T.R.
INONU UNIVERSITY
GRADUATE SCHOOL OF NATURE AND APPLIED SCIENCES

MMP-9 AND MMP-2 GENE EXPRESSION LEVELS IN BIPOLAR DISORDER: EVALUATING RELATIONSHIPS WITH CLINICAL FEATURES AND CHILDHOOD TRAUMATIC EXPERIENCES

MASTER THESIS

ZİLAN KOP

Department of Molecular Biology and Genetics

Supervisor: Prof. Dr. Ceren ACAR

JULY 2024

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İNÖNÜ ÜNİVERSİTESİ
FEN BİLİMLERİ ENSTİTÜSÜ MÜDÜRLÜĞÜ

MMP-9 and MMP-2 Gene Expression Levels in Bipolar Disorder: Evaluating Relationships with Clinical Features and Childhood Traumatic Experiences

Tezi Hazırlayan: ZİLAN KOP
Yüksek Lisans Tezi

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To my brothers and sister

WORD OF HONOR

I hereby declare that this study titled "MMP-9 and MMP-2 Gene Expression Levels in Bipolar Disorder: Evaluating Relationships with Clinical Features and Childhood Traumatic Experiences", which I have submitted as a master's thesis, has been written by myself without any help that would contradict scientific ethics and traditions, and that all the sources I have used consist of those cited both in the text and in the bibliography in accordance with the method, and I confirm this with my honor.

Zilan KOP

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SYMBOLS AND ABBREVIATIONS

APA	**:** American Psychiatric Association
AP	**:** Antipsikotik
BD	**:** Bipolar Disorder
BD-I	**:** Bipolar I Disorder
BD-II	**:** Bipolar II Disorder
BBB	**:** Blood-Brain Barrier
CNS	**:** Central Nervous System
DNA	**:** Deoxyribonucleic acid
DSM-V	**:** Diagnostic and Statistical Manual of Mental Disorders-V
ECM	**:** Extracellular matrix
MMPs	**:** Matrix metalloproteinases
MMP-2	**:** Matrix metalloproteinase-2
MMP-9	**:** Matrix metalloproteinase-9
mRNA	**:** Messenger ribonucleic acid
qPCR	**:** Quantitative polymerase chain reaction

ÖZET

Yüksek Lisans Tezi

BİPOLAR BOZUKLUKTA MMP-9 VE MMP-2 GEN İFADE DÜZEYLERİ: KLİNİK ÖZELLİKLER VE ÇOCUKLUK ÇAĞI TRAVMATİK DENEYİMLERİ İLE İLİŞKİNİN DEĞERLENDİRİLMESİ

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Moleküler Biyoloji ve Genetik Anabilim Dalı

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Danışman: Prof. Dr. Ceren ACAR

ECM tüm doku ve organlarda hücre dışı ortamını oluşturan karmaşık bir ağdır. Beynin yapısal bileşeni olarak da görev yapan ECM, nöronal işlev ve beynin çevresel uyaranlara tepkisi için önemli iki süreç olan nöroplastisite ve nöroinflamasyonun düzenlenmesinde önemli bir rol oynar. ECM düzenlemesinin önemli bir yönü, ECM'nin bozulması ve yeniden şekillendirilmesinden sorumlu bir enzim ailesi olan MMP'lerdir. MMP'ler arasında MMP-9 ve MMP-2 beyinde önemli bir rol oynamaktadır. MMP-9, ECM proteinlerini parçalayarak ve sinapsların yapısını yeniden şekillendirerek sinaptik plastisitenin modüle edilmesinde rol oynar. MMP-2 sinir sistemi gelişimi sırasında hücre göçünü ve akson büyümesini düzenler. ECM, MMP'ler ve bunların nöroplastisite ve nöroinflamasyondaki rolleri de bipolar bozuklukla ilişkilendirilmiştir. BD, epizodik ruh hali değişimleri ile karakterize ciddi bir akıl hastalığıdır. Çalışmalar, ECM bileşimi ve MMP aktivitesindeki değişikliklerin bipolar bozukluğu olan hastalarda meydana gelebileceğini ve bozukluğun patofizyolojisi ile ilişkili olabileceğini göstermiştir. Bu çalışma, moleküler biyoloji ve psikiyatri arasındaki arayüzde temel soruları ele almaktadır. Çalışmanın amacı, BD'li ve sağlıklı bireylerde MMP-9 ve MMP-2 ifade düzeylerini karşılaştırmak ve bu gen ifadeleri ile klinik özellikler ve çocukluk çağı travmatik deneyimleri arasındaki ilişkiyi araştırmaktır. Sonuçlar, MMP-9 ve MMP-2 gen ifade düzeylerinde BD'li ve sağlıklı bireyler arasında anlamlı bir fark olmadığını göstermiştir. Ayrıca, klinik özellikler ve çocukluk çağı travmatik deneyimleri ile MMP-9 ve MMP-2 gen ifade düzeyleri arasında anlamlı bir ilişki bulunmamıştır.

Anahtar Kelimeler: Gen ifadesi, MMP-9, MMP-2, bipolar bozukluk, çocukluk çağı travması

ABSTRACT

Master Thesis

MMP-9 AND MMP-2 GENE EXPRESSION LEVELS IN BIPOLAR DISORDER: EVALUATING RELATIONSHIPS WITH CLINICAL FEATURES AND CHILDHOOD TRAUMATIC EXPERIENCES

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ECM is a complex network that forms the extracellular environment in all tissues and organs. Serving as a structural component of the brain, the ECM plays an important role in the regulation of neuroplasticity and neuroinflammation, two processes important for neuronal function and brain response to environmental stimuli. An important aspect of ECM regulation is MMPs, a family of enzymes responsible for ECM degradation and remodeling. Among MMPs, MMP-9 and MMP-2 play an important role in the brain. MMP-9 is involved in the modulation of synaptic plasticity by degrading ECM proteins and structural reorganization of synapses. MMP-2 regulates cell migration and axon growth during the development of the nervous system. ECM, MMPs and their roles in neuroplasticity and neuroinflammation have also been associated with bipolar disorder. BD is a serious mental illness characterized by episodic mood swings. Studies have shown that alterations in ECM composition and MMP activity can occur in patients with BD and may be related to the pathophysiology of the disorder. This study addresses fundamental questions at the interface between molecular biology and psychiatry. The aim of the study was to compare MMP-9 and MMP-2 expression levels in individuals diagnosed with BD and healthy controls and to investigate the relationship between these gene expressions and clinical features and childhood traumatic experiences. The study showed that no significant difference in gene expression levels between individuals with BD and healthy controls. There was also no significant relationship between clinical characteristics and childhood traumatic experiences and gene expression levels.

Keywords: Gene expression, MMP-9, MMP-2, bipolar disorder, childhood trauma

1. INTRODUCTION

Psychiatric disorders, also known as mental disorders, are a heterogeneous group of mental health conditions that include behavioral, emotional or cognitive dysfunctions associated with clinically significant distress or impairment in one or more areas affecting mood, thought and behavior (Elleker & O'Neill, 2014; APA, 2022). These disorders are not easily controlled by the individual. Bipolar disorder (BD), which was previously referred to as manic-depressive disorder, is characterized by the occurrence of alternating periods of elevated emotional moods as mania or hypomania and depressed moods as depression (Smith D. J., et al., 2012). Such fluctuations in mood can impact various aspects of an individual's functioning, including sleep, energy levels, behavior, judgement, and cognitive clarity. Mood fluctuations may occur with some frequency, ranging from less than once a year to more than once a year. BD affects approximately 1-3% of the world population (Rowland & Marwaha, 2019). Although the disorder typically presents in late adolescence or early adulthood, it may be seen in any age group (Rowland & Marwaha, 2019). Although the course and type of BD may vary by gender, there is no significant difference in prevalence between males and females (Rowland & Marwaha, 2019). Given the high morbidity rate, the increased risk of comorbid conditions, and the in-suicide risk in severe cases, it is of great importance to diagnose and treat BD as early as possible (Rowland & Marwaha, 2019). A strong hereditary component is postulated for BD (Smoller & Finn, 2003). The available evidence indicates that those with a first-degree relative with BD are at increased risk of developing the disorder themselves (Smoller & Finn, 2003). A multitude of genetic studies have identified a multitude of risk genes associated with the disorder (Craddock & Sklar, 2013). Neuroimaging studies have demonstrated the presence of abnormal structural and functional entities in the brains of individuals diagnosed with bipolar disorder (Pereira, et al., 2017). Neurotransmitter imbalances, particularly those involving serotonin, dopamine and norepinephrine, are believed to play a role in the pathophysiology of the disorder (Pereira, et al., 2017). It has been demonstrated that stressful life events, trauma and significant psychosocial stressors can act as triggers or exacerbators of BD symptoms (Erten, et. al., 2014). Furthermore, substance abuse and disturbances in sleep patterns have been identified as contributing factors. In this context, the etiology of BD is complex and multifactorial, including genetic, biological and environmental factors.

