T.R.
USKUDAR UNIVERSITY
INSTITUTE OF SCIENCE

DEPARTMENT OF MOLECULAR BIOLOGY

MOLECULAR BIOLOGY PROGRAM

MASTER'S DEGREE THESIS

INVESTIGATION OF MTHFR AND MIR137 GENE POLYMORPHISMS IN BIPOLAR DISORDER PATIENTS

Mais Iter

Thesis Advisor

Prof. Dr. Muhsin Konuk

ISTANBUL-2024

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SUMMARY

Bipolar disorder is a complex mental illness characterized by alternating episodes of mania and depression, and while its precise causes remain uncertain, genetic factors are crucial in its development. This study focuses on two significant genes, MTHFR and MiR137, which have been implicated in the disorder. The MTHFR gene is involved in folate metabolism and neurotransmitter synthesis, while MiR137 regulates numerous genes related to neurodevelopment. The aim of this thesis is to explore the associations between MTHFR rs1801133 C677T and MiR137 rs1625579 polymorphisms in bipolar disorder patients, with the goal of advancing the understanding of the disorder's genetics and facilitating personalized diagnostic and treatment approaches. The research involved analyzing blood samples from 20 bipolar disorder patients who applied to the Psychiatry outpatient clinic of Istanbul NP Brain Hospital. DNA was extracted using the Invitrogen genomic DNA kit, and genotyping was performed with TaqMan Genotyping Assays on a Thermo Fisher QuantiStudio 5 Real-Time PCR system. Results showed that for the MTHFR rs1801133 polymorphism, the genotype distribution was 60% GG, 30% GA, and 5% AA, with allele frequencies of 77.5% G and 22.5% A. For the MiR137 rs1625579 polymorphism, the genotypic distribution was 25% GG, 40% GT, and 35% TT, with allele frequencies of 45% G and 55% T. The findings reveal a higher prevalence of the GG genotype and G allele for the MTHFR polymorphism. In contrast, the MiR137 polymorphism shows a higher prevalence of the T allele. Notably, the potential risk associated with the MiR137 polymorphism is significantly greater, with 75% of patients carrying the risk genotype compared to 40% for MTHFR. This suggests a stronger genetic predisposition related to MiR137 in the bipolar disorder cohort. Overall, this research highlights the importance of genetic variations in MTHFR and MiR137 in the context of bipolar disorder. The study contributes to an understanding of the disorder's genetic basis and supports the development of personalized diagnostic and therapeutic strategies. These insights are valuable for advancing personalized medicine approaches and enhancing treatment efficacy for bipolar disorder patients.

Key words: bipolar disorder, MTHFR gene, MiR-137 gene, polymorphism.

ABSTRACT

Background:

Bipolar disorder, marked by alternating episodes of mania and depression, has a significant genetic component. The MTHFR gene, involved in folate metabolism and neurotransmitter synthesis, and the MiR137 gene, regulating many genes impacting neurodevelopment, are key candidates for genetic studies in bipolar disorder. Polymorphisms in these genes can influence susceptibility to the disorder. This thesis explores the association between MTHFR rs1801133 C677T and MiR-137 rs1625579 variations in bipolar disorder patients, aiming to enhance understanding of the disorder's biology and support personalized diagnostic and treatment strategies through pharmacogenomics test.

Methods: blood samples were collected from 20 patients with bipolar disorders. Invitrogen, TaqMan SNP genomic DNA kit was used to obtain DNA from blood (Invitrogen, USA). Genotyping analyses were performed using TaqMan Genotyping Assays (Applied Biosystems Foster City, CA, USA) kits on Thermo Fisher Quanti station 5 Real-Time PCR system (Thermo Scientific, Waltham, Massachusetts, USA).

Results: The distribution of genotypes for MTHFR rs polymorphism in the study population was 60% GG, 30% GA, and 5% AA. Allele frequencies were 77.5% G and 22.5% A. For MIR137 rs polymorphism, the genotypes were distributed as 25% GG, 40% GT, and 35% TT, with allele frequencies of 45% G and 55% T. The study reveals a higher prevalence of the GG genotype and G allele in the MTHFR polymorphism, while the MIR137 polymorphism shows a higher prevalence of the T allele. Notably, the potential risk for MIR137 is higher, with 75% of patients carrying the risk genotype compared to 40% for MTHFR. This indicates a significant genetic predisposition associated with the MIR137 polymorphism in the studied population.

Keywords: bipolar disorder, MTHFR gene, MiR-137 gene, polymorphism.

DECLARATION

I declare that I have obtained all the information and documents in this study within the framework of academic rules, that I have presented all visual, audio and written information and results in accordance with the rules of scientific ethics, that I have not falsified the data I have used, that I have referred to the sources I have used in accordance with scientific norms, that my thesis is original, except for the cases cited as sources, that it was produced by me and that it was written according to the Üsküdar University Institute of Science and Technology Thesis Writing Guide

MAIS ITER

10-08-2024

FOREWORD

I am honored to conclude my master's thesis, the result of a year of dedicated effort. This is the perfect moment to express my deep gratitude to those who have supported me throughout this academic journey. First and foremost, I extend my heartfelt appreciation to Prof. Dr. Muhsin Konuk for his invaluable guidance at every stage of my research. His unwavering support, from selecting the topic to executing the study, enabled me to realize my potential as a graduate student. I am also deeply thankful to Dr. Tayfun Gözler for this patience and for his steadfast encouragement during the challenging phases of my thesis work I am indebted to the esteemed members of my thesis monitoring committee for their expertise and insights. Their valuable feedback significantly shaped my research, and their constructive criticism was crucial in refining my study. This academic journey has been enriched by the mentorship and support of these respected individuals, and I am truly grateful for their contributions to the success of my master's thesis

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INDEX OF ABBREVIATION

MTHFR- Methylenetetrahydrofolate Reductase

MIR-137- MicroRNA-137

BB- bipolar disorder

BD- bipolar disorder

PCR- polymerase chain reaction

DNA- deoxyribo nucleic acid

FDA- Food and Drug Administration

BD-bipolar disorder

SNP- Single nucleotide polymorphism

PGX- Pharmacogenetics test

1. INTRODUCTION

Bipolar disorder is a complex mental illness characterized by alternating episodes of mania and depression (Phillips and Kupfer, 2013). These episodes can significantly impact the individual's mood, energy levels, and ability to function in daily life. While the exact etiology of bipolar disorder remains elusive, accumulating evidence suggests a significant genetic contribution to its development and progression (Soronen et al., 2008).Understanding the genetic underpinnings of bipolar disorder is crucial for elucidating its pathophysiology, identifying potential biomarkers for early detection and diagnosis, and informing personalized treatment strategies .Biomarkers such as genetic variations associated with bipolar disorder can help in the early identification of individuals at risk (Sigitova et al., 2017).

Among the many genes implicated in bipolar disorder, the methylenetetrahydrofolate reductase (MTHFR) gene and the microRNA-137 (MIR137) gene have emerged as promising candidates for genetic studies in bipolar disorder.

The investigation into MTHFR and MiR137 gene polymorphisms in bipolar disorder patients is essential as these genetic variations have been implicated in the susceptibility to schizophrenia, affective disorders, and other psychiatric conditions (Zintzaras, 2006).

Studies have extensively explored MTHFR polymorphisms in various medical conditions, including psychiatric diseases like bipolar disorder (Levin & Varga, 2016). Meta-analyses have specifically linked MTHFR gene polymorphisms to common psychiatric disorders such as bipolar disorder, highlighting the importance of understanding these genetic variations in the context of mental health (Gilbody et al., 2006).

