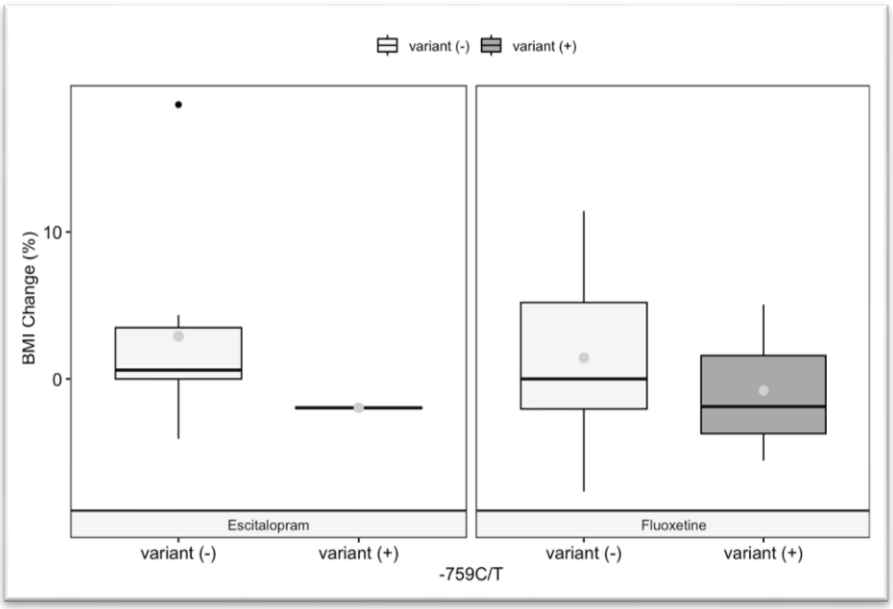

Figure 3.12. Distribution of BMI change (%) among participants by administered SSRI and presence or absence of the variant allele for the -759G/C polymorphism of the 5-HT2CR. No significant BMI change was observed based on variant allele presence or the administered SSRI. A trend of weight loss was noticeable in variant allele carriers in both SSRI groups. Black dots indicate outliers; gray dots indicate the mean.

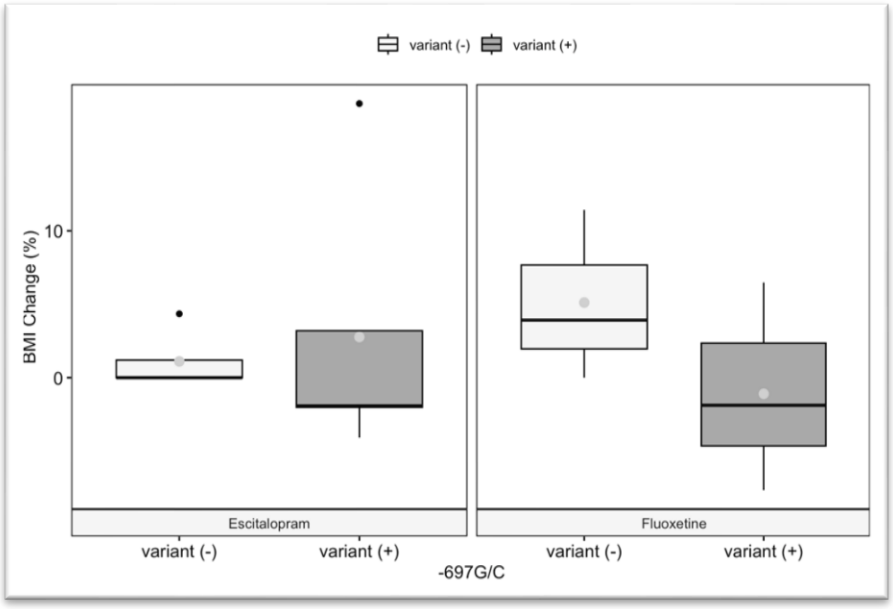

Figure 3.13. Distribution of BMI change (%) among participants by administered SSRI and presence or absence of the variant allele for the -697G/C polymorphism of the 5-HT2CR. No significant BMI change was observed based on variant allele presence or the administered SSRI. A trend of weight loss was noticeable in variant allele carriers in both SSRI groups, with non-carriers in the fluoxetine group tending to gain weight. Black dots indicate outliers; gray dots indicate the mean.

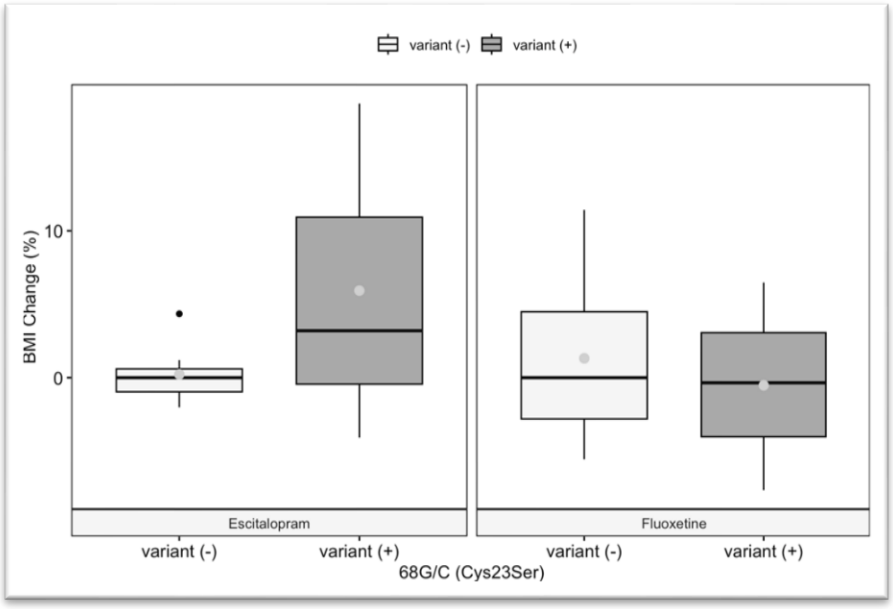

Figure 3.14. Distribution of BMI change (%) among participants by administered SSRI and presence or absence of the variant allele for the 68G/C (Cys23Ser) polymorphism of the 5-HT2CR. No significant BMI change was observed based on variant allele presence or the administered SSRI. A trend of weight gain was noticeable in variant allele carriers in the escitalopram group. Black dots indicate outliers; gray dots indicate the mean.