Gene expression includes the process of transcribing deoxyribonucleic acid (DNA) from a gene into messenger ribonucleic acid (mRNA) and then translating mRNA into functional gene products. Many external stimuli and regulatory mechanisms affect this process, adding to the ever-changing character of cellular activity. Gene expression research identifies genes that show significant changes in expression in different situations, such as diseased and healthy tissues (Dehghan, 2018). They explain the underlying molecular processes and regulate genes under different physiological or pathological conditions (Dehghan, 2018). In addition, they address cellular function and causes of disease by studying interactions between genes and their expression patterns (Dehghan, 2018). In this context, psychiatric disorders are polygenic and involve the interference of numerous genes. Gene expression research allows these small effects to be aggregated to identify important aspects. Gene expression levels provide a dynamic representation of changes in response to environmental and internal stimuli. This contrasts with the static nature of DNA sequence data. This provides an understanding of how these elements influence disease.

Matrix metalloproteinases (MMPs) are a group of zinc-dependent endopeptidases that degrade various components of the extracellular matrix ECM (Chaudhary, et. al., 2013). These components are essential for numerous biological processes (Cabral-Pacheco, et. al., 2020). MMPs have a wide range of functions beyond the degradation of the ECM. Neuroplasticity which the brain's ability to reorganize itself by forming new neural connections is affected by MMPs (Beroun, et. al., 2019). They modulate the ECM surrounding neurons, affecting synaptic plasticity, dendritic spine morphology and cell adhesion (Beroun, et. al., 2019). Neuroinflammation is linked to MMPs, a common feature of numerous psychiatric disorders (Leonardo & Pennypacker, 2009). In the CNS, MMPs serve two functions. High levels of MMPs can cause neuronal death and neurodegeneration. This occurs during normal brain repair and remodeling. This balance is crucial for maintaining CNS homeostasis and is often disrupted by psychiatric disorders.

The study, "MMP-9 and MMP-2 Gene Expression Levels in Bipolar Disorder: Evaluating Relationships with Clinical Features and Childhood Traumatic Experiences," addresses several fundamental problems at the intersection of molecular biology, psychiatry, and clinical psychology. First, BD is a complex and multifaceted psychiatric condition characterized by alternating periods of mania and depression, posing significant challenges for accurate diagnosis and effective treatment. The etiology of BD is complex and poorly understood, potentially involving a combination of genetic predispositions, environmental factors, and neurobiological mechanisms. Among the proposed biomarkers, MMPs, particularly matrix metalloproteinase-9 (MMP-9) and matrix metalloproteinase-2 (MMP-2), have attracted attention for their role in neuroinflammation, synaptic plasticity, and the integrity of the blood-brain barrier (BBB) (Rempe, et. al., 2016). However, the expression levels of these enzymes in individuals with BD and their potential links to clinical features such as severity, duration, and response to treatment have not been fully elucidated. Furthermore, childhood trauma is increasingly recognized as a critical factor influencing the course and prognosis of BD (Janiri, et. al., 2015). However, the underlying biological mechanisms mediating this relationship require further investigation. This thesis aims to address the knowledge gaps by assessing the gene expression patterns of MMP-9 and MMP-2 in BD patients, examining their association with various clinical presentations, and investigating how these molecular markers are related to childhood trauma histories. In this way, the research aims to improve our understanding of the pathophysiological basis of BD and identify potential biomarkers that can inform individualized therapeutic strategies.

1.1. Bipolar Disorder

Bipolar disorder (BD) is a serious and complex mood disorder associated with cognitive, physical and behavioral symptoms accompanied by changes in a person's activity or energy (Jain & Mitra, 2024). A complex and multifaceted psychiatric disorder, BD is a testament to the complex interplay between genetics, environment and neurobiology, with a notable genetic component in shaping mental health (Jain & Mitra, 2024). The condition is characterized by the occurrence of recurrent episodes of depression, which may be accompanied by periods of mania and/or hypomania (Jain & Mitra, 2024). These episodes are characterized by high energy, hyperactivity and increased self-confidence. However, they can also be interspersed with periods of normal mood.

1.2. History

The concept of BD has its origins in two of the oldest human diseases, defined as mania and melancholia (Angst & Marneros, 2001). These two extremes of mood were documented very early in history through the work and opinions of ancient Greek physicians and philosophers. Hippocrates (460-337 BC) was the first to systematically document and analyze these emotional states, based on his own clinical observations (Angst & Marneros, 2001). Hippocrates recognized the brain as the organ of mental functions and disorders. In his renowned work, "On the Sacred Disease," Hippocrates proposes a theory that human emotions, thoughts, and behaviors are controlled by the brain (Angst & Marneros, 2001). He further asserts that the brain is the source of pleasures, joys, sorrows, and worries. Hippocrates postulates that when the brain is diseased, the individual's emotional and mental experiences are disrupted, potentially leading to the emergence of various disorders. Another first-century Greek physician, Aretaeus of Cappadocia, argued that mania and melancholia were biological changes, not innate personality traits or specific reactions to situations (Angst & Marneros, 2001). In this context, the differences between mania and melancholia were not only psychological but also biological.

In 1851, Jean-Pierre Falret established the first idea of a psychiatric disorder he called "folie circulaire (circular insanity)" (Angst & Marneros, 2001). This disorder was characterized by free intervals of varying length between continuous cycles of depression and mania. In 1863, Karl Kahlbaum attempted to characterize mood disorders, proposing the first nosologically classification of psychoses (Angst & Marneros, 2001). Emil Kraepelin, influenced by the work of Karl Kahlbaum and Jean-Pierre Falret, tried to define all mood disorders and unify mania, depression, and all psychotic states (Angst & Marneros, 2001). Kraepelin's work made a significant contribution to the understanding of what is now known as schizophrenia and bipolar disorder.

In the 1950s, the first edition of the Diagnostic and Statistical Manual of Mental Disorders (DSM) was published with the objective of standardizing and categorizing mental disorders, thereby providing a common framework for diagnosis, research, treatment, and education (Angst & Marneros, 2001). The evolution of psychiatric understanding and diagnostic precision has facilitated the periodic updates to the DSM. This journey underscores the necessity to adapt diagnostic frameworks to more accurately reflect the environmental and genetic complexity of mental health conditions, with the goal of improving patient care and outcomes.

1.3. Clinical Features

Bipolar and Related Disorders are included in the Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V) between the schizophrenia spectrum and other psychotic and depressive disorders. They are considered to bridge the two diagnostic classes in terms of symptomatology, family history, and genetics. As outlined in the DSM-V, BD is divided into several types, with the main classification based on specific patterns and severity of manic, hypomanic, and depressive episodes. All three periods involve significant changes in mood, energy, and activity levels (APA, 2022).

The DSM-V, published by the American Psychiatric Association (APA) and utilized in the classification of psychiatric disorders, subdivides BD into BD type I, BD type II, cyclothymic disorder, substance/drug-induced bipolar, bipolar due to another medical condition, other specified bipolar, unspecified bipolar, and related disorders (APA, 2022).