The MTHFR gene is associated with homocysteine metabolism, which is relevant in the context of bipolar disorder, the MTHFR gene encodes an enzyme involved in folate metabolism, a pathway critical for neurotransmitter synthesis and DNA methylation, processes implicated in mood regulation and neuronal function. Polymorphisms in the MTHFR gene have been associated with altered enzyme activity and folate levels, potentially influencing susceptibility to bipolar disorder and treatment response(Permoda-Osip et al., 2014).The etiology of bipolar disorder has also been linked to MiR137, a gene that encodes a microRNA and is linked to neuropsychiatric disorders like autism spectrum disorder and schizophrenia (Cheng et al., 2018; Cortabitarte et al., 2018; Curtis & Emmett, 2018; Loohuis etal.,2017). Within biological networks, miRNAs may control the expression of many genes, and dysregulation of miRNAs may be the cause of the molecular alterations linked to mental disorders (Miller & Wahlestedt, 2010). According to Loohuis et al. (2017), miR-137 has been identified as a crucial gene network hub that contributes to the pathophysiology of schizophrenia. MiR-137 has been connected to the control of genes related to neurite outgrowth in bipolar illness, which is essential for brain plasticity (Kidnapillai et al., 2020). Additionally, miR-137 has been associated with anxiety-like behaviors Yan et al. (2019) and has been found to impact synaptic plasticity and neurodevelopment (Cheng et al., 2018).

In this thesis, we aim to investigate if there is a significant association between MTHFR rs1801133 C677T and MiR-137 rs1625579 genetic variations in bipolar disorder patients. By comprehensively examining these genetic variations in bipolar disorder, we seek to advance our understanding of the disorder's underlying biology and pave the way for personalized approaches to diagnosis and treatment.

2. GENERAL INFORMATION

2.1 Overview of Bipolar Disorder

Extreme mood fluctuations, ranging from manic episodes (high or agitated mood) to depressive episodes (Lee et al., 2018), are the hallmark of bipolar disorder, a complicated mental health illness (Daneshjou et al.,2017). These mood fluctuations have the potential to seriously impair a person's functioning as well as their relationships, career, and general quality of life. Studies have revealed a seasonal fluctuation in bipolar disorder symptoms, with depressive symptoms increasing in the months around the winter solstice and manic symptoms peaking around the fall equinox (Ahmed et al., 2013). According to Phillips and Swartz (2014), bipolar illness may be explained as a neurological circuitry malfunction involving reward processing, emotion processing, and control pathways.

According to Adu et al. (2022), there is a 1-2% lifetime prevalence of bipolar disorder, with a larger risk in those with a family history of illness. Estimates of the prevalence of bipolar disorder vary; bipolar I disorder ranges from 0.3% to 1.5%, while bipolar II disorder ranges from 1.0% to 2.0% (Lin et al., 2014). According to Swartz and Fagiolini (2012), bipolar disorder is a multisystemic disorder that affects not only mood regulation but also immune function and cardiovascular state. Its severe and chronic character is highlighted by its ranking among the top 10 major causes of disability worldwide (Harvey et al., 2015). For those with BD, workplace functioning is linked to functional and cognitive impairment, a higher risk of suicide, and a high rate of relapses that are chronic.

Bipolar disorder is a complex disorder that involves multiple factors, including genetic susceptibility, environmental factors, biological system failure, and abnormality in brain networks. In the onset and course of BD, hereditary variables, epigenetic processes, and anomalies in signaling pathways all play important roles (Manji Husseini K et al., 2011).

Moreover, bipolar disorder has been shown to be highly heritable, with at-risk individuals experiencing a shift from an asymptomatic phase to a crippling condition (Keramatian et al., 2021). Bipolar disorder polygenic risk scores have been demonstrated to share genetic architecture with major depressive disorder and schizophrenia and to accurately predict the disorder in separate samples (Tansey et al., 2013). Genetic loading has been linked to clinical characteristics of bipolar spectrum illness in people with major depressive disorder, indicating the significance of genetic liability in bipolar disorder (Wiste et al., 2014). Research on the genetic overlap between major depressive disorder, schizophrenia, bipolar disorder, and other affective disorders has shown that these ailments are predisposed to one another by common genetic variables (Mitchell et al., 2011).

Figure 1 Bipolar Disorder symptoms

St Patrick’s Mental Health Services (SPMHS) , 2022

2.1.1 Genetic Basis of Bipolar Disorder

Bipolar disorder is a complex psychiatric condition with a significant genetic component.
There is a strong genetic component to bipolar disorder, with a heritability estimate of approximately 80-90%. (Prata et al.2008). Additionally, twin studies have provided further evidence for the genetic basis of bipolar disorder (McGuffin et al., 2001) For example, a study by McGuffin et al found a 42% concordance for major depression in monozygotic twins and a 20% concordance in dizygotic twins, suggesting a significant genetic contribution. Another study implicated chromosome 17 in not only bipolar disorder but also moyamoya disease, suggesting a common neurobiological basis (Bhere et al.,2012).

Genetic studies have shown a strong genetic contribution to the development of bipolar disorder. Recent research in the field of genetics has shed light on the intricate molecular pathways associated with bipolar disorder (Ogden et al., 2004). Studies have identified specific genetic variations that are linked to the susceptibility of developing the disorder (Smith et al., 2011).

Genetic factors play a crucial role in the pathogenesis of bipolar disorder, with some studies demonstrating that different subtypes of bipolar disorder may have distinct genetic contributions towards illness severity and cycling frequency (Gitlin & Malhi, 2020).

Additionally, Studies have indicated that schizophrenia and bipolar disorder partially share common genetic etiologies, challenging the traditional separation between these two disorders (Lichtenstein et al., 2009), studies have suggested a positive association between the DAOA gene and both schizophrenia and bipolar disorder, further supporting the genetic overlap between these affective disorders (Ahmadi et al., 2015).

There is a shared genetic etiology between bipolar disorder and other psychiatric disorders such as schizophrenia, major depressive disorder, autism spectrum disorders, and attention-deficit/hyperactivity disorder (Lee et al., 2013).

Furthermore, the interaction between genetic predisposition and environmental factors has been a key focus of investigation. Understanding these complex interplays is crucial in unraveling the underlying mechanisms of bipolar disorder (Nürnberger et al., 2014).

Advancements in genetic technology have enabled researchers to delve deeper into the genetic architecture of bipolar disorder. Genome-wide association studies and next-generation sequencing techniques have provided valuable insights into the specific genetic markers and pathways involved in the disorder (Kuo et al., 2021). In addition to adding to our understanding of bipolar disease from a molecular perspective, these results may pave the way for the creation of focused therapies and interventions.

Personalized medicine has also been made possible by the discovery of genetic biomarkers linked to bipolar disorder. A patient's genetic profile can help medical practitioners better customize treatment plans based on the patient's specific genetic predispositions, which will ultimately result in more efficient and individualized care Wang, Z. et al. (2019).

As our understanding of the genetic basis of bipolar disorder continues to evolve, it becomes increasingly clear that an integrative approach encompassing genetics, neuroscience, and clinical psychiatry is essential for comprehensively addressing the complexities of this condition (McMahon et al., 2020).