The weight changes in relation to presence or absence of variant alleles for the 5-HT2CR polymorphisms -759C/T, -697G/C, and 68G/C (Cys23Ser) in both the escitalopram or fluoxetine groups were compared with the Mann-Whitney *U* test. None of the polymorphic sites showed a significant difference in weight change for either SSRI group. The p-values were as follows: p=0.15 (*U*=14, 95% CI=(-2.16, 20.69)) for -759C/T, p=0.53 (*U*=16, 95% CI=(-18.67, 6.27)) for -697G/C, and p=0.64 (*U*=8, 95% CI=(-20.59, 5.28)) for 68G/C (Cys23Ser) in escitalopram group; and p=0.67 (*U*=13, 95% CI=(-8.80, 13.31)) for -759C/T, p=0.18 (*U*=17, 95% CI=(-5.06, 16.99)) for -697G/C, and p=0.83 (*U*=12, 95% CI=(-10.22, 12.75)) for 68G/C (Cys23Ser) for fluoxetine group.

The weight changes between escitalopram and fluoxetine groups were analyzed among each corresponding allele of the 5-HT2CR polymorphisms -759C/T, -697G/C, and 68G/C (Cys23Ser) with the Mann-Whitney *U* test. None of the polymorphic sites showed a significant difference in weight change for either SSRI group. The p-values were as follows: p=0.8 (*U*=2, 95% CI=(-7.08, 3.64)) for -759T, p=0.76 (*U*=20, 95% CI=(-6.98, 13.61)) for -697C, and p=0.75 (*U*=4, 95% CI=(-4.08, 18.67)) for 68C (Ser23) carriers; and p=0.73 (*U*=31.5, 95% CI=(-46.48, 7.69)) for -759T, p=0.43 (*U*=4.5, 95% CI=(-11.43, 4.35)) for -697C, and p=1 (*U*=5, 95% CI=(-2.02, 4.35)) for 68C (Ser23) non-carriers.

CHAPTER 4

DISCUSSION

The significant global escalation in the prevalence of psychiatric disorders has highlighted the issue as a major health problem that needs to be addressed. Major depressive disorder and anxiety disorders lead the list of mental health conditions as the most common ones by a substantial difference (WHO, 2022; McGrath et al. 2023). In the last decade, SSRIs, the first-line medication for aforementioned disorders, have been among the most frequently prescribed drugs on the market. Unfortunately, these therapeutic drugs also come with serious adverse effects.

Weight change, which usually appears as weight loss in acute terms, turns into weight gain in prolonged antidepressant use impacts patient experience in two ways: physically, such as deteriorating health and fitness, and psychologically, such as body dysmorphia. Both aspects reduce quality of life and need addressing. Similar to psychiatric diseases, obesity is undeniably on the rise in the world and Türkiye at substantial rates (TÜİK Kurumsal, n.d.; OECD, n.d.). The serotonin 2C receptor is a prominent serotonin receptor subtype regarding appetite regulation (Voigt & Fink, 2014). The activity of 5-HT2CR is also associated with psychiatric disorders such as major depressive disorder, anxiety disorders, and substance use disorders, in addition to obesity.

The primary mechanism of serotonin reuptake inhibition is common among various SSRI substances; however, their overall pharmacological profiles and physiological effects differ in several subtle ways. Cumulatively, this creates large differences in physiological response unique to patients considering variations in their genetic makeup. In this project, our aim was to investigate if there is any relationship between the serotonin 2C receptor polymorphisms -759C/T (rs3813929), -697G/C (rs51847), 68G/C (Cys23Ser, rs6318) and weight change associated with SSRI use.

Suitable outpatients who applied to the Psychiatric Service of Necmettin Erbakan University, Faculty of Medicine were included with the assistance of medical personnel. Blood samples from the participants were collected to identify their alleles for 5-HT2CR polymorphisms -759C/T, -697G/C, and 68G/C (Cys23Ser) using the PCR-RFLP method. Patient height and weight data before and 4 weeks after the treatment were recorded to calculate their BMI. Another objective of this study was to compare weight change patterns and 5-HT2CR polymorphisms in patients using different SSRIs to investigate if their distinct pharmacological profiles have any effect. Hence, SSRI prescription was not restricted to any specific substance. Our goal in this research was to advance personalized medicine in SSRI use by developing a procedure that could help reduce and prevent weight change as an adverse effect in patients. If certain genotypes or genotype-SSRI combinations produce unwanted adverse reactions, this procedure could provide a way to predict these outcomes which is currently unavailable. Additionally, the findings from this research may provide insights into how SSRIs cause weight gain.

It was found that to achieve at least 80% statistical power, a minimum sample size of 46 was required for demographic characteristic analyses, 77 for weight change analyses between variant allele carriers and non-carriers, and 42 for comparing weight change based on administered medication. These values were determined among the many statistical tests conducted in each section when determining sample size requirements for statistical analyses in our research with G*Power software (Kang, 2021). In this project, a total of number of 61 patients were accepted to the study. However, 34 patients who initially agreed to participate in the study did not attend the second session, 4 weeks after treatment started without providing any reasons. The requirement to draw blood likely contributed to restricting our participant pool from the clinic's potential applicants. Without any reward at the end, many of the potential participants may have shied away from undergoing such a painful and uncomfortable procedure. Additionally, the month of Ramadan in the Islamic calendar coincided with our timeline for participant

recruitment which necessitated an earlier conclusion to participant recruitment for the study.