1.3.1. Bipolar I disorder (BD-I)

A mood disorder characterized by at least one recurrent episode of full-blown mania or mixed symptoms of both manic and depressive symptoms (APA, 2022). The main characteristic of a manic episode is a period of abnormal, persistently elevated, expansive, or irritable mood and persistently increased activity or energy for most of the day, almost every day for at least 1 week (or for any period if hospitalization is required), as detailed in Figure 1.3.1 (APA, 2022).

1.3.2. Bipolar II disorder (BD-II)

A mood disorder characterized by a clinical course of recurrent mood episodes, which may include one or more major depressive episodes and at least one hypomanic episode (APA, 2022). To meet the diagnostic criteria, the major depressive episode listed in Figure 1.3.3 must last at least two weeks, and the hypomanic episode listed in Figure 1.3.2. must last at least four days. The presence of a manic episode during the course of the illness excludes the diagnosis of BD-II. The primary distinction between BD-I and BD-II is the severity of manic episodes. In BD-II, manic episodes are less severe and are referred to as hypomanic episodes (APA, 2022).

1.3.3. Cyclothymic disorder

A mood disorder characterized by chronic fluctuations with multiple episodes of hypomanic and depressive symptoms that do not meet the criteria for a major depressive episode (APA, 2022). Individuals with cyclothymic disorder should have persistent periods of multiple hypomanic and depressive symptoms during the first two years (one year in children or adolescents) and periods of stable mood with no symptoms lasting no longer than two months (APA, 2022).

A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased goal-directed activity or energy, lasting at least 1 week and present most of the day, nearly every day (or any duration if hospitalization is necessary).

B. During the period of mood disturbance and icreased energy or activity, three (or more) of the following symptoms (four if the mood is only irritable) are present to a significant degree and represent a noticeable change from usual behavior:

1. Inflated self-esteem or grandiosity.
2. Decreased need for sleep (e.g., feels rested after only 3 hours of sleep).
3. More talkative than usual or pressure to keep talking.
4. Flight of ideas or subjective experience that thoughts are racing.
5. Distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli), as reported or observed.
6. Increase in goal-directed activity (either socially, at work or school, or sexually) or psychomotr agitation (i.e., purposeless non-goal-directed activity).
7. Excessive involvement in activities that have a high potential for painful consequences (e.g., engaging in unrestrained buying spress, sexual indiscretions, or foolish business investments).

C. The mood disturbance is sufficiently severe to cause marked impairment in social or occupational functioning or to necessitate hospitalization to prevent harm to self or others, or there are psychotic features.

D. The episode is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication, other treatment) or to another medical condition.

Figure 1.3.1. Diagnostic criteria for manic episodes according to DSM-V **(APA, 2022)**.

A. A distinct period of abnormally and persistently elevated, expansive, or irritable mood and abnormally and persistently increased activity or energy, lasting at least 4 consecutive days and present most of the day, nearly every day.

B. During the period of mood disturbance and increased energy and activity, three (or more) of the following symptoms (four if the mood is only irritable) have persisted, represent a noticeable change from usual behavior, and have been present to a significant degree:

1. Inflated self-esteem or grandiosity.
2. Decreased need for sleep (e.g., feels rested after only 3 hours of sleep).
3. More talkative than usual or pressure to keep talking.
4. Flight of ideas or subjective experience that thoughts are racing.
5. Distractibility (i.e., attention too easily drawn to unimportant or irrelevant external stimuli), as reported or observed.
6. Increase in goal-directed activity (either socially, at work or school, or sexually) or psychomotor agitation.
7. Excessive involvement in activities that have a high potential for painful consequences (e.g., engaging in unrestrained buying sprees, sexual indiscretions, or foolish business investments).

C. The episode is associated with an unequivocal change in functioning that is uncharacteristic of the individual when not symptomatic.

D. The disturbance in mood and the change in functioning are observable by others.

E. The episode is not severe enough to cause marked impairment in social or occupational functioning or to necessitate hospitalization. If there are psychotic features, the episode is, by definition, manic.

F. The episode is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication, other treatment).

Figure 1.3.2. Diagnostic criteria for hypomanic episodes according to DSM-V **(APA, 2022)**.

A. Five (or more) of the following symptoms have been present during the same 2-week period and represent a change from previous functioning; at least one of the symptoms is either (1) depressed mood or (2) loss of interest or pleasure.

1. Depressed mood most of the day, nearly every day, as indicated by either subjective report (e.g., feels sad, empty, or hopeless) or observation made by others (e.g., appears tearful). (**Note:** In children and adolescents, can be irritable mood.)
2. Markedly diminished interest or pleasure in all, or almost all, activities most of the day, nearly every day (as indicated by either subjective account or observation).
3. Significant weight loss when not dieting or weight gain (e.g., a change of more than 5% of body weight in a month), or decrease or increase in appetite nearly every day. (**Note:** In children, consider failure to make expected weight gain.)
4. Insomnia or hypersomnia nearly every day.
5. Psychomotor agitation or retardation nearly every day (observable by others; not merely subjective feelings of restlessness or being slowed down).
6. Fatigue or loss of energy nerly every day.
7. Feelings of worthlessness or excessive or inappropriate guilt (which may be delusional) nearly every day (not merely self-reproach or guilt about being sick).
8. Diminshed ability to think or concentrate, or indecisiveness, nearly every day (either by subjective account or as observed by others).
9. Recurrent thoughts of death (not just fear of dying), recurrent suicidal ideation without a specific plan, or a suicide attempt or a specific plan for committing suicide.

B. The symptoms cause clinically significant distress or impairment in social, occupational, or other important areas of functioning.

C. The episode is not attributable to the physciological effects of a substance or another medical condition.

Figure 1.3.3. Diagnostic criteria for major depressive episode according to DSM-V **(APA, 2022)**.

1.4. Epidemiology

BD is a condition that can significantly affect an individual's quality of life and typically emerges during early adulthood. Consequently, a significant proportion of patients must contend with this condition throughout their lives. In this context, due to its early onset and lifelong chronicity, BD is responsible for a greater number of disability-adjusted life years (DALYs) lost than all types of diseases like cancer or major neurological conditions such as epilepsy and Alzheimer's disease (Feigin, et. al., 2020).

The epidemiology of BD remains relatively stable, with prevalence rates varying depending on the population studied and the diagnostic criteria employed. Nevertheless, BD affects approximately 40 million individuals worldwide, representing one of the most prevalent mental health disorders. While most epidemiological reports have indicated that BD-I targets approximately 1% of the adult population, more recent approaches have suggested that as many as 4% of individuals may be affected by bipolar spectrum disorders, including BD-II and cyclothymia (Harrison, et. al., 2019). The average age of onset for BD is estimated to be in the early twenties (Sit, 2004). However, cases range from 20 to 30 years. It is estimated that over half of patients with BD do not require treatment. Individuals with

BD have a reduced life expectancy of approximately 12 to 14 years. They also experience a 1.6 to 2-fold increase in cardiovascular mortality. This occurs on average 17 years earlier than in the general population. People with BD are more likely to have metabolic syndrome, obesity, smoking and type 2 diabetes (Sit, 2004). This makes them more likely to die early. People with BD are 80 times more likely to die by suicide than the general population. 15-20% of people with BD die by suicide (Sit, 2004).