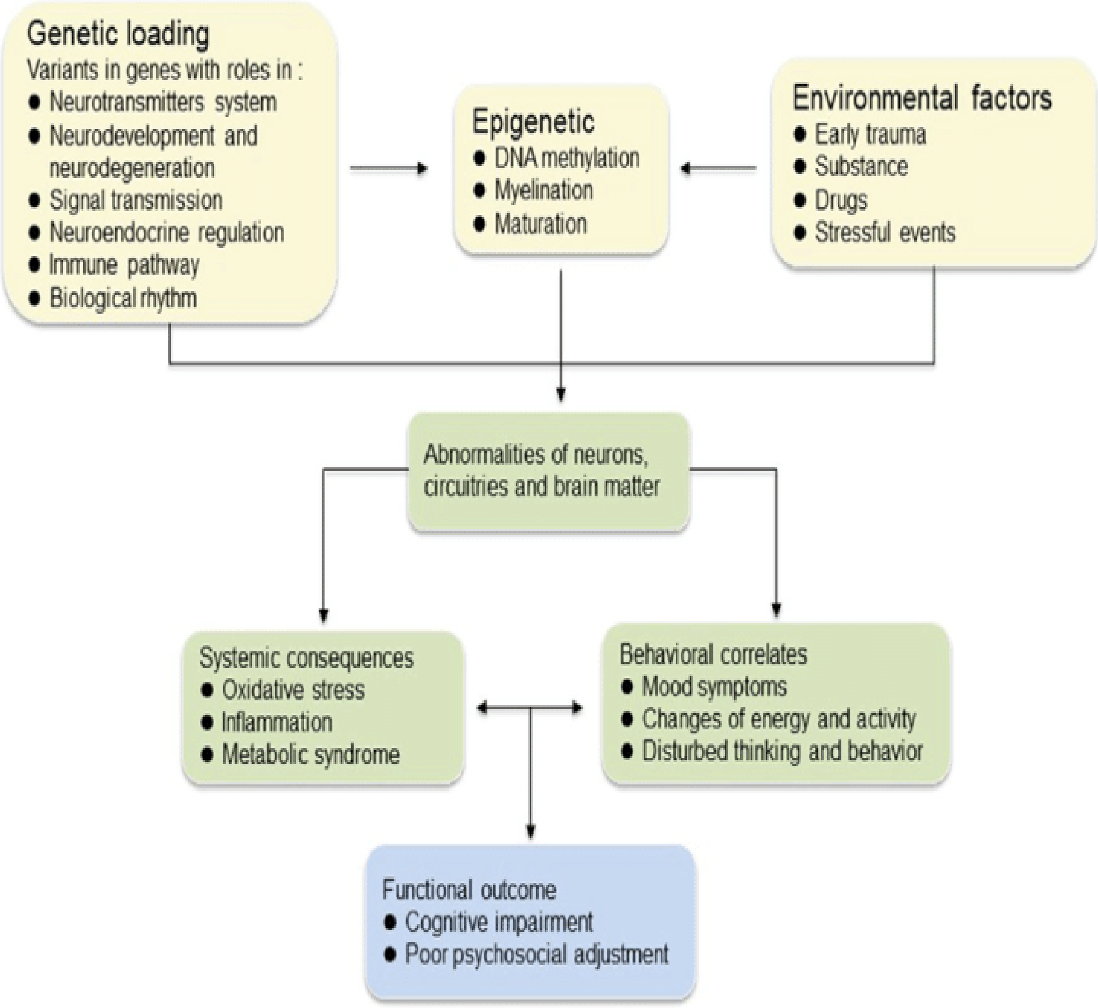

Figure 2 Multifactorial model of etiology and pathogenesis for bipolar disorder (Zouwei Wang , 2019) .

2.2 Single Nucleotide Polymorphisms

Single nucleotide polymorphisms (SNPs) are changes in a single base at a specific position in the genome, in most cases with two alleles. SNPs are found at a frequency of about one every 1,000 bases in humans [Kruglyak, 1997].

SNPs are a common form of genetic variation occur at a frequency of at least 1% in a general population based on a single nucleotide (adenine, thymine, guanine or cytosine) alteration that occurs (Thomas et al., 2011).

SNPs can be found in coding and non-coding regions of the genome (F. Zhang & Lupski, 2015) and have minimal, moderate, or negative effects on a person's health and development. In addition, Because SNPs occur abundantly with even spacing along the human genome, they offer significantly greater potential to be used as biomarkers for diagnosing genetic diseases (Shastry, 2007).

An individual can determine whether certain SNPs are present in his genome by using various techniques, such as genotyping (J. Zhang et al., 2020). The relative simplicity of SNP genotyping technologies and the abundance of SNPs in the human genome have made them very popular in recent years. The first projects using SNPs as markers for genotyping have been shown by Wang et al., (1998).

Single nucleotide polymorphisms (SNPs) are variations in a single nucleotide in a DNA sequence that can affect an individual's susceptibility to various disorders, including bipolar disorder. Studies have identified specific SNPs associated with bipolar disorder, such as those within the G72 gene (Zuliani et al., 2009). These genetic variations have been linked to alterations in brain structures like the temporal lobe and amygdala in individuals with bipolar disorder (Zuliani et al., 2009). Furthermore, investigations have explored combinations of SNPs and their association with bipolar disorder, revealing significant clusters of genetic variants linked to the condition (Mellerup et al., 2017; Mellerup et al., 2015). The genetic complexity of bipolar disorder is highlighted by the involvement of multiple genes and their polymorphisms, such as those in the FYN kinase gene (Szczepankiewicz et al., 2009). Moreover, genes related to the circadian system, like PERIOD3, have been found to influence the onset of bipolar disorder, emphasizing the role of genetic variations in circadian rhythm regulation (Nievergelt et al., 2006; Dallaspezia et al., 2011). The intricate interplay of genetic factors, including SNPs and haplotypes, underscores the multifactorial nature of bipolar disorder and the importance of understanding the genetic underpinnings of this complex condition.

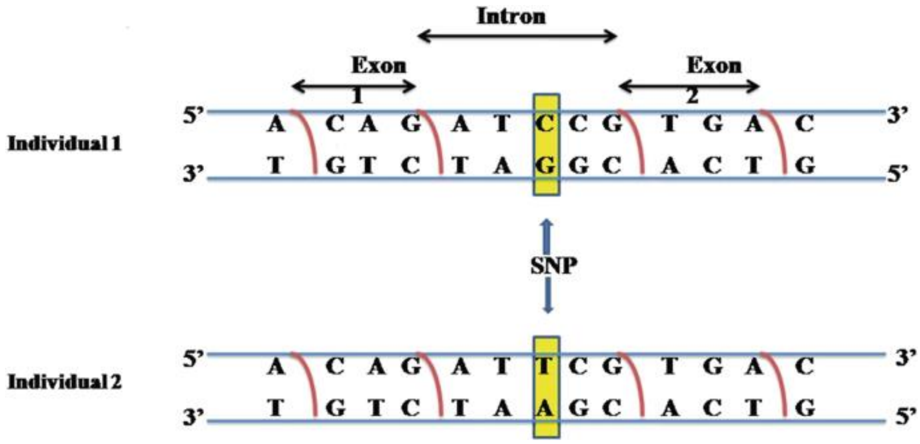

Figure 3 single-Nucleotide polymorphism.

(2022) Handbook of DNA profiling.

2.3 Methylenetetrahydrofolate Reductase (MTHFR) Gene

MTHFR is a protein involved in folate metabolism is encoded by the Methylenetetrahydrofolate Reductase (MTHFR) gene, which has 11 exons and is found on chromosome 1p36.3. (Kałużna et al., 2017; Qi et al., 2003). The production of DNA, RNA, and proteins depends on the folate metabolism pathway, which is mostly gene. Furthermore, it plays a critical role in a number of biological processes, including methylation patterns, carcinogenesis, and DNA biosynthesis (Verma et al., 2022).