Another limit during the research timeline was optimizing the timing of the second weight measurement. SSRI antidepressants are known to produce significant weight change side effects in long-term use (Mastronardi, 2010). Initially, serotonin reuptake inhibition in the acute phase can lead to appetite suppression causing patients to experience minor or no weight changes in the first weeks. After receptor modifications and rewiring of certain networks start to take place, gradual weight gain is usually observed (Richelson, 2001; Sharp & Collins, 2023). As a matter of fact, the same antidepressant that causes weight gain may cause weight loss in the acute phase. In an exemplary study conducted with fluoxetine, sertraline, and paroxetine, 4.3% of patients experienced significant weight change of 7% or more as weight loss in the acute phase (6-8 weeks), whereas 17.9% of patients experienced significant weight change as weight gain in the long-term phase (16-48 weeks) (Sussman et al., 2001). Therefore, 4 weeks may not be sufficient to observe significant weight changes, although such changes may become evident in long-term use. Additionally, if SSRI-associated weight changes are related to 5-HT2CR desensitization via mRNA editing and the 5-HT2CR polymorphisms in question play a role in the process, 4 weeks is not enough to detect these alterations.

As discussed earlier, the discontinuation of SSRI treatment is a common and detrimental issue (Kelly et al., 2008; Cascade et al., 2009). Apart from the side effects that can impact patients' lives from the start, such as insomnia, drowsiness, nausea, and sexual dysfunction, psychiatric medications are often viewed negatively in our society. Relying on a substance that must be taken regularly and externally is not appealing to some. Also, there is usually a lack of understanding about the necessity of long-term use to improve symptoms, as well as the importance of quitting antidepressants in a controlled manner under medical supervision. Our initial aim for the second measurement time was 10-12 weeks after SSRI treatment started. Out of those 27 participants who showed up, only a few returned for subsequent sessions. Considering long-term patient follow-up has

proven to create complications and the data collection schedule is restricted, 4 weeks after treatment starts was decided as the second measurement point. Ultimately, with a sample size of 27, our analyses have insufficient statistical power to reflect the actual phenomenon in the Turkish population accurately. Therefore, we conducted all analyses without excluding the outliers to avoid losing valuable data. However, when we removed the outliers, there was no change in terms of statistical significance.

Determining the first onset of depression and anxiety disorders in psychiatric patients is challenging, because symptoms may have started years before an official diagnosis. However, Solmi et al. (2021) conducted a large-scale meta-analysis on the age at onset of mental disorders with a sample size of over 700,000 across 192 studies worldwide. They found that the median age at the onset of depressive disorders was 31 (IQR=(21, 46)) years with onset defined as the first diagnosis. The statistics for generalized anxiety disorder was 34 (IQR=(23, 48)) years of age as median. When they analyzed the data that defined first onset by age of first symptoms instead of diagnosis, the median age shifted from 31 to 26 years for depressive disorders, while it shifted from 35 to 39 years for generalized anxiety disorder. In our study population, according to the age distribution it was symmetrical with almost equal mean and median. The median age was 35 overall, 33.50 for females, and 38 for males, indicating that our participants were slightly older than Solmi et al. (2021) reported. The higher median age of male applicants might suggest they seek psychiatric help later than females after their symptoms start.

The sex ratio among the study participants was notably different with female patients outnumbering males by two-thirds. Even so, the finding is striking, it is a highly prevalent phenomenon in psychiatric help-seeking. Social norms often discourage men from acknowledging their need for help, as help-seeking behavior is associated with fragility, weakness, and a lack of masculinity (Pattyn et al., 2015). Murphy (1998) reports men’s suicide rates are four times higher than women’s, while women are diagnosed with major depressive disorder at twice the

rate of men. It seems men's reluctance to admit their need for help results in unrecognized depression, which is often excluded from medical studies and contributes to a significant increase in suicide. To overcome the epidemic of mental illnesses, it is not enough to find ways to treat patients; we also need to understand how to reach them. Besides differences between sexes, there are additional factors influencing help-seeking behavior. In a recent research on this topic, Güney et al. (2024) analyzed data from the Türkiye Health Survey of 2016, 2019, and 2022. They found that variables such as age, education level, health, and employment status are also influential on the odds of seeking professional aid for mental disorders. They also reported a similar ratio of female applicants to our findings with 71% in their study compared to 67% in ours. In our study, such variables were not recorded as they were unrelated to study objectives. However, this issue is a serious concern that needs to be addressed.

Aside from its impact on psychiatric medicinal treatment, one major concern of SSRI-associated weight change is its potential contribution to the increasing obesity rate among both Türkiye and the global population. The BMI values of the study population were concerning, with a ratio of 59% overweight and 33% obese. Both sexes had similar baseline BMI values and comparable ratios of overweight or obese individuals. The overall and sex-grouped distributions were symmetrical with almost exact mean and median values. Our findings were significantly higher than TÜİK and OECD data. Yet, a recent publication on the noteworthy negative impact of obesity on health and the economy in Türkiye reported similar statistics, referencing the WHO European Regional Obesity Report for 2022, which indicates that 61% of the Turkish population is overweight, while 32.1% is obese (Gogas Yavuz et al., 2024). This either suggests that individuals with psychiatric disorders tend to have more unhealthy body weights or, despite our small sample size possibly not representing the Turkish population accurately, the already alarming rates of obesity could be even more severe based on WHO data.