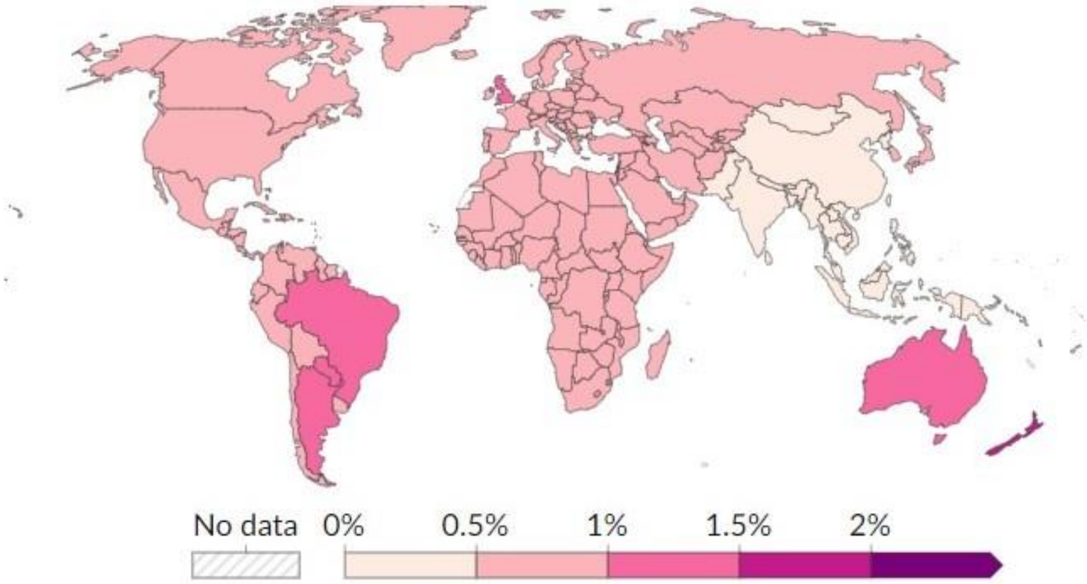

Figure 1.4. Bipolar disorder prevalence. Estimated share of males vs. females who have bipolar disorder. IHME Global Burden of Disease (2019)

The epidemiology of BD may result in inconsistent findings across populations. Several studies have demonstrated that BD varies according to sociodemographic variables (Rowland & Marwaha, 2019). Factors such as geographical region, cultural differences, economic conditions, age, gender, educational level, and socioeconomic status may influence the prevalence of BD (Rowland & Marwaha, 2019). For instance, BD-II is known to be more prevalent in women, whereas BD-I may be more common in men (Rowland & Marwaha, 2019). Alternatively, traumatic events experienced intensely in a particular geographical region may cause BD to be more prevalent in that region. Furthermore, high levels of stress in certain socioeconomic groups may increase the risk of BD in these groups. Consequently, the epidemiology of BD may differ between populations and may be shaped by a combination of sociodemographic variables and childhood traumatic events.

1.5. Etiology

The etiology of BD is a complex and multifaceted issue that has long engaged researchers and clinicians. Several factors are being investigated by researchers, including genetic predisposition and neurochemical dysregulation, which may contribute to the onset of this disorder. One significant factor is genetic predisposition, as research has demonstrated that subjects with a family history of BD are at an increased risk of developing the disorder (O'Connell & Coombes, 2021). In addition to genetic factors, environmental factors such as stress, trauma, or sleep disturbances also play a role in the development of BD. Nevertheless, the precise etiology of BD remains elusive, necessitating further investigation.

1.5.1. Genetics

BD exhibits a clear familial clustering, which indicates a strong genetic component. Twin cohort and adoption studies have demonstrated that genetic factors account for 60% to 80% of the susceptibility to the disorder (O'Connell & Coombes, 2021). A positive family history is a risk factor for the development of BD. The risk of a child developing BD is elevated by approximately 10 to 15 percent if one parent suffers from the disorder (Ozdemir, et. al., 2016). Furthermore, research indicates that the risk is further elevated if both parents are affected by BD.

Several gene loci and genes associated with BD have been identified. The *CACNA1C* gene encodes a calcium channel that plays a key role in the transmission of signals in nerve cells. Mutations or variations in this gene have been associated with increased susceptibility to BD (Craddock & Sklar, 2013). The *ANK3* gene encodes ankyrin G, a protein important for maintaining the structural stability of neurons. Alterations in *ANK3* have been linked to the pathophysiology of BD (Leussis, et. al., 2012). *BDNF* (Brain-Derived Neurotrophic Factor) is an essential protein that promotes the survival and growth of neurons. Studies have demonstrated that alterations in the expression or function of *BDNF* lead to impairments in neuronal plasticity observed in bipolar patients (Autry & Monteggia, 2012).

1.5.2. Molecular biology

The molecular biological mechanisms contributing to BD involve a variety of cellular signaling pathways and processes. Second messenger pathways, such as those involving cyclic adenosine monophosphate (cAMP), have been demonstrated to play a role in the pathogenesis of BD (D’Onofrio, et. al., 2017). These signaling pathways affect a range of cellular functions, including gene expression and neuronal excitability. Abnormalities in the function of ion channels, particularly calcium and potassium channels, can affect neuronal excitability and thus the risk of manic and depressive episodes (Imbrici, et. al., 2013). Another crucial aspect of BD is impairment in neuroplasticity, which refers to the brain's capacity to reorganize itself in response to novel experiences. Modifications in neurogenesis and synaptic plasticity can influence the stability of mood and emotional responses.

1.5.3. Environment

The development and course of BD are influenced by a multitude of environmental factors, including psychosocial stressors, childhood trauma, substance abuse, sleep patterns, and social support (Marangoni, et. al., 2018). Among these, psychosocial stressors have been the subject of extensive investigation. Studies have demonstrated that stressful life events, such as the loss of a loved one, financial difficulties, or interpersonal conflicts, frequently precede the onset of the first bipolar episode. Such events can act as triggers for the onset of the disorder in individuals with a genetic predisposition to the disorder.

Research indicates that early adverse experiences, such as childhood trauma, can increase the risk for a variety of mental disorders, including BD (Aldinger & Schulze, 2016; Brown, et. al., 2005; Etain, et. al., 2008). Neurobiological changes caused by trauma can lower the threshold for triggering disorder-specific symptoms. Another important environmental factor is substance abuse. Drug and alcohol use not only exacerbates existing symptoms of BD but also promotes the development of the disorder. Substances such as alcohol can destabilize mood and exacerbate both depressive and manic phases. Sleep is another crucial environmental factor that can influence mood and energy balance in individuals with BD. Irregular sleep patterns, frequently precipitated by shift work or frequent travel, can significantly contribute to the symptoms of BD. Furthermore, an unbalanced diet and a lack of physical activity can increase the risk of mood swings.

BD is influenced by many factors, including genetics, biology, and the environment. The identification of specific genes and the study of their function and related molecular pathways have increased our understanding of the biological basis of this disorder. Future research, particularly focusing on the integration of genetic, epigenetic, and environmental factors, is critical to developing more effective treatment.

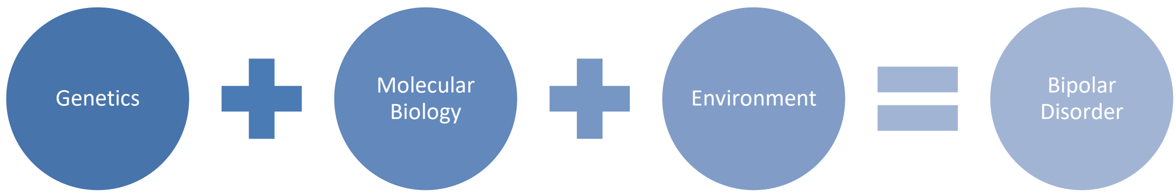

Figure 1.5. Etiology of bipolar disorder. Bipolar disorder is a complex heterogeneous disorder that is influenced by interactions between genetics, molecular biology and environment.

1.6. Traumatic Life Experiences

In the DSM-V, a trauma or trauma-related condition is typically defined in the context of trauma and stressor-related disorders as an encounter with stimuli that the person is unable to cope with, comprehend, and process in a short period of time, which negatively affects his/her self-functions and disrupts his/her mental balance (APA, 2022).