The product of this gene's enzymatic conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTHF), a cofactor for the remethylation of homocysteine to methionine, is an enzyme that is essential to the metabolism of folate. This activity yields methionine, which is essential for

controlling genes and maintaining cellular health. Dysfunctional MTHFR, caused by the C677T polymorphism, results in reduced enzyme activity, affecting folate metabolism. This genetic variation leads to impaired FAD binding, significantly reducing MTHFR activity. Homozygotes for this variant have only 30% enzyme activity compared to wild-type homozygotes, while heterozygotes retain 65% activity.C677T transition is a common mutation in the coding region of the MTHFR gene that causes an alanine to valine (Al222Val) amino-acid substitution (Frosst et al., 1995). This mutation is associated with MTHFR activity and homocysteine levels. Homozygosity for the mutation (TT) predisposes to significantly elevated plasma homo- cysteine levels (Frosst et al., 1995), and hyperhomocys teinemia can be a risk factor for premature cardiovascular disease (Kang et al., 1991) and neural tube defect (van der Put et al., 1995). (Iemitsu et al., 2010 ; Pazarbaşı et al., 2023; Brezovska-Kavrakova et al., 2013; Gokalp et al., 2010).

The C677T polymorphism, in particular, has been associated with altered enzyme activity and increased risk for different diseases (Wang et al., 2013; Sohn et al., 2009). Research has shown that MTHFR deficiency can lead to DNA hypomethylation, potentially initiating cancer development and influencing tumor progression (Li et al., 2012). MTHFR polymorphisms have been associated with conditions like breast cancer, Parkinson's disease, acute lymphoblastic leukemia, and hypertension (Verma et al., 2022; Vallelunga et al., 2013; Kałużna et al., 2017; Fan et al., 2022; Hosseini et al., 2011). The impact of the MTHFR gene extends to brain function and neurodevelopment, highlighting its significance beyond traditional metabolic pathways (Wang et al., 2015). Moreover, studies on MTHFR gene polymorphisms have been conducted in various populations, including Han Chinese, Koreans, and Iranians, demonstrating its relevance across different ethnic groups (Wang et al., 2015; Fan et al., 2022; Hosseini et al., 2011). The gene's influence on conditions like depression, schizophrenia, and renal function underscores its broad involvement in complex disorders (Fung et al., 2011; Kang et al., 2010; Gaysina et al., 2008). In conclusion, the polymorphisms of the MTHFR gene have significant implications in health and disease, affecting enzyme activity, folate metabolism, and disease susceptibility across diverse populations. Understanding the role of MTHFR gene variants is crucial for personalized medicine approaches and targeted interventions in conditions influenced by folate metabolism and methylation processes (Balkan MedJ et al., 2013).

The methylenetetrahydrofolate reductase (MTHFR) gene is located on chromosome 1p36.3 (Goyette et al., 1994) and catalyzes the conversion of 5,10-methylenete- trahydrofolate to 5-methyltetrahydrofolate, Deficient activity of MTHFR may also possibly be associated with psychiatric conditions such as schizophrenia (Freeman et al., 1975) and affective disorders (Reynolds and Stramentinoli, 1983). Another polymorphism of the MTHFR gene is located at position A1298C (Glu429Ala) (Weisberg et al., 1998). The A1298C polymorphism in combination with the C677T base pair change may be associated with MTHFR activity and homocysteine levels (Van der Put et al., 1998). Functional polymorphisms in the MTHFR gene, including C677T and A1298C, have been linked to various health conditions (Vallelunga et al., 2013; Kałużna et al., 2017; Li et al., 2012).

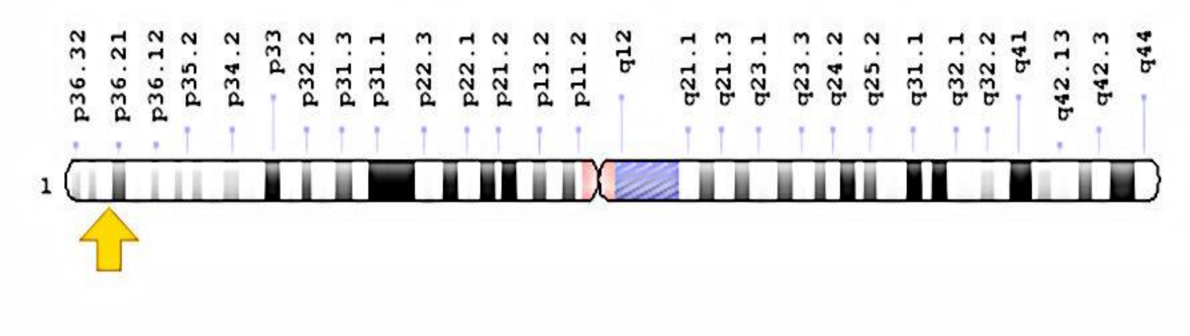

Figure 4

MTHFR is a gene make the MTHFR enzyme and is located on the short (p) arm of chromosome 1 at position 36.3.

2.3.1 MTHFR rs1801133 polymorphism in BD

The rs1801133 polymorphism, a common variant in the MTHFR gene, involves a substitution of cytosine (C) for thymine (T) at position 677. This genetic variation has been associated with reduced enzymatic activity of MTHFR, leading to decreased production of 5-methyltetrahydrofolate. As a consequence, individuals with the rs1801133 polymorphism may experience altered folate metabolism and impaired methylation processes (Waseeem et al.,_2016).

The MTHFR C677T rs1801133 variant has been extensively studied in relation to various psychiatric disorders, including bipolar disorder. Research has shown a significant association between the MTHFR C677T variant and bipolar disorder (Gilbody et al., 2006; Peerbooms et al., 2011; Zintzaras, 2006; Jönsson et al., 2008). This variant, which results in a substitution of alanine with valine, has been linked to a reduction in MTHFR enzyme activity, impacting folate metabolism and potentially contributing to the pathophysiology of bipolar disorder (Kaya et al., 2016; Owen et al., 2011; Ramos-Esquivel et al., 2020).

The potential influence on methylation processes is particularly significant because methylation is a fundamental mechanism for the regulation of gene expression. Changes in DNA methylation patterns can impact the expression of genes involved in various cellular functions, including those related to neurotransmitter synthesis and neural development. Therefore, highlighting on the implications of the MTHFR rs1801133 polymorphism provides valuable insights into the molecular mechanisms underlying not only folate metabolism but also broader aspects of cellular function, with potential implications for cond1`itions such as bipolar disorder. Chen et al. (2009) conducted an association study on the C677T MTHFR gene polymorphism in bipolar disorder within the Chinese population. Their research includes a meta-analysis of genetic association studies, enhancing the study's breadth. Focusing on a specific gene variant, C677T, underscores the targeted exploration of genetic factors. The findings from this study can contribute insights into the genetic susceptibility of individuals to bipolar disorder in the Chinese population.

In the exploration of the genetic underpinnings of bipolar disorder, the intricate relationship between folate metabolism, genetic variations, and the potential influence on DNA methylation processes assumes paramount significance (Stanger, 2002). Exhaustive review lays a robust foundation by elucidating the multifaceted roles of folate and vitamin B12 in cellular processes, including DNA synthesis and methylation. This serves as a crucial backdrop for comprehending how genetic variants, such as the MTHFR rs1801133 polymorphism, might disrupt these fundamental processes, thus potentially contributing to the complex etiology of bipolar disorder. A common mutation in methylenetetrahydrofolate reductase not only identifies MTHFR rs1801133 as a potential genetic risk factor but also sheds light on the functional consequences of this polymorphism. Beyond its vascular implications, their findings underscore the intricate interplay between altered enzymatic activity of MTHFR, disrupted folate metabolism, and potential impacts on broader methylation processes. This elucidation is pivotal in contextualizing the role of MTHFR in bipolar disorder, especially considering its vascular and neurobiological components Frosst, et al. (1995).