The relationship between obesity and depressive or anxiety disorders is more complex than weight gain associated with SSRI use. Both are serious medical

issues that have been drastically rising over the last decade. Additionally, SSRIs, the most commonly prescribed medication for depression and anxiety disorders, cause weight gain, thereby affecting the obesity rate. Yet, another aspect of the issue is that symptoms of depression and anxiety are more prevalent in obese individuals, and the intensity of these symptoms increases in correlation with BMI (Balkoca et al., 2023). Thus, to address the growing issue, the need for combined therapeutic approaches for both obesity and psychiatric disorders should be considered in the future.

As previously mentioned, SSRI antidepressants are prescribed for a wide range of psychiatric disorders, such as major depressive disorder, generalized anxiety disorder, obsessive-compulsive disorder (OCD), panic disorder, and phobias among others (Chu & Wadhwa, 2023). The dosage of the medication may vary based on the diagnosis and can be gradually increased from smaller starting doses to maintenance doses after a few weeks (Sheffler et al., 2023). The prescription doses were determined according to the most recent literature relevant to patients' diagnoses (WHOCC, n.d.; Sheffler et al., 2023). However, in some cases, the starting dose may result in little to no improvement in patients due to being at subtherapeutic levels. Considering the participants of our study met with psychiatrists only twice, 4 weeks apart, during the course of data collection, there was no drug dose titration. This further complicates detecting physiological changes caused by SSRIs.

Since the 5-HT2CR gene is located on the X chromosome, males have only one copy of the gene, while females have two. However, this does not necessarily mean that females will produce twice the amount of the receptor protein as males. A natural process called X-inactivation ensures that one of the X chromosomes is randomly silenced for transcription in each cell to balance the dosage of the protein (Sun et al., 2022). In heterozygous individuals, this can create mosaicism where some cells express the common allele while others express the variant allele. Due to this genetic complexity, participants of the study were compared based on the presence or absence of the variant allele. In the same way as the established

research discussed earlier on this topic, we followed this methodology in this project (Godlewska et al., 2009; Ryu et al., 2007; Yuan et al., 2000).

In our project, demographic characteristics, such as age, sex, and baseline BMI, of participants with the presence or absence of the variant alleles of the serotonin 2C receptor -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms were compared. No statistically significant differences were detected, except for baseline BMI values between variant allele -697C carriers and non-carriers ($p=0.04$). Although the distributions were asymmetrical and skewed to a degree, they did not significantly deviate from a normal distribution. The baseline BMI was notably higher in -697C variant allele carriers with a mean difference of 5.16 kg/m^2 and a median difference of 7.77 kg/m^2 compared to non-carriers. The only research that reported baseline BMI differences between -697G/C polymorphic alleles found the variant allele C significantly less common in Japanese men with a BMI of 28 kg/m^2 or greater than those with lower BMI (Yuan et al., 2000). However, when the study population was split into diabetic and non-diabetic groups, statistical significance was lost.

Available research on 5-HT2CR polymorphisms related to body weight has predominantly focused on antipsychotic use in patients with schizophrenia or other psychotic disorders. In Godlewska et al.'s (2009) study on the -697G/C polymorphism and olanzapine-induced weight gain in schizophrenic patients a significant difference was found between groups, with a 10% protection observed in variant allele carriers. However, baseline BMI values did not differ between variant allele carriers and non-carriers. Both findings in the literature that associate the -697C variant allele with weight change suggest it is more common in individuals resistant to weight gain. On the contrary, our results were the opposite. Considering the previous research was not conducted on healthy controls, it is possible that these findings do not reflect the general population. Along with these, although not significant, there was a trend in baseline BMI differences between variant allele carriers and non-carriers. We observed that both the mean and median values were higher in carriers than in non-carriers for each. These interesting and

novel findings should be explored in future studies with larger sample sizes, and ideally with a healthy control group. If similar results were replicated and found to be significant in a sample representing the general population, the underlying mechanism might involve diminished 5-HT2C receptor activity due to the disruptive effects of the -759C/T and -697G/C promoter polymorphism variant alleles on transcription, and the reduced intracellular calcium release associated with the 68C (Ser23) variant receptor (McCarthy et al., 2005; Hill & Reynolds, 2007; Hill & Reynolds, 2011; Land et al., 2019).

No patients were found with the TT genotype at the -759 site or the CC genotype at the 68 site. Due to the small sample size and low counts for multiple genotypes, difference assessments among genotypes, and deviations from Hardy-Weinberg equilibrium could not be calculated. Additionally, linkage disequilibrium could not be calculated because of zero counts in several genotypes. Although -759C/T and -697G/C polymorphisms are located in close proximity and were previously reported to be in linkage disequilibrium by Godlewska et al. (2009) and Ryu et al. (2007), we could not detect this in our study. There are no reports of linkage disequilibrium between 68G/C (Cys23Ser) polymorphism and the other two promoter polymorphisms. This highlights the importance of analyzing the effects of these polymorphisms together. Instead of only comparing alleles individually, defining the haplotypes of the patients and comparing as demonstrated by Gunes et al. (2009), McCarthy et al. (2005), and Yuan et al. (2000), might produce more accurate and meaningful results. Unfortunately, our small sample size limited us from pursuing this approach. Even though it was among our initial considerations, we realized that several haplotypes would have zero counts and the rest would be too few to detect any significant differences by the end of the data collection.

Allele frequencies are naturally expected to vary among different populations. When considering SNPs linked to certain disorders or medical conditions, the frequencies in patient groups or study populations may differ from those in the local population Therefore, differences in our allelic distribution data compared to previous research are expected. However, despite our small sample size, this

project still provides valuable insights considering the limited research on these polymorphisms in the Turkish population.