The DSM-V defines traumatic event as actual or threatened death, serious injury or exposure to sexual violence (APA, 2022). The way individuals respond to traumatic experiences varies considerably. The way trauma is perceived is contingent upon an individual's life experiences, personality traits, and existing coping skills. This exposure can occur in a variety of ways, including direct experience, witnessing the event happening to others, learning that the traumatic event occurred to a close family member or close friend, and repeated or excessive exposure to the details of the traumatic event. The long-term effects of these experiences can be diverse, including cognitive, emotional, and psychosocial developmental disorders. Nevertheless, it is crucial to acknowledge that individual perceptions and processing of traumatic events may vary, suggesting that not all individuals who experience such events will develop mental health disorders.

1.6.1. Childhood traumatic experiences

Children can be affected by many traumatic events that can harm their mental health. Childhood trauma is a phenomenon as old as human history. It is defined in the DSM-V as a form of stress resulting from serious events that physically or emotionally threaten or harm a child (APA, 2022). In the classification of childhood traumatic experiences, neglect is divided into two groups: physical and emotional. In contrast, abuse is divided into three groups: sexual, physical, and emotional.

1.6.1.1. Physical and emotional neglect

Physical neglect is explained as the harm caused to an individual under the age of 18 because of inadequate care in the fulfillment of basic life needs, including nutrition, shelter, clothing, and hygiene. The absence of nurturing and caregiving experiences during the first three years of life may result in difficulties in forming secure attachments and establishing close relationships in the future. Additionally, it may hinder the development of emotional competencies such as empathy, compassion, and sympathy in children (Taner & Gökler, 2004; Şar, 1998).

Emotional neglect is defined as a lack of sufficient attention and affection, as well as the absence of the necessary support and supervision for social development. It also encompasses the failure to impart social rules and norms. Emotional neglect and abuse represent the most prevalent form of child trauma, and in one study, it was determined that 81.6% of children had experienced emotional neglect (Taner & Gökler, 2004; Bahar, et. al., 2009).

1.6.1.2. Physical, emotional and sexual abuse

Physical abuse is defined as the intentional application of physical force by a parent, guardian, or other individual responsible for the care of a child in a manner that causes harm to the child's development and health. It is the most easily identifiable form of abuse and the most prevalent, with prevalence rates ranging from 15% to 75% (Taner & Gökler, 2004; Lipman, et. al., 2001).

Emotional abuse is defined as the imposition of demands that are inappropriate for the child's age and capacity, resulting in psychological damage to the child through the deprivation of love and attention, the denial of social relations, and the constant denigration of the child's capacities and desires. Emotional abuse, which is more prevalent than physical abuse, is more challenging to recognize, comprehend, and legally substantiate. It has been demonstrated that emotional neglect and abuse are present in 90% of cases involving physical abuse and neglect (Taner & Gökler, 2004; Tıraşçı & Gören, 2007).

Sexual abuse is defined as the act of an adult deceiving, intimidating, or threatening a child with incomplete sexual development to meet their own sexual desires, wishes, or demands. This definition also encompasses instances where the age difference between the two children is over four years. It has been reported that sexual abuse, which is four times more common in girls than in boys, is seen at an average rate of 10-40% in childhood. Approximately 53% of these children are under the age of 14 (Terr, 1991; Aktepe, 2009).

1.6.2. Childhood traumatic experiences and bipolar disorder

Childhood trauma, and other adverse experiences, has been associated with a range of long-term psychological and physiological difficulties. Emerging studies suggest a significant correlation between early traumatic events and the onset and severity of BD (Erten, et. al., 2014). A study of the impact of specific forms of childhood trauma and abuse on the clinical outcomes of adult patients with BD revealed that emotional abuse was experienced by 37% of the subjects, physical abuse by 37%, emotional neglect by 24%, and physical neglect by 12% (Erten, et. al., 2014). A study conducted in Turkey aimed to investigate the effect of childhood trauma on the clinical characteristics and quality of life of BP-I patients (Erten, et. al., 2014). The results indicated that physical abuse was reported in 25.9% of cases, emotional abuse in 26.7%, and sexual abuse (11.2% by a stranger, 9.5% within the family) in 20.7% of cases (Erten, et. al., 2014). Physical neglect and emotional neglect were evaluated together and found in 39.7% of patients with BD (Erten, et. al., 2014).

The relationship between childhood traumatic experiences and BD is complex, involving genetic, biological, and clinical dimensions. Genetic studies have highlighted the importance of gene-environment interactions in understanding this relationship. A study examined the role of epigenetic changes in individuals with a history of childhood trauma and BD (Perlis et al., 2014). The researchers discovered that trauma could result in alterations to the methylation of genes associated with stress response and mood regulation, which might increase the likelihood of developing BD. A comprehensive genome-wide association study (GWAS) identified numerous gene-environment interactions between specific genetic variants (e.g., *BDNF* and *FKBP5*) and childhood trauma (Binder et. al., 2008). The interactions were found to influence the risk and severity of BD. Conversely, biological research indicates the existence of mechanisms such as hypothalamus-pituitary-adrenal (HPA) axis dysregulation and neuroinflammation. Research revealed that childhood trauma results in long-term dysregulation of the HPA axis, which has a pivotal role in the stress response (Murphy, et. al., 2022). Individuals diagnosed with BD and a history of childhood trauma exhibited elevated cortisol levels, which are indicative of HPA axis hyperactivity. A study focused on biomarkers of neuroinflammation and neurotrophic factors (such as *TNF-α* and *BDNF*) (Tian, et. al., 2021). The study revealed that individuals with BD who had experienced childhood trauma exhibited increased levels of pro-inflammatory markers and diminished levels of neurotrophic factors. This suggests the existence of a biological pathway linking trauma and BD. From a clinical perspective, childhood trauma has been shown to influence the severity, course, and treatment response of BD. In a study, the clinical outcomes of bipolar patients with and without a history of childhood trauma were evaluated (Quidé, et. al., 2020). The study revealed that childhood trauma was associated with an earlier onset of BD, an increased frequency of mood episodes, rapid cycling, and a greater comorbidity with anxiety disorders.

Future studies should continue to elucidate these intricate connections, with an emphasis on the development of tailored therapeutic approaches that address the underlying trauma to improve outcomes for individuals with BD.

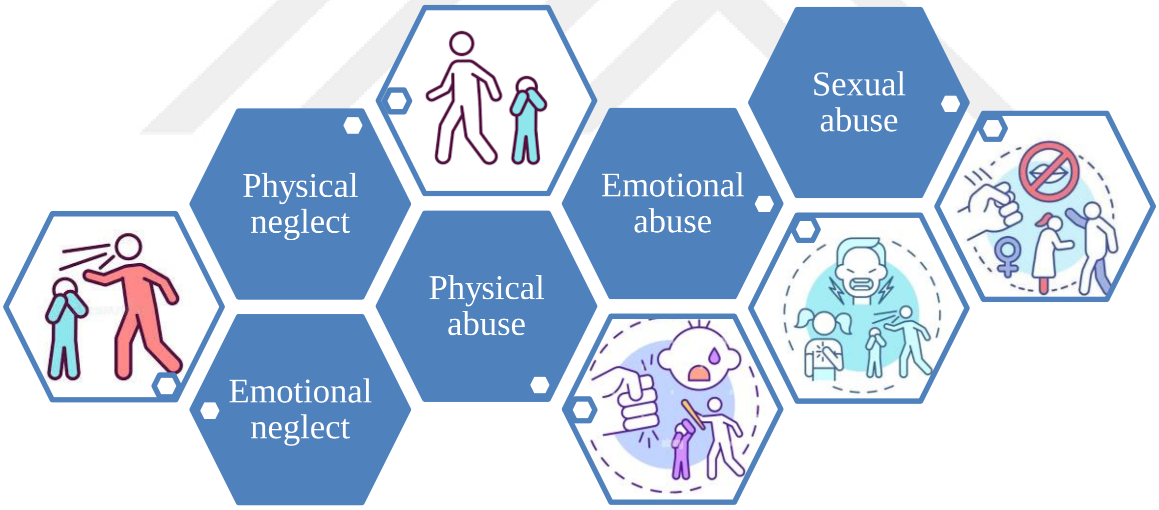

Figure 1.6.1. Types of childhood traumatic experiences

1.7. Extracellular Matrix (ECM)

All cells in the body are embedded in a complex extracellular matrix (ECM) that influences their survival, development, shape, polarity and migratory behavior (Alberts, et. al., 2002). The composition and density of the ECM can vary greatly depending on the type of tissue in which it is found (Alberts, et. al., 2002). The ECM is a three-dimensional network structure responsible for forming the external environment of cells and tissues in the body. It performs various functions and is produced mainly by the cells themselves. It is comprised of a multitude of macromolecular constituents, including, but not limited to, proteins and polysaccharides. The ECM has several components. These components are generally organized into a three-dimensional structure that can be divided into two main forms. These two main forms are called basement membrane and interstitial matrix. While there are differences between these two forms, the three-dimensional structural plan is broadly similar.