Additionally, Bird, et al.,2002) research provides valuable insights into how altered DNA methylation patterns, potentially influenced by MTHFR rs1801133, could contribute to the dysregulation of genes associated with mood disorders

Moreover, individuals with bipolar disorder have been found to have higher odds of hyperhomocysteinemia, a condition associated with the MTHFR C677T polymorphism (Ezzaher et al., 2011). The MTHFR gene, particularly the C677T variant, has been implicated in various diseases beyond psychiatric disorders, such as diabetes, cancer, and cardiovascular conditions (Chauhan et al., 2012; Zhou et al., 2015; Frikha et al., 2020). Additionally, the MTHFR C677T variant has been linked to cognitive dysfunction, mild cognitive impairment, and hypertension (Sun et al., 2021; Fan et al., 2021). Overall, the MTHFR C677T rs1801133 variant plays a crucial role in various health conditions, including bipolar disorder. Its impact on folate metabolism and homocysteine levels underscores its significance in the pathogenesis of bipolar disorder and other related disorders. Further research into the mechanisms by which these variant influences disease susceptibility and progression is warranted to better understand and potentially target these conditions for improved management and treatment outcomes.

2.4 MicroRNA (MiR-137) Gene

MicroRNAs (miRNAs) are small non-coding RNA molecules that play a crucial role in post-transcriptional gene regulation by targeting messenger RNAs (O'Brien et al., 2018). They are typically transcribed from DNA sequences into primary miRNAs, which are then processed into precursor miRNAs and finally mature miRNAs (Huibregtse et al., 2021). These mature miRNAs can modulate the expression of multiple genes simultaneously, making them important regulators of various biological processes (Kwast et al., 2019). It is a microRNA gene that produces a small noncoding RNA molecule known as miR-137. MiR-137 is involved in various physiological activities in the human body, including neuronal development, synaptic plasticity, and neurogenesis (Lu et al., 2017).

Furthermore, it has been thoroughly researched in relation to bipolar disorder, schizophrenia, and other neuropsychiatric illnesses (Sun et al., 2020). MiR-137 has been connected to cognitive deficits in those who have it, as well as an elevated risk of schizophrenia. It is recognized that it controls the growth and activity of neurons, impacting the connections between brain regions and the structural alterations in areas linked to schizophrenia. A sequence of processing stages known as miRNA biogenesis results in mature miRNAs that have the ability to suppress the expression of genes (Taylor, 2015). These miRNAs have the ability to function as biomarkers in a number of illnesses, such as epilepsy, cancer, and respiratory conditions, indicating their potential for both diagnosis and treatment (Brown et al., 2014; Henshall, 2017; Sessa & Hata, 2013).

Additionally, miRNAs have been implicated in stem cell differentiation, immune responses, and vascular biology, demonstrating their diverse roles in different physiological and pathological conditions (Hof et al., 2019; Baraban et al., 2021). The genetic and functional analyses have implicated microRNA 499A in bipolar disorder development (Tielke et al., 2022), further highlighting the role of microRNAs in the pathogenesis of mood disorders.

Understanding the biogenesis and functions of miRNAs, including MIR137, provides valuable insights into their roles in health and disease, offering potential avenues for targeted therapeutic interventions.

In the pursuit of unraveling the intricate genetic landscape of bipolar disorder, the miR-137 rs1625579 polymorphism emerges as a compelling focus, linking genetic variations to neurodevelopmental processes and the manifestation of this complex psychiatric condition. MiR-137, a microRNA, plays a pivotal role in gene expression regulation, particularly during neurodevelopmental stages critical for shaping the neural circuits underlying mood regulation.

The rs1625579 polymorphism within MIR137 has been identified as a potential modulator of this regulatory process. Its association with neurodevelopmental pathways makes it a promising candidate for understanding the genetic basis of bipolar disorder, a condition characterized by aberrations in mood regulation.

Investigation on the impact of a miR-137 susceptibility variant on brain function in individuals at high genetic risk for schizophrenia or bipolar disorder, using advanced neuroimaging techniques provided insights into how this genetic variation influences brain activity, contributing to our understanding of the neurobiological aspects of susceptibility to these psychiatric disorders (McDonald et al., 2004). The study's focus on individuals with elevated genetic risk highlights the relevance of miR-137, potentially paving the way for targeted interventions. The research lays a foundation for further exploration of the intricate interplay between genetic factors and brain function in severe psychiatric disorders. Whalley et al.'s (2012). A sequence analysis of miRNA genes associated with schizophrenia and bipolar disorder uncovered two functional variants in the miR-137 gene. These variants, found to impact mature miR-137 expression, were associated with reduced levels of the mature miRNA. This reduction influenced gene sets related to synaptogenesis and neuronal transmission; processes linked to psychiatric disorders. The study underscores the role of miR-137 in the etiology of psychiatric conditions, emphasizing its importance in nervous system development and synaptic function as mentioned by Strazisar et al. (2014).

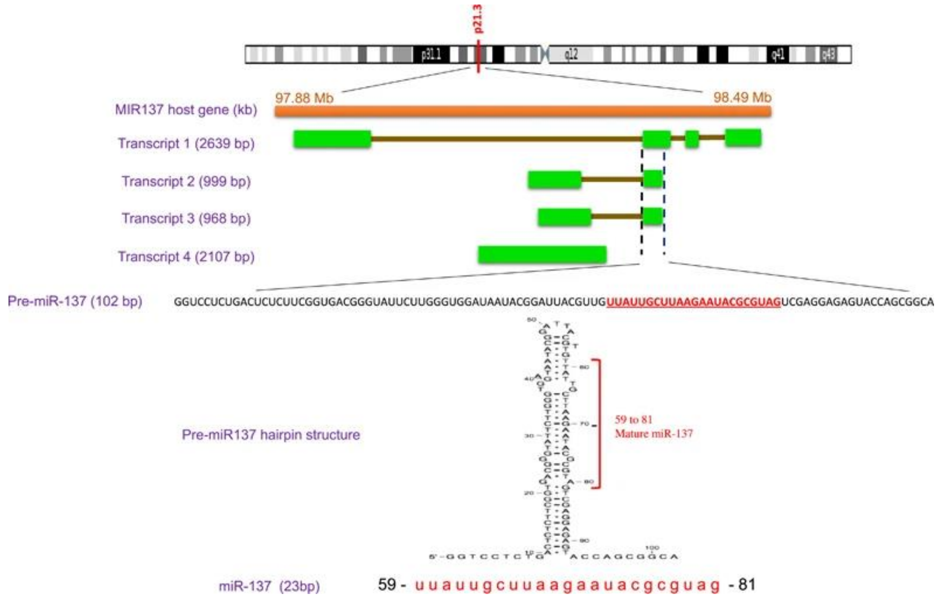

Figure 5The miR-137 gene is located on the short arm of chromosome 1 in humans, spanning 61Kb. it generates four transcripts. the primary transcript (pri-mir) is 102 nt long and is cleaved to produce a mature miRNA (miR-137) that is 23 nt in length. (Ebrahim Mahmoudi , 2016)

2.4.1 MiR-137 rs1625579 polymorphism in BD

MicroRNA-137 is a gene that encodes a microRNA, a small non-coding RNA molecule that plays a crucial role in regulating gene expression. It has been observed that genetic variation in the MIR137 gene, specifically the rs1625579 polymorphism, can influence the transcription processes (Kandratsenka et al., 2018).

The rs1625579 polymorphism in the miR-137 gene, localizing on chromosome 1p22, is strongly associated with the etiology of neuropsychiatric diseases, such as schizophrenia and bipolar disorder (Senousy et al., 2020; Kim et al., 2012). Research has shown that the miR-137 gene, which encodes a microRNA involved in synaptic function, is associated with psychiatric disorders (Strazisar et al., 2014). However, the specific rs162579 variant's impact on bipolar disorder is not well understood.