For the -759C/T polymorphism, the percentage of variant allele -759T carriers was 23.3% in a control group from the Greek population (Iordanidou et al., 2008), 22% in psychotic disorder patients from the Netherlands (Mulder et al., 2006), 29.4% in psychiatric patients from India (Das et al., 2017), 37% in a control group from Southern England (Pooley et al., 2003), 13.1% in schizophrenia patients from Korea (Ryu et al., 2007), and 26.2% in schizophrenia patients from Poland (Godlewska et al., 2009). For the -697G/C polymorphism, the percentage of variant allele -697C carriers was 44% in psychotic patients in the Netherlands (Mulder et al., 2006), 47.7% in schizophrenia patients from Poland (Godlewska et al., 2009), and 14.8% in men from Japan including obese and diabetic individuals (Yuan et al., 2000). For the 68G/C (Cys23Ser) polymorphism, the percentage of variant allele 68C (Ser23) carriers was 13% in a control group from England (Hu et al., 2003), 9% in a control group from Spain (Gutiérrez et al., 1996), 8% in a control group from Sweden (Westberg et al., 2002), 16.8% in normal weight children from Germany (Lentes et al., 1997), and 13% in combined control groups from Austria and Canada (Praschak-Rieder et al., 2005).

There is only one published study on the 5-HT2CR -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms in the Turkish population. Yücel et al. (2015) report allele frequencies of 28.8% for -759T, 52.5% for -697C, and 28.8% for 68C (Ser23) in healthy controls. Our study population had 26% for -759T, 52% for -697C, and 22% for 68C (Ser23) alleles. There were no prominent differences in our -759C/T allele distribution compared to Yücel et al.'s study or other global populations. However, Korean and Southern English populations show distinct differences from the rest of the world. The distribution of -697G/C alleles generally reported as closely distributed to each other compared to other polymorphisms, except for the data from Japan presented by Yuan et al. (2000).

In our study, the -697C variant allele was found to be more common among participants compared to what is reported in the literature. Surprisingly, Yücel et al. (2015) reported similar findings, with the -697C variant allele being more common in their larger Turkish sample (n=135). Moreover, this frequency increased to 60.7% in migraine patients. Considering the notable differences in the distribution of -697G/C polymorphic alleles and baseline BMI in the Turkish population, as well as rare variant allele frequency in the Japanese population, the necessity for further investigation has become apparent. Additionally, the -759C/T allele distribution in the South England and Korean populations also diverges from the rest of the world, suggesting the influence of local, population-specific factors. These observations hint at potential environmental or genetic interactions that could explain the distinct patterns seen in our study. Future research focusing on haplotype and gene-gene interaction analysis, detailed lifestyle information records from patients, and genetic comparisons across different populations can help in understanding the underlying mechanisms. These approaches could help identify the components contributing to these variations and their implications for conditions such as obesity, psychiatric disorders, and the effects of psychiatric medications on weight change.

Second weight data was collected after 4 weeks of SSRI treatment, and patients' % BMI change during the experimental period was calculated. No statistically significant differences among any of the variant allele carriers and non-carriers, or between sexes for serotonin 2C receptor -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms were found. BMI change ratios for both sexes were skewed and distributed non-normally. Only the -759T (p=0.32) and 68C (Ser23) (p=0.7) allele distributions could be assumed as normal distributions. Therefore, comparing and commenting according to means were not reliable. Due to the small sample size, skewed data, and unequal variances and sample sizes the Mann-Whitney *U* test was used for statistical analysis. Though there were no significant differences between the alleles, a trend was observed. All non-carrier patients had a median value of 0, while the variant allele carriers had at least a 1.20% BMI change in the positive or

negative direction. The largest weight change in mean was observed in the variant allele 68C carriers. Additionally, the same allele was the only one with a positive median value among all with. The largest weight change in the median was observed in the variant allele -759T carriers. On the contrary to baseline BMI statistics, where all variant carriers tended to score higher, carriers of the -759C/T or -697G/C promoter polymorphism variant alleles lost more weight than non-carriers, except for the 68G/C (Cys23Ser) polymorphism. This pattern in baseline BMI and weight change differences based on the presence of the -759C/T and -697G/C promoter polymorphisms, though not statistically significant, suggests that these differences could become more evident in a future study with a larger sample size and longer treatment duration. In addition, the observed weight loss in carriers of the promoter polymorphism variant alleles was unexpected, due to their presumably lower transcription rates compared to non-carriers. Future research might benefit from quantifying expression levels and investigating potentially neglected genetic interactions or compensatory mechanisms.

In the studies we used as a guide for our research, antipsychotic-induced or antidepressant-associated weight changes were compared by dividing the subjects into two groups based on changes in their BMI. The cut-off values selected to identify significant weight changes were 5%, 7%, and 10% (Ryu et al., 2007; Godlewska et al., 2009; Uguz et al., 2015). As discussed earlier, a 4-week SSRI treatment period was insufficient to cause significant weight changes of up to 10%. Therefore, we used 5% and 7% cut-off values in our study. Since weight loss is common in the acute phase of SSRI treatment, we also compared -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms on the negative side using cut-off values of -5% and 7%. No significant weight changes were found in any of the weight change categories for any of the SNPs. We speculate that the insufficient period between weight change comparisons along with the limited sample size, obscured our ability to determine the hypothesized effect of serotonin 2C receptor polymorphisms on SSRI-associated weight change, if such an effect exists.

Consequently, our results for this section are inconclusive and should be revisited with a larger study population and longer treatment period.

Escitalopram, sertraline, and paroxetine alone made up 52.4% of all antidepressant prescriptions in Turkey, according to 2014 IMS Health Turkey Medical Index data which was gathered from quarterly surveys of 705 medical (Adam et al., 2019). This study also reported that among all antidepressants, 48.2% were prescribed for depression, 33.5% for anxiety, and 2.4% for OCD. Similarly, Demirhan Keleş et al. (2024) presented IQVIA/Turkey data from 2018 to 2022 listing escitalopram, sertraline, fluoxetine, and paroxetine as the most consumed antidepressant drugs by unit box in Türkiye, respectively. Although we did not restrict SSRI prescription drugs to any particular subset, these four substances were the ones prescribed to our study participants. However, due to insufficient sample sizes in paroxetine and sertraline users, we excluded them from the section comparing weight change differences between variant allele carriers and non-carriers of the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms based on the administered SSRI substance.