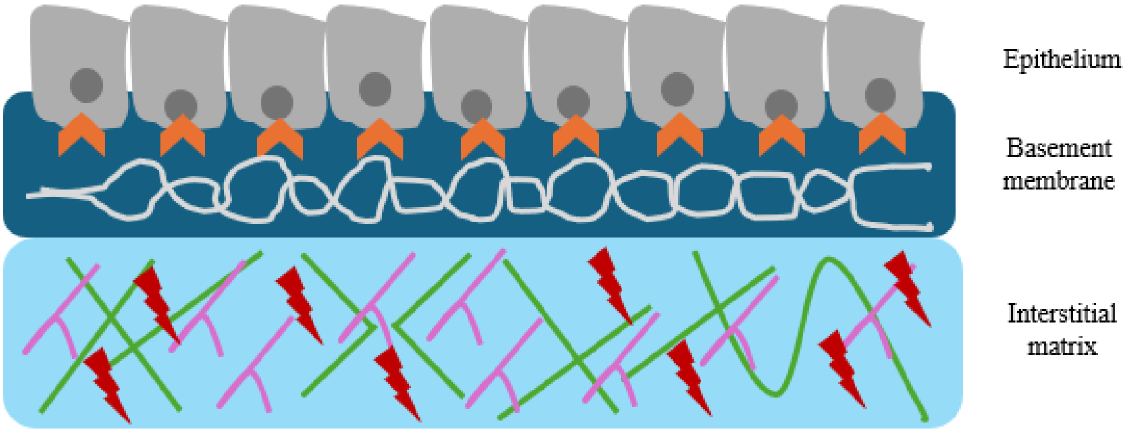

Figure 1.7. ECM are generally organized into two main forms: the basement membrane and interstitial matrix

Various collagens form the skeleton of both the basement membrane and the interstitial matrix and non-structural proteins bind to this skeleton. Thus, these non-structural proteins communicate with the surrounding cells via integrins. The basement membrane is a thin and dense layer that serves as a support for epithelial, endothelial, and some nervous tissues. The basement membrane structure consists primarily of laminin, type IV collagen, proteoglycans and entactin, whereas the interstitial matrix consists primarily of fibrillar proteins such as collagen and elastin, which provide the skeletal structure. In addition, gel-forming proteins such as fibronectin and hyaluronic acid are responsible for maintaining mechanical stability and water retention.

ECM has been determined to play a role in many physiological and pathological processes beyond acting as a passive scaffold in cells and tissues. ECM provides mechanical stability and flexibility for organs such as skin, lungs and blood vessels, and by binding to cell receptors such as integrins, it has a profound effect on numerous cellular processes including cell adhesion, migration, survival, proliferation and differentiation, which are crucial for the vital activities of the cell. It also has an important role in the organization of granulation tissue involved in the wound healing process and in the formation of scars. It has also been determined that it acts as a reservoir for growth factors and cytokines that can affect cell communication and behavior. It has also been established that the ECM has a crucial role in the pathogenesis of neurological diseases. The ECM acts as a structural framework for neurons, regulating various cellular processes including cell migration, adhesion, differentiation and communication. The BBB, which plays a role in regulating the exchange of molecules between the brain and the surrounding tissue, is also protected by the ECM (Daneman & Prat, 2015). Molecules such as collagens, proteoglycans and glycoproteins in the structure of the ECM provide a stable environment for brain cells, in which the functions of brain cells can easily take place.

In essence, the ECM is a foundational element that delineates the physical and functional characteristics of human tissues. Its role extends far beyond that of a simple support scaffold, as it is involved in the active modulation of cellular behaviors and functions in both health and disease.

1.7.1. Matrix metalloproteinases

Matrix metalloproteinases (MMPs), also known as matrixins, constitute a large family of ECM proteins (Wang, X., et. al., 2018). These proteins are zinc-dependent and include collagens, elastin, gelatin, matrix glycoproteins, proteoglycans, and remodeling endopeptidases (Wang, X., et. al., 2018). They have the capacity to degrade both matrix and non-matrix proteins (Wang, X., et. al., 2018). MMPs are multi-domain proteins, that can be active or inactive depending on the tissue inhibitors of metalloproteinases (TIMPs). In general, MMPs have a propeptide sequence about 80 amino acids long, a catalytic zinc-containing metalloproteinase domain 170 amino acids long, a linker peptide of variable length and a haemopexin domain about 200 amino acids long (Cui, et. al., 2017). MMPs are typically classified according to their substrate organization and structural domains, with the following categories being distinguished: collagenases, gelatinases, stromelysins, matrilysins, membrane-type (MT)-MMPs, and other MMPs (Cui, et. al., 2017).

1.7.1.1. MMP-2 and MMP-9

Gelatinases are a specific subgroup of MMPs that degrade gelatin and type IV collagen. These proteins are integral parts of the basement membrane and other components within the ECM (Cui, et. al., 2017). The gelatinases are named gelatinase A (MMP-2) and gelatinase B (MMP-9). Both gelatinase A and B easily digest denatured collagen and gelatins. MMP-2 and -9 behave like other proteinases in the MMP family, but differ in the way they bind to collagen. This domain is required for gelatin binding (Cui, et. al., 2017).

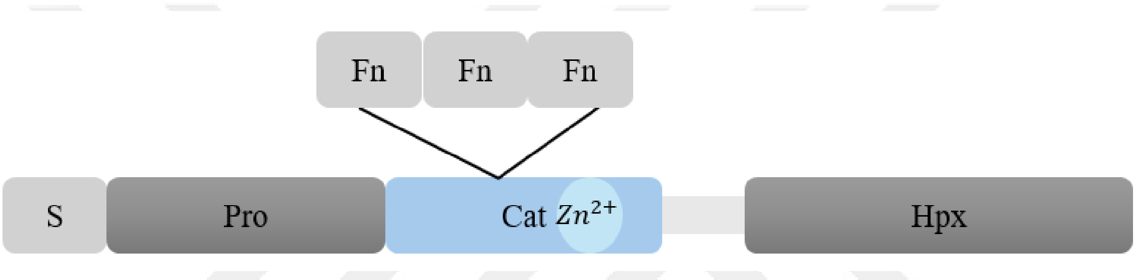

Figure 1.7.1.1. Domain structure of MMP-2 and MMP-9. S, signal peptide; Pro, propeptide; Cat, catalytic domain; Zn, active domain zinc; Hpx, hemopexin domain; Fn, fibronectin domain.