The mir137 gene is involved in neurodevelopment and has been found to be downregulated in individuals with bipolar disorder. This downregulation of the mir137 gene may lead to dysregulation in neuronal processes and contribute to the development of bipolar disorder by playing a crucial role in regulating brain development and function, the rs1625579 polymorphism has been linked to changes in the activation and functional connectivity of brain regions such as the dorsolateral prefrontal cortex and the posterior cingulate cortex (Zhang et al., 2018; Guella et al., 2013).

There is a direct molecular link between miR-137 e and other neuropsychiatric disorders like autism and schizophrenia, indicating a shared genetic basis among these conditions (Devanna et al., 2014).

This polymorphism has been identified as a risk variant for schizophrenia in various populations, including European and Chinese populations (Zhang et al., 2018; Lu et al., 2017). The genetic correlation between schizophrenia and bipolar disorder has been reported to be high, indicating a shared genetic susceptibility (Lee et al., 2013).

The T allele of rs1625579 has been consistently linked to a higher risk of schizophrenia compared to the G allele (Liu et al., 2017; Taha et al., 2021). Furthermore, the T allele has been associated with a decrease in miR-137 expression in the cerebral cortex, suggesting a potential mechanism for its involvement in the disorder (Ma et al., 2014).

There Also an impact of rs1625579 on brain morphology and function, with associations found between this polymorphism and alterations in white matter density, reduced hippocampal volume, and larger lateral ventricles in schizophrenia patients (Mahmoudi et al., 2020).

Studies suggest that variations in miR-137 e may be implicated in the vulnerability to bipolar disorder, possibly influencing the intricate balance of neurotransmitter systems and neural pathways associated with mood regulation.

(Khavari & Cairns et al., 2020) present experimental evidence linking MIR137 to the regulation of adult neurogenesis, dendritic development, and neuronal maturation, which are crucial processes in mood regulation and psychiatric disorders.

Furthermore, (Sun et al. 2020) highlight that miR-137 has been identified as a risk gene for schizophrenia, bipolar disorder, and autism spectrum disorder, emphasizing its significance in the pathophysiology of these conditions.

Variations in miR-137 e play a significant role in the vulnerability to bipolar disorder and other neuropsychiatric conditions by influencing neurodevelopmental processes, gene expression, and neural pathways critical for mood regulation.

3. MATERIAL-METHOD

3.1Patient selection

This study is a prospective cross-sectional study. Patients with bipolar disorder Between the ages of 18 and 65, who applied to the Psychiatry outpatient clinic of Istanbul NP Brain Hospital, were admitted to the psychiatric ward or followed up as outpatients, and were independently diagnosed with bipolar disorder according to the DSM-5 diagnostic criteria by a senior psychiatry assistant and a psychiatrist during a clinical interview.

3.2 Ethics statement

Üsküdar University Unitential Research of Ethics Assembly 27/03/2020 the meeting of 03 on the date of 03 "Genotype-phenotype in neuropsychiatric diseases Assessment of the Relationship "decide that this study research project is an ethically eligible are given.

3.3. Sample collection.

A total of 20 patients between the ages of 18-65 who were admitted to the psychiatry outpatient clinic, hospitalized in the psychiatry service or followed up on an outpatient unit diagnosed with bipolar disorder according to DSM-5 diagnostic criteria by a senior psychiatry resident and a psychiatrist independently of each other, and who were in manic episode, depressive episode and remission were included. While blood samples were collected from patients diagnosed with bipolar disorder, which will constitute our sample, in a tube containing 2% ethylene dimethyl tetra acetic acid (EDTA) in 2 cc, the patients were informed about the project and their voluntary consent was signed in written before the study.

All samples were processed in NP Istanbul Brain Hospital Üsküdar University, Faculty of Medicine, Laboratory of Molecular Genetics and Molecular Diagnoses section.

3.4. Equipment

-20 °C freezer Arçelik (Türkiye).

- Quant Studio 3 (Thermofisher, USA).

- Inolab WTW pH Meter (Germany).

- Microfuge 16 Microcentrifuge, Beckman Coulter (USA).

- Radway AS 220 / C / 2 Precision Scale (Poland).

- SBH130 Water Bath, Block Heater (UK).

- Stuart Vortex (England).

- Thermo Scientific Smart 2 Pure 3 Distilled Water Device (U.S.A.).

- Thermo Scientific Automatic Micropipettes, Eppendorf Research Plus (USA).

- Refrigerator Vestel (Türkiye) 3.1.2. Chemical Substances Used.

3.5. Material

- Swab DNA Isolation Kit (Thermofisher Scientific Invitrogen, USA).

- TaqMan SNP Genotyping Assays, Human, SM (Thermofisher, USA).

- TaqMan Universal Master Mix II, UNG (uracil DNA glycosylase) (USA).

- Distilled Water (dH2O).

- Fast 96-well Reacton plate.

-optical adhesive covers.

-Micropipette and Micropipette Tips.

-Eppendorf Tube.

3.6. DNA isolation

The Invitrogen Genomic DNA used for the collection of genomic DNAs from the blood (Invitrogen, USA).

The following protocol was applied to the DNA collection (Miller et al. 1988).

200 µL blood sample was taken into 1.5 ml Eppendorf tubes

200 µl of Binding Buffer and 20 µl of Proteinase and RNAase were added to them and mixed well with the help of vortex

The mixture was left to incubate at 55 °C for 10 minutes

To ensure that the DNAs precipitate after incubation, 100 µl of ethanol was added to each tube and mixed with the help of a vortex

Collection tubes were removed as many as the number of patients and a filtered tube was placed in each of them

This prepared mixture was transferred to collection tubes with filters

The mixture in the filtered tubes was centrifuged at 11000 rpm for 3 min

After centrifugation, the collection tubes were discarded, and the filtered tubes were transferred to the new collection tubes

500 µl of Wash buffer-1 was added to each tube and centrifuged for 3 min at 14000 rpm

After centrifugation, the collection tubes were discarded, and the filtered tubes were transferred to the new collection tubes

500 µl of Wash buffer-2 was added to each tube and centrifuged for 3 min at 14000 rpm

After centrifugation, the collection tubes were discarded, and the filtered tubes were taken into 1.5ml Eppendorf tubes

100 µl of elution buffer will be added to each tube and centrifuged for 2 min at 14000 revolutions.

After centrifugation, the filters will be discarded, and the resulting DNA samples will be written on each sample by measuring the concentration in nanodrop. The DNA samples were then stored at -20 degrees Celsius for study.

3.7. Thermo Fisher Quanti study 5 MIR137 and MTHFR gene polymorphism genotyping with Real-Time PCR kit

Genotyping analyses were performed using the Thermo Fisher Quanti Study 5 Real-Time PCR system (Thermo scientific, Waltham, Massachusetts, USA) Taqman Genotyping Assays (Applied Biosystems Foster City, CA, USA) kits. The DNA sample was determined by fluorescence radiation using specific probes that hybridized in the annealing step of the Amplicon PCR cycle.

3.8. Preparation of Samples for Real-Time PCR

Before the reaction mixture was prepared, the Thermo Fisher Quanti stution 5 Real-Time PCR device was turned on and the device was self-tested. Then, a protocol to be used in the analysis of MTHFR and MIR137 gene polymorphisms was installed in the system. The patient list was entered, and the system was made ready for study. The reaction kits required for PCR were removed from −20ºC and thawed. The genotyping protocol for MTHFR (rs1801133) and MIR137 (rs1625579) SNPs analyzed in our study was published in the Dmitrzak-Weglarz et al. (2015) and applied as follows:

PCR mxi was prepared by adding 5 µL master mix (Applied Biosystems, TaqPath™ USA), 3.75 µL of distilled water, 0.50 µL of Taqman Genotyping Assays and 1 µL (10 ng) of DNA, with a total of 10 µL. A 96-well 0.1mL reaction plate (Applied Biosystems, MicroAmp* Fast 96-Well Reaction Plate) was added to the prepared mixture. Positive and negative control were added to the two wells in the plate. For the negative control, the same amount of distilled water was added instead of the DNA in the mix mixture, and for the positive control, the Heterozygous sample DNA, the result of which was previously known, was added. Then, the prepared plate is tightly covered with sealing foil (Applied Biosystems, Optical Adhesive Covers, USA). The wells in the plate were checked for air bubbles. If an air bubble has formed, the air bubble caused by the vibration movement applied to the plate has been destroyed. Finally, the program was started by making the necessary checks on the PCR program.