The primary therapeutic mechanism for all SSRIs is the inhibiton of serotonin reuptake in presynaptic neurons, as suggested by their name. However, these pharmaceutical substances vary in efficacy, potency, metabolism, and affinity for other neurotransmitter systems. Therefore, the interaction between serotonin 2C receptor polymorphic alleles and SSRIs may differ depending on the specific antidepressant, leading to variations in adverse effects. For instance, cytochrome P450 2D6 polymorphisms affect the rate of paroxetine and fluoxetine metabolism (Leonard, 1992). To expand further, paroxetine is the most potent serotonin reuptake inhibitor among all SSRIs and has a high affinity for blocking norepinephrine reuptake (Hyttel, 1993; Nemeroff and Owens, 2004). While both fluoxetine and sertraline downregulate β-adrenergic receptors, sertraline stands out with its dopamine reuptake inhibition (Leonard et al., 1992; Nemeroff and Owens, 2004). Returning to the topic, Gill et al. (2020) classified escitalopram in the medium-risk group (>7% and 0.5-1.4 kg weight gain) for acute or long-term use,

whereas fluoxetine was categorized as low risk (no weight change or weight loss). In their cross-sectional study of patients taking antidepressants for 6 to 36 months., Uguz et al. (2015) reported that 48.7% of escitalopram users put on significant weight (7% body weight), compared to only 6.9% of fluoxetine users.

In our study, escitalopram users did not significantly differ in weight change from fluoxetine users, either overall or for the corresponding alleles at each polymorphic site. Also, weight change differences between the variant allele carriers and non-carriers of the -759C/T, -697G/C, and 68G/C (Cys23Ser) polymorphisms in both groups did not reach statistical significance. Among the escitalopram group, both individuals carrying the -759T variant allele lost weight, a trend also observed in the fluoxetine group. On the contrary, non-carriers of the -759T variant allele in both groups tended to shift to the positive side of the weight change scale. In both the escitalopram and fluoxetine groups, -697C variant allele carriers shared a weight loss trend. However, an outlier among the escitalopram users shifted the mean above zero. Non-carriers of the -697C and 68G/C (Cys23Ser) variant alleles tended to stay neutral or gain weight, with -697C carriers in the fluoxetine groups showing noticeable weight gain. Interestingly, carriers of the 68C (Ser23) variant allele showed an opposite pattern, with escitalopram users gaining noticeable weight, while those in the fluoxetine group lost weight. The results from Chapter 3.3.2 were consistent with BMI changes observed without specifying any particular SSRI. However, an exception was found among 68G/C (Cys23Ser) coding region polymorphism variant allele carriers in the fluoxetine group, who were inclined to lose weight, unlike the others. Also, while non-carriers of the 68G/C (Cys23Ser) polymorphism in the overall population or among escitalopram users either lost or maintained their weight, those in the fluoxetine group tended to maintain or gain weight. Although these findings are noteworthy, they were not statistically significant.

Among all SSRIs included in the study, only fluoxetine has a high affinity to the serotonin 2C receptor (Ni & Miledi, 1997). It is also the only one in the low-risk category with a possibility to cause weight loss in contrast to other SSRIs (Gill et

al., 2020). These divergences from overall study population and the escitalopram group may reinforce the hypothesis that the differential effects of fluoxetine in weight change are related to its interaction with 5-HT2CR and the receptor's role in SSRI-associated weight change. Moreover, given that serotonin 2C receptor mRNA editing has been observed in rats with chronic fluoxetine administration, its link with SSRI-associated weight change can be assumed, particularly for fluoxetine use. Additionally, the transition of the 23rd amino acid from Cys to Ser may provide insights into the binding site of fluoxetine on the 5-HT2C receptor, an underexplored subject.

CHAPTER 5

CONCLUSION

Substantial increase in obesity and psychiatric disorder incidences has reached epidemic levels both globally and locally, in Türkiye. Although these medical conditions seem unrelated, they contribute to the growth rate and severity of each other. The most common psychiatric disorders are depressive and anxiety disorders by a significant margin. SSRI antidepressants are the first-line pharmacological treatment, as well as one of the most prescribed medications of all. Weight gain stands out as one of the most detrimental side effects of SSRIs by increasing the risk of treatment discontinuation and obesity. Despite the advancements in neuroscience, we do not have a detailed picture of SSRI-associated weight change to take preventive measures.

Neurotransmitter serotonin and the serotonergic system have a crucial role in the maintenance of many physiological functions, including mood and satiety. Antipsychotics, another group of psychiatric medications that cause weight gain, have been shown to exert this adverse reaction due to their affinity for serotonin 2C receptors. Moreover, its single-nucleotide polymorphisms have been demonstrated to have protective effects over antipsychotic-induced weight gain. Although most SSRIs do not exhibit high affinity towards the serotonin 2C receptor, they inhibit the reuptake of serotonin in the synaptic cleft. Hence, increased synaptic concentration triggers changes in the activity of the serotonin receptors indirectly. Yet, there is no research conducted on the role of the serotonin 2C receptor polymorphisms in SSRI-associated weight change.