MMP-2 is one of the most studied members of the endopeptidase family. MMP-2, also termed gelatinase-A or type IV collagenase, has a gene locus on chromosome 16q13-q21 (Cui, et. al., 2017). MMP-2 is synthesized as a 72 kDa pro-MMP-2 and requires an activation to fulfil its enzymatic function in the body. Activation of MMP-2 is generally mediated by removal of the pro-domain by MT-MMPs or other proteinases (Lafleur, et. al., 2001). Activation of MMP-2 is generally mediated by removal of the pro-domain by MT-MMPs or other proteinases (Lafleur, et. al., 2001). MMP-2 facilitates cell migration and ECM reorganization by degrading denatured collagen and native collagen. This function is vital in wound healing and organ development processes (Parks, 2004). MMP-2 degrades ECM components for endothelial cell migration and the formation of new blood vessels, including both physiological processes such as wound healing and pathological conditions such as tumor growth. The role of such MMP-2 in angiogenesis has also been well described (Egeblad & Werb, 2002). MMP-2 ensures the correct formation of tissues and organs during embryonic development. Thus, it takes part in ECM remodeling (Page-McCaw, et. al., 2007). MMP-2 facilitates cancer metastasis by its capacity to disrupt the basement membrane, allowing tumor cells to invade surrounding tissues and enter the bloodstream (Deryugina & Quigley, 2006). MMP-2 has been associated with various cardiovascular diseases including atherosclerosis and myocardial infarction by contributing to the degradation of vascular ECM (Newby, 2012). MMP-2 has been implicated in rheumatoid arthritis and osteoarthritis through the breakdown of cartilage and subsequent joint destruction (Cabral-Pacheco, et. al., 2020). Further insights into potential therapeutic interventions may be gained by continued investigation of the structural features of MMP-2, its regulatory mechanisms and roles in disease.

MMP-9 or gelatinase-B is also a type IV collagenase that has a gene locus on chromosome 20q11.2-q13.1 (Cui, et. al., 2017). The MMP9 is secreted as a 92kDa zymogen (Christensen & Shastri, 2015). Cleavage of ProMMP-9 results in the active enzyme, having a molecular weight of approximately 82kDa. MMP-9 plays a role in ECM remodeling by degrading denatured collagens and native collagens, particularly types IV and V. This is of significance during wound healing, angiogenesis, and embryonic development (Overall & López-Otín, 2002). MMP-9 modulates immune responses by facilitating leukocyte migration through the basement membrane and ECM. It affects inflammatory pathways by processing cytokines and chemokines (Van den Steen, et. al., 2002). MMP-9 plays a role in tumor progression by degrading ECM barriers, increasing tumor cell invasion and metastasis. Additionally, it promotes angiogenesis and tumor growth by releasing ECM-bound growth factors such as vascular endothelial growth factor (VEGF) (Quintero-Fabián, et. al., 2019). In cancer, MMP-9 facilitates tumor invasion, metastasis, and angiogenesis. Elevated levels of MMP-9 are associated with unfavorable prognoses in diverse cancers, including those of the breast, colon, and stomach (Deryugina & Quigley, 2006). MMP-9 exacerbates tissue damage by degrading ECM components and modulating inflammatory mediators in chronic inflammatory conditions such as rheumatoid arthritis, asthma and inflammatory bowel disease (Hu, et al., 2007). MMP-9 contributes to neuroinflammation and neuronal damage in neuroinflammatory and neurodegenerative diseases such as multiple sclerosis (MS) and Alzheimer's disease (AD) by disrupting the BBB (Rosenberg, 2002). Therefore, understanding the regulatory mechanisms and pathways of MMP-9, a versatile MMP, may be important for developing targeted therapies for MMP-9-related diseases.

1.7.1.2. Evidence in neurological and psychiatric disorders

The activities of MMP-2 and MMP-9 in the CNS go beyond the degradation of the ECM and include roles in synaptic plasticity, neuronal development and communication between neurons. This process facilitates cellular migration, differentiation, and synaptic modulation within the CNS. These processes are of great importance for the maintenance of neuronal functionality and plasticity, influencing learning, memory, and the response to injury. Both MMP-2 and MMP-9 regulate the structural integrity of the ECM by degrading collagen, gelatin, and other ECM components. This remodeling is essential for processes such as synaptic plasticity, axonal growth, and neuronal migration during development and after injury (Yong, et al., 2001). MMP-2 and MMP-9 regulate synaptic plasticity by remodeling the perisynaptic ECM, which affects synaptic strength and connectivity. For example, MMP-9 has been found to play a crucial role in the formation of long-term potentiation (LTP), a cellular correlate of learning and memory. It has also been shown that MMP-9 can degrade ECM proteins such as agrin and brevican which can alter synaptic signaling (Wiera, et. al., 2021). MMP-2 and MMP-9, during development, facilitate neurite outgrowth by cleaving inhibitory ECM molecules and form pathways that direct neurons to their synaptic targets (Nagy, et al., 2007). The remodeling of dendritic spines that occurs at synapses is required for adaptive changes in neural circuits underlying learning and memory formation. This remodeling is mediated by MMP-9 (Wang, X., et al., 2008). MMP-9 modulates synaptic transmission by degrading cell adhesion molecules, including neural cell adhesion molecule (NCAM). This, in turn, affects synaptic adhesion and plasticity, thereby demonstrating how MMP activity translates into altered neuronal communication dynamics (Dityatev, et al., 2021). MMP-2 and MMP-9 regulate neuroinflammatory responses and leukocyte migration across the BBB. By disintegrating the basal lamina, they facilitate the entry of immune cells into the CNS, an important process in both disease pathology and repair mechanisms (Rosenberg, 2002). In the study conducted by Nagy et al., it was documented that the induction of LTP in hippocampal neurons was accompanied by an increase in MMP-9 activity (Nagy, et al., 2007). Inhibition of MMP-9 led to impaired LTP and memory formation in rodents, suggesting that MMP-9 plays an important role in the relationship between synaptic modification during learning. A study showed that MMP-2 is upregulated after ischemic injury (Zhou, et. al., 2022). This upregulation contributes to ECM degradation and facilitates neurovascular remodeling. Thus, this process is essential for tissue recovery and reorganization after stroke. Precise regulation of MMP-2 and MMP-9 enzymes is vital for synaptic plasticity, neurodevelopment and response to injury. Understanding these mechanisms may provide the basis for the development of therapeutic strategies aimed at regulating MMP activity in neurological diseases.

The role of MMP-2 in the CNS has attracted considerable research interest, especially in the context of neurological and psychiatric disorders. A study by Montagne et al. showed that MMP-2 activity was increased in the brains of patients diagnosed with AD (Montagne et al., 2015). This increase was found to be associated with the disruption of the BBB and the accumulation of amyloid-beta (Aβ) plaques. The findings demonstrate the role of MMP-2 in BBB permeability and its potential contribution to Aβ pathology, thus emphasizing its importance in AD progression. In a study by Yong et al., it was reported that MMP-2 levels were increased in MS lesions (Yong et al., 2001). MMP-2 activity is associated with myelin destruction and axonal damage and therefore contributes to exacerbation of the disease. These findings suggest that MMP-2 may play a role in mediating inflammation and tissue damage in MS and therefore may be a potential target for therapeutic intervention. A study demonstrated that MMP-2 activity increased following traumatic brain injury (TBI) and correlated with ECM disruption and neuronal apoptosis (Lee, et. al., 2012). A study by Lepeta et al. demonstrated that patients with schizophrenia exhibited altered MMP-2 activity, which was linked to synaptic plasticity and cognitive functions (Lepeta & Kaczmarek, 2015). These findings indicate that MMP-2 dysfunction may be a contributing factor to the synaptic and cognitive deficits observed in schizophrenia. A study demonstrated that individuals with major depressive disorder (MDD) exhibited elevated plasma MMP-2 levels, which correlated with the severity of depressive symptoms (Omori, et. al., 2020). The elevated levels of MMP-2 observed in patients with MDD indicate a potential involvement in neuroinflammatory pathways and stress-induced neural changes. The collective findings of these studies indicate that MMP-2 exerts a dual influence on both physiological and pathological processes within the CNS. Its capacity to regulate the integrity of the BBB, the composition of the ECM, and synaptic function exemplifies its extensive influence on brain health. In neurological diseases such as Alzheimer's disease, multiple sclerosis, and traumatic brain injury, MMP-2 contributes to pathogenesis primarily through disruption of the ECM, facilitating neuroinflammation and tissue damage. In psychiatric disorders such as schizophrenia and MDD, aberrant MMP-2 activity impairs synaptic plasticity and cognitive functions, thereby implicating it in disease mechanisms.