3.9. Statistical Analysis of Data

IBM SPSS Statistics for Windows, Version 25.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA) package program was used in the statistical analysis of the data obtained from the genotyping results. Sociodemographic findings and genotype results of the patients were given as (n and %) for categorical data, and as Mean±SD and Median (min-max) for numerical data. Mann Whitney u test, one of the nonparametric tests, was used in age and genotype comparisons. Fisher's Exact test was used to compare categorical variables. $p<0.05$ was considered statistically significant.

4. RESULT

Table 1 characteristics features of each patient

No	Age	Gender	Disease
1	49	Female	Bipolar
2	75	Male	Bipolar
3	19	Female	Bipolar
4	49	Female	Bipolar
5	31	Male	Bipolar
6	55	Female	Bipolar
7	27	Male	Bipolar
8	47	Female	Bipolar
9	55	Female	Bipolar
10	53	Female	Bipolar
11	57	Female	Bipolar
12	16	Male	Bipolar
13	23	Female	Bipolar
14	33	Male	Bipolar
15	50	Male	Bipolar
16	43	Female	Bipolar
17	53	Female	Bipolar
18	41	Female	Bipolar
19	16	Female	Bipolar
20	73	Male	Bipolar

Table 2 distribution of characteristics features of patients

Variables	**N**	**%**
Age		
Mean ± SD	43.25 ± 17.29	
Median (min-max)	48.0 (16-75)	
Gender		
Female	13	65,0
Male	7	35,0

As seen in Table 2, the average age of the patients was determined as 43.25 ± 17.29 and the median age was 48.0 (16-75). The minimum age of the patients was 16 and the maximum age was 75. 65% (n = 13) of the patients were determined to be female and 35% (n = 7) were male.

Table 3 genotype and allele distributions of MTHFR, MIR137 polymorphisms

Variables	**N**	**%**
Genotype-MTHFR (rs1801133)		
GG-Normal	12	60.0
GA-Risk	7	30.0
AA-Risk	1	5.0
Allele-MTHFR (rs1801133)		
G	31	77.5
A	9	22.5
Genotype-MIR137 (rs1625579)		
GG-Normal	5	25.0
GT-Risk	8	40.0
TT-Risk	7	35.0
Allele-MIR137 (rs1625579)		
G	18	45.0
T	22	55.0

As seen in Table 3, the distribution of genotypes belonging to MTHFR polymorphism was determined as 60% (n = 12) GG, 30% (n = 7) as GA and 5% (n = 1) as AA. When allele distributions were examined, 77.5% (n=31) was determined to be G allele and 22.5% (n=9) was determined to be A allele.

The distribution of genotypes belonging to MIR137 polymorphism was determined as 25% (n = 5) GG, 40% (n = 8) as GT and 7% (n = 7) as TT. When allele distributions were examined, 45% (n = 18) were determined to be G allele and 55.5% (n = 22) were determined to be T allele.

Table 4 distribution of risks in terms of polymorphisms

MTHFR (rs1801133)	**N**	**%**
Normal	12	60.0
Risk	8	40.0
MIR137 (rs1625579)	0	0.0
Normal	5	25.0
Risk	15	75.0

As seen in Table 4, 60% (n=12) of the patients have normal genotype and 40% (n=8) have risk genotype in terms of MTHFR (rs1801133) polymorphism. In terms of MIR137 (rs1625579) polymorphism, 25% (n=5) of the patients have normal genotype and 75% (n=15) have risk genotype.

Table 5 comparison of MITHFR genotypes in terms of age and gender

Variables	**MTHFR (rs1801133)**		**p**
	Normal **GG**	**Risk** **GA+AA**	
Age, Mean ± SD	49.41 ± 13.50	34.00 ± 19.02	0.071[b]
Age, Median (min-max)	51.5 (23-75)	32.0 (16-73)	
Gender, n (%)			
Female	9 (%75.0)	4 (%50.0)	0.356[a]
Male	3 (%25.0)	4 (50.0)	

a:Fisher's Exact test, b:Mann Whitney u test, p<0.05

As seen in Table 5, MTHFR (rs1801133) genotypes did not show a significant difference in terms of Age (p = 0.071) and Gender (p = 0.356).

Table 6 comparison of MIR137 genotypes i terms of age and gender

Variables	MIR137 (rs1625579)		p
	Normal **GG**	**Risk** **GT+TT**	
Age, Mean ± SD	44.40 ± 13.10	42,86 ± 18.87	0.845[b]
Age, Median (min-max)	50.0 (23-55)	47.0 (16-75)	
Gender, n (%)			
Female	4 (%80.0)	9 (%60.0)	0.613[a]
Male	1 (%20.0)	6 (40.0)	

a:Fisher's Exact test, b:Mann Whitney u test, p<0.05

As seen in Table 6, MIR137 (rs1625579) genotypes did not show a significant difference in terms of Age (p = 0.845) and Gender (p = 0.613).

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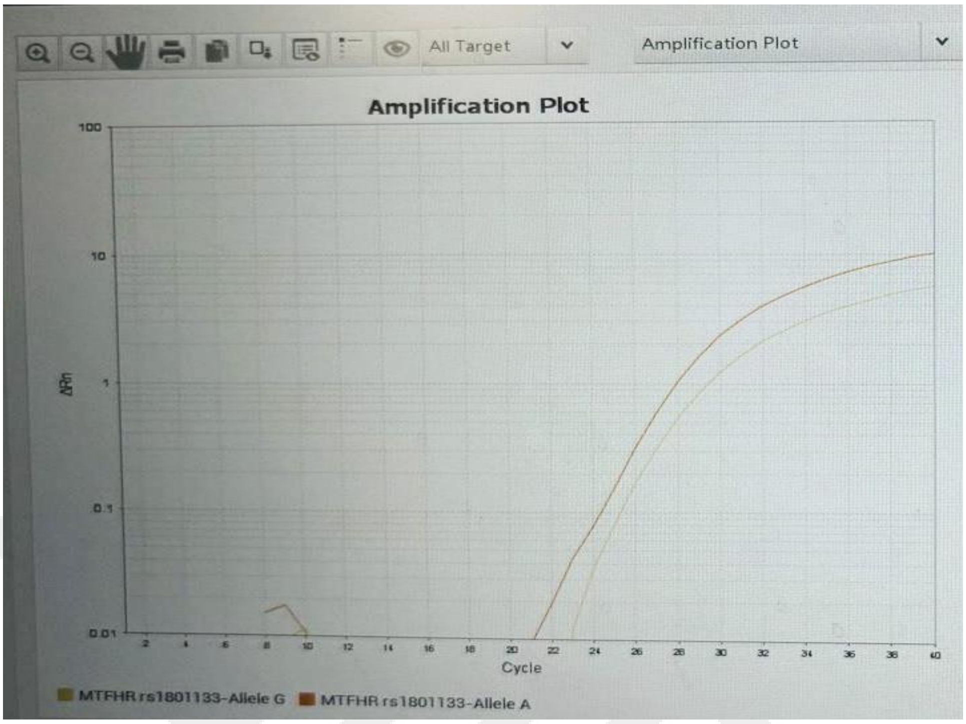

Figure 6 the real-time PCR amplification plot 1

An example of what the results appear in the real time PCR, the yellow line is referred to as allele G and the orange one refers to allele A when the line over the threshold point it will represent the presence of that specific allele therefore the genotype.