In this project, our aim was to determine whether the alleles of the -759C/T (rs3813929), -697G/C (rs518147), and 68G/C (Cys23Ser, rs6318) single-nucleotide polymorphisms of the *HTR2C* gene, which are most commonly

associated with weight change in the literature, affect weight change with SSRI use. We included patients prescribed SSRIs from the Psychiatry Service at Necmettin Erbakan University, Faculty of Medicine, who had not received psychiatric treatment recently and did not have any psychotic disorder or chronic medical conditions. We utilized the PCR-RFLP, a reliable and affordable method, to determine patients' alleles due to its suitability for clinical settings, considering its potential for personalized SSRI selection. Weight measurements were collected at the start of the study and four weeks after the treatment. Demographic characteristics of the study population were compared between variant allele carriers and non-carriers for each polymorphic site. Additionally, allelic distributions were analyzed. Weight changes were compared between variant allele carriers and non-carriers both unconditionally and by grouping based on cut-off values of 5%, 7%, -5%, and -7% BMI changes. Weight changes were also analyzed between the escitalopram and fluoxetine groups.

The number of male applicants was considerably lower than that of females, and their ages were higher. The rates of overweight and obese individuals among the participants were concerning and higher than available data. The frequency of the -697C variant allele was higher in our sample compared to non-carriers, which is a previously reported phenomenon in another study conducted in the Turkish population. There was a significant difference in baseline BMI values between the -697G/C polymorphism variant allele carriers and non-carriers. Despite the insufficient sample size to detect statistically significant differences, some trends were observed. All variant allele carriers patients had higher BMI than non-carriers for each polymorphic site. Carriers of the -759T and -697C variants alleles tended to lose weight, while carriers of the 68C (Ser23) variant allele gained weight, and non-carriers of the variant alleles maintained their weight. Among fluoxetine users, those with the 68C (Ser23) variant allele diverged from both escitalopram users and the overall population by losing weight, while the rest tended to gain weight.

Gathering sufficient participants was the most challenging aspect of this project. The requirement for patient adherence and the necessity of blood donation made it

difficult to increase our sample size. Although SSRIs typically start to exert their weight change effects in the long term, extending the weight measurement period beyond four weeks would have further reduced the study population due to discontinuation. Another limitation was the lack of demographic data other than age and sex for further analysis.

In conclusion, our project revealed a novel finding: carriers of the -697C variant allele had a considerably higher BMI compared to non-carriers. Additionally, this study validated previous research showing that the frequency of the -697C variant allele was higher in the Turkish population. This study is significant as it is the only research to investigate the -759C/T, -697G/C, and 68G/C (Cys23Ser) serotonin 2C receptor polymorphisms in relation to SSRI-associated weight change. Furthermore, noticeable patterns in weight change were observed based on the alleles and administered SSRI.

Future research with a larger sample size, longer treatment duration, and a healthy control group is necessary to provide more detailed insights into the topic. Such studies should consider comparisons with different populations, different drug doses, haplotype analysis, and molecular assays for expression levels, mRNA editing, and gene-gene interaction in their research design.

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APPENDICES

A. VOLUNTEER CONSENT FORM

NECMETTİN ERBAKAN ÜNİVERSİTESİ
GÖNÜLLÜ KATILIMCI OLUR FORMU

Bu metin bölümümüzce yürütülen hastalığınızla ilgili araştırma çalışmasına katılımınızla ilgili olarak hazırlanmıştır. Sizi Necmettin Erbakan Üniversitesi Meram Tıp Fakültesi Psikiyatri A.D. ve ODTÜ Moleküler Biyoloji ve Genetik AD. tarafından yürütülen "Antidepresan İlişkili Kilo Değişiminde Serotonin 2C Reseptör Polimorfizmlerinin Rolünün İncelenmesi" başlıklı araştırmaya davet ediyoruz. Bu araştırmanın amacı HTR2C geni -759C/T ve -697G/C Polimorfizmlerinin SSRI Kaynaklı Kilo Değişimiyle İlişkisinin araştırılmasıdır.

Bu çalışmaya katılmak tamamen GÖNÜLLÜLÜK esasına dayanmaktadır.

Bu formu okuyup onaylamanız, araştırmaya katılmayı kabul ettiğiniz anlamına gelecektir. Ancak, çalışmaya katılmama veya katıldıktan sonra herhangi bir anda çalışmayı bırakma hakkına da sahipsiniz. Ayrıca araştırmacı tarafından gerek görüldüğünde araştırma dışı bırakılabilirsiniz.

Bu çalışmadan elde edilecek bilgiler tamamen araştırma amacı ile kullanılacak olup KİŞİSEL BİLGİLERİNİZ GİZLİ TUTULACAKTIR; ancak verileriniz yayın amacı ile kullanılabilir.

Eğer araştırmanın amacı ile ilgili verilen bu bilgiler dışında, şimdi veya sonra daha fazla bilgiye ihtiyaç duyarsanız, araştırmacıya şimdi sorabilir veya aşağıdaki iletişim bilgilerinden ulaşabilirsiniz.

Çalışmaya toplam 108 gönüllü katılacaktır. Gönüllü hastalardan yalnızca bir defa tam kan örneği alınacaktır. Kan örneği araştırmanın yürütüldüğü diğer merkez olan ODTÜ'ye ulaştırılacak ve HTR2C geni -759C/T ve -697G/C Polimorfizmi incelenecektir. Kan örneğinizden başka bir analiz yapılmayacak ve uygun şekilde artan imha edilecektir. Çalışmanın başlangıcında ve 10-12 hafta sonra iki kez olmak üzere kilo, boy ve bel çevresi ölçümü yapılacaktır. Araştırma 24 haftada tamamlanacaktır.