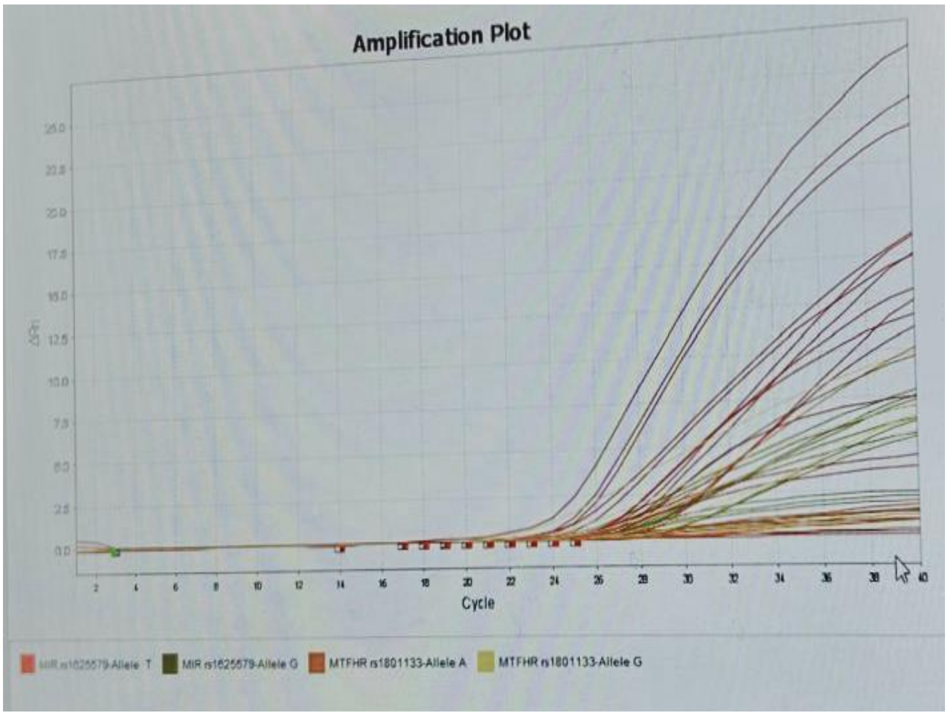

Figure 7 the real-time PCR amplification plot 2

5. Discussion and Conclusion

Recent evidence suggests that there is a clear and significant genetic contribution to BD (Adolfo S. , et al.2006 & Kato T. , et al.2007), Several genes have been investigated for association with BD , one of them MTHFR but, to the best of our knowledge, No research has been done to far that specifically examines potential relationships with MIR137 gene` polymorphisms and BD .The present study investigating the SNP variants of the MTHFR and MiR137 genes in individuals with Bipolar disorder suggests that the homozygous MiR137 rs1625579 A/G SNP and MTHFR C677-T rs18011**33** T/G may play a role in the biological susceptibility to Bipolar disease. Moreover, this is the first study to explore the association between BP disorder and MTHFR and MIR137 genes polymorphism in a Turkish population. Our research showed an association between the MTHFR C677-T rs1801133 polymorphism and BD as BD patients have higher frequencies of the A allele for the GA, and AA genotypes. This finding matches the findings of Kempisty et al., who suggest that BD may be associated to the 1p36.3 locus on MTHFR (C677T) gene. Additionally, the T allele was more common in BD patients, and Gilbody et al. and Jönsson et al.'s meta-analysis on BD indicated an almost significant correlation with MTHFR C677T. Furthermore, carriers of the A allele and AA genotype have a slightly statistically significant increased risk of developing serious mental illnesses, including BD, according to a meta-analysis by (Peerbooms et al.,2011). However, meta-analyses by (Zintzaras et al.,2006) discovered no significant association between the risk of developing BD and any allele of the C677T polymorphism. Chen et al.'s link study in the Chinese population and their meta-analysis also revealed no apparent variations in genotype distribution or allele frequencies between BD patients and controls. Regarding the single nucleotide polymorphism (SNP) rs1625579 within the MIR137 gene and bipolar, our study shows as BD patients have higher frequencies of the T allele for the GT, and TT genotypes. This result is partially in accordance with Warburton et al., 2014 which has been strongly linked Mir137 gene rs1625579 to schizophrenia. However, there is not much evidence regarding bipolar disorder. This SNP has shown susceptibility to schizophrenia in populations of European ancestry (Lu et al., 2017). Furthermore, the rs1625579 SNP has been linked to the internal MIR137 promoter, supporting allele-specific expression and its role in schizophrenia (Warburton et al., 2015). Furthermore, two studies have shown that the rs1625579 polymorphism in MIR137 is has potential association with bipolar disorder (Kim et al., 2012; Senousy et al., 2020). The MIR137 gene, which encodes microRNA-137, plays a crucial role in neuronal development and post-transcriptional regulation in the nervous system (Kandratsenka et al., 2018; Cheng et al., 2018). The risk genotype of rs1625579 has been linked to hyperactivation in the dorsolateral prefrontal cortex, a region implicated in schizophrenia (Erp et al., 2014). Additionally, the loss of microRNA-137 has been shown to impact the homeostasis of potassium in neurons, further emphasizing its role in neural function (Sun et al., 2020). The rs1625579 polymorphism in the MIR137 gene is significantly associated with both schizophrenia and bipolar disorder. These genetic variant influences brain function, neural connectivity, and may contribute to the pathophysiology of these psychiatric disorders. Further research into the mechanisms by which MIR137 affects neuronal processes could provide valuable insights into the development and treatment of bipolar disorder. The studies indicate a potential association between the MTHFR gene polymorphisms, including C677T, and bipolar disorder while the relationship between MIR137 rs1625579 polymorphism and bipolar is less clear and requires further investigation. Studies have shown that men with the MTHFR gene C677T polymorphism are at a greater risk of schizophrenia, while women are at a higher risk of bipolar affective disorders (Kevere et al., 2012). However, this study examined 20 bipolar disorders samples. The average age of the patients was determined as 43.25 ± 17.29, 65% were female and 35% were male. Although the percentage of women is higher than men there was no significant difference in terms in age and gender. Our study showed Mir137 rs16279 polymorphism associated with more risk for BPD, 60% (n=12) of the patients have normal genotype and 40% (n=8) have risk genotype in terms of MTHFR (rs1801133) polymorphism. On the other hand, showed a smaller number of risks between MTHFR polymorphism rs180133 and the risk for BPD. In terms of MIR137 (rs1625579) polymorphism, 25% (n=5) of the patients have normal genotype and 75% (n=15) have risk genotype, therefore notably, the potential risk associated with the MiR137 polymorphism is significantly greater, with 75% of patients carrying the risk genotype compared to 40% for MTHFR. This suggests a stronger genetic predisposition related to MiR137 in the bipolar disorder cohort. The results obtained are important but has several` limitations. Firstly, the sample size in the study was quite small, only 20 patients limiting the generalizability of the results. Therefore, studies on larger sample groups are needed. In this way, the results can be made more reliable and valid. Secondly, the unavailability of studies in Turkish population examining this gene and any other genetic studies in general. This makes comparison with previous studies from Turkish population so difficult Therefore, we compare the present results with results in other (Caucasian) populations. Thirdly, the clinical significance of the associations between MTHFR and Mir137 genetic polymorphisms and their predicted phenotypes has not been emphasized. More studies should be done to reveal the relationship between MIR137 rs1625579 polymorphism and MTHFR gene C677T polymorphism and bipolar disorder.

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