Yardımcı Araştırmacı/Sorumlu Araştırmacı Tarafından Doldurulacak	
Katılımcının kişisel bilgilerinin gizli tutulacağını, katılımcının çalışma kapsamında sağlayacağı tüm verilerin etik kurallara göre işleneceğini ve bu etik kuralların ihlali durumunda, ortaya çıkacak tüm sorumluluğu kabul ettiğimi beyan ederim.	
Unvanı, Adı-Soyadı:	.....
Tarih:	.....
İmza:	

Yetişkin Katılımcının Kendisi tarafından doldurulacak

□ Yukarıda yer alan ve araştırmadan önce katılımcıya verilmesi gereken bilgileri okudum ve katılmam istenen çalışmanın kapsamını ve amacını, gönüllü olarak üzerime düşen sorumlulukları anladım.
□ Çalışma hakkında yazılı/sözlü açıklama araştırmacı tarafından yapıldı ve kişisel bilgilerimin özenle korunacağı konusunda yeterli güven verildi.
□ Bu koşullarda, araştırmaya kendi isteğimle, hiçbir baskı ve telkin olmaksızın katılmayı kabul ediyorum.

Adı-Soyadı: **veya Katılımcı Kodu:**	
Tarih:	
İmza:	
İletişim Bilgileri:	

Bu form, katılımcının kendisi/velisi/vasisi tarafından imzalandıktan sonra araştırmacıya teslim edilecektir. Ayrıca talep edildiği takdirde, bu formun bir nüshası katılımcıya verilecektir.

B. ETHICAL COMMITEE APPROVAL

UYGULAMALI ETİK ARAŞTIRMA MERKEZİ
APPLIED ETHICS RESEARCH CENTER

ORTA DOĞU TEKNİK ÜNİVERSİTESİ
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15 MAYIS 2023

Konu: Değerlendirme Sonucu

Gönderen: ODTÜ İnsan Araştırmaları Etik Kurulu (İAEK)

İlgi: İnsan Araştırmaları Etik Kurulu Başvurusu

Sayın Doç. Dr. Tülin YANIK ve Prof.Dr. Mehmet AK

"HTR2C geni -759C/T ve -697G/C Polimorfizmlerinin SSRI Kaynaklı Kilo Değişimiyle İlişkisinin Araştırılması" başlıklı araştırmanız İnsan Araştırmaları Etik Kurulu tarafından uygun görülerek **0280-ODTUİAEK-2023** protokol numarası ile onaylanmıştır.

Bilgilerinize saygılarımla sunarım.

Prof. Dr. Ş. Halil TURAN
Başkan

Prof.Dr. İ. Semih AKÇOMAK
Üye

Doç. Dr. Ali Emre Turgut
Üye

Doç. Dr. Şerife SEVİNÇ
Üye

Dr. Öğretim Üyesi Murat Perit ÇAKIR
Üye

Dr. Öğretim Üyesi Süreyya ÖZCAN KABASAKAL
Üye

Dr. Öğretim Üyesi Müge GÜNDÜZ
Üye

C. COMPLETE DATA OF THE RESEARCH PARTICIPANTS

Table C.1. Complete data of the research participants.

Patient	Age (years)	Sex	Height (m)	Weight 1 (kg)	Weight 2 (kg)	ΔBMI (%)	SSRI	-759	-697	68
2	35	F	1.58	75	89	18.67	Escitalopram	CC	CC	GC
4	42	M	1.85	102	106	3.92	Fluoxetine	C-	G-	C-
5	52	F	1.52	60	60	0	Escitalopram	CC	GG	GG
8	39	F	1.65	45	45	0	Escitalopram	CC	GG	GG
9	33	F	1.72	92	96	4.35	Escitalopram	CC	GG	GG
11	43	M	1.70	65	64.5	-0.77	Sertraline	C-	G-	G-
12	19	F	1.60	48	48	0	Paroxetine	CT	CC	GG
13	54	M	1.74	97.5	97	-0.51	Paroxetine	T-	C-	G-
14	33	M	1.75	79	83	5.06	Fluoxetine	T-	C-	G-
15	64	F	1.52	54	57.5	6.48	Fluoxetine	CC	CC	GG

Δ indicates change.

Table C.1. (cont.) Complete data of the research participants.

Patient	Age (years)	Sex	Height (m)	Weight 1 (kg)	Weight 2 (kg)	ΔBMI (%)	SSRI	-759	-697	68
16	24	F	1.69	90	85	-5.56	Fluoxetine	CT	CC	GG
17	38	M	1.75	63	73.5	16.67	Sertraline	C-	G-	G-
18	37	M	1.68	53	50	-5.66	Paroxetine	C-	G-	G-
20	42	M	1.78	89	89	0	Sertraline	C-	G-	G-
22	33	F	1.56	49	49	0	Escitalopram	CC	GG	GG
23	42	F	1.77	70	78	11.43	Fluoxetine	CC	GG	GG
25	34	F	1.70	78	72	-7.69	Fluoxetine	CC	CC	GC
26	42	F	1.78	99	97	-2.02	Escitalopram	CT	CC	GG
27	19	M	1.88	143	142.5	-0.35	Fluoxetine	C-	C-	C-
31	26	F	1.67	85	83.4	-1.88	Fluoxetine	CT	CC	GG

Δ indicates change.

Table C.1. (cont.) Complete data of the research participants.

Patient	Age (years)	Sex	Height (m)	Weight 1 (kg)	Weight 2 (kg)	ΔBMI (%)	SSRI	-759	-697	68
33	23	F	1.58	50	48	-4	Sertraline	CC	GG	GG
39	28	F	1.72	89	89	0	Fluoxetine	CC	GG	GG
40	35	F	1.73	83	84	1.20	Escitalopram	CC	GG	GG
44	22	F	1.62	52	51	-1.92	Escitalopram	CT	CC	GG
53	48	F	1.60	62.5	64.5	3.20	Escitalopram	CC	CC	GC
59	27	M	1.68	98	94	-4.08	Escitalopram	C-	C-	C-
61	33	F	1.63	107	103	-3.74	Fluoxetine	CC	GC	GG

Δ indicates change.