The role of MMP-9 in neurological and psychiatric diseases has been a significant area of investigation. MMP-9 has been linked to the pathogenesis of AD due to its involvement in the aggregation of Aβ and vascular remodeling. Studies have demonstrated that elevated levels of MMP-9 in cerebrospinal fluid (CSF) and brain tissues are associated with increased Aβ accumulation and BBB disruption (Wang, X., et. al., 2014; Yan, P., et. al., 2006). Studies on models of amyotrophic lateral sclerosis (ALS) have demonstrated that levels of MMP-9 are elevated in spinal cord tissues of patients, where it is associated with neuroinflammation and motor neuron destruction (Łukaszewicz-Zając, 2014). This indicates that MMP-9 is involved in the progression of the disease. Emerging evidence suggests a potential link between MMP-9 and MDD. Studies have demonstrated that elevated serum MMP-9 levels are present in patients with MDD, suggesting that MMP-9 may serve as a biomarker for inflammatory processes associated with depression (Bobińska, et. al., 2016). MMP-9 has been linked to the pathophysiology of schizophrenia through its involvement in synaptic plasticity and remodeling. Genetic studies have identified associations between MMP-9 polymorphisms and susceptibility to schizophrenia (Rybakowski, et. al., 2009). Additionally, elevated levels of MMP-9 have been observed in the plasma of patients with schizophrenia, with levels correlating with disease severity. Further research is necessary to elucidate the precise mechanisms by which MMP-9 contributes to these disorders and to develop targeted therapies that can reduce its deleterious effects without disrupting its normal physiological roles.

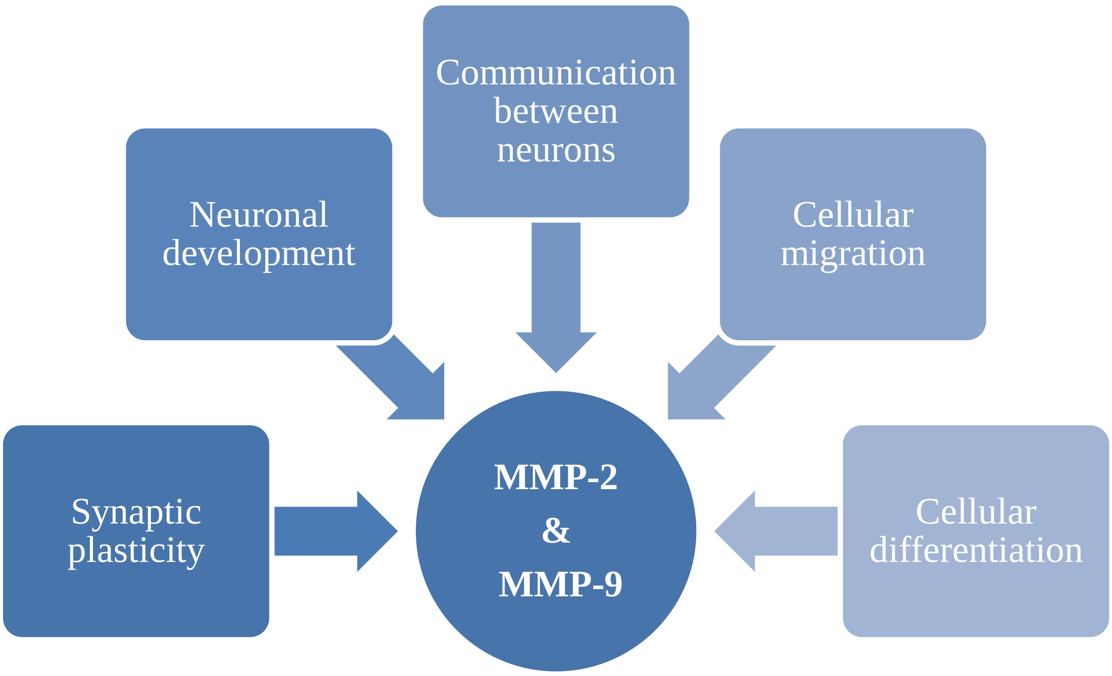

Figure 1.7.1.2. The role of MMP-2 and MMP-9 in neurological and psychiatric disorders.

2. MATERIAL/METHOD

2.1. Research Type

The study is a case-control, clinical observational study.

2.2. Place and Time of Research

This study was conducted between July 2023 and April 2024 by the Ambulatory Service Unit of the Psychiatry Department at Maltepe University Faculty of Medicine Hospital and Koç University Translational Medicine Research Centre, under the leadership of Inönü University Faculty of Arts and Sciences.

The patient and healthy volunteer groups were interviewed using information from the study titled “Oxidative DNA Damage and Base Removal Repair in First Degree Relatives of Individuals with Bipolar Disorder”, which was approved by the Maltepe University Clinical Research Ethics Committee (number 2019/900/48) on 24 July 2019 at Maltepe University Faculty of Medicine, Department of Psychiatry. Approval for the use of blood samples from volunteers in future studies has been obtained, along with voluntary consent forms approved by the ethics committee.

The procedures for purifying whole blood RNA, synthesizing cDNA, and performing qPCR were carried out at Koç University Translational Medicine Research Center. The study was funded by “Inönü University” under the TUSEB 2022-A2-YL call code 31044, titled “MMP-2 Gene Expression Level in Bipolar Disorder: Evaluating Relationships with Clinical Features and Childhood Traumatic Experiences”. The statistical analysis was conducted at Inönü University Faculty of Medicine Department of Biostatistics and Medical Informatics.

2.3. Population and Sample of the Research

In order to compare the gene expression levels of bipolar patients and the control group, the minimum number of samples to be taken per group was calculated as 17 when the effect size was predicted as 1 with 95% confidence level (α=0.05) and 80% power (β=0.20).

The subjects were recruited from Maltepe University Faculty of Medicine, Department of Psychiatry. All participants were between the ages of 18-60, able to give written informed consent, of both sexes, and were subjected to a SCID-V interview in which

DSM-V diagnostic criteria were asked. The inclusion and exclusion criteria for the patient and healthy volunteer groups are shown in Table 2.3.

Table 2.3. Inclusion and exclusion criteria of the study.

	Inclusion Criteria	Exclusion Criteria
Patient Volunteer Group (n=20)	• To be between 18-60 years old, • Being diagnosed with 'bipolar disorder type 1' according to SCID-V, • Being euthymic for at least 4 months (Young Mania Rating Scale<7, Hamilton Depression Scale-17<7), • To have at least primary school education, • Signing the informed consent form.	• For all groups, having a known decompensated systemic medical disease, diabetes mellitus, rheumatological disease, active infection or a serious neurological disease, using antioxidant-containing treatments, supportive products that may affect oxidative parameters, serious impairment in routine laboratory findings, lifelong mental retardation, having conditions affecting cognitive functions (delirium, dementia, epilepsy, etc.), being diagnosed with alcohol and substance abuse or addiction in any period of life, being pregnant or lactating.
Healthy Volunteer Group (n=20)	• To be between 18-60 years old, • To have at least primary school education, • Signing the informed consent form.	• For healthy volunteers, the addition of any past or current axis I psychiatric diagnosis after the SCID-V interview.

2.4. Study Material

Ethics Committee Approval

The material used in the research is nucleic acid (RNA) samples obtained from blood samples taken from patient and healthy volunteer groups.

2.5. Data Collection and Evaluation Tools

Consent and Informing: All participants who participated in the study were given an informed consent form approved by the Maltepe University Clinical Research Ethics

Committee before the clinical interviews, detailed information about the study was given, and then the signed consent of all participants was obtained.

Diagnostic Criteria: Diagnoses were made with the standardized interview SCID-V (Structured Clinical Interview for DSM-V Axis Disorders) of the DSM-V, which is the classification of the American Psychiatric Association. The original English version of the scale was developed in 2015 (First, 2015). The scale was adapted into Turkish by Dr. Müge Elbir et al. in 2019 with permission from the author (Elbir, et. al., 2019).

Disease Severity Evaluations: Patients with bipolar disorder who were euthymic for at least 4 months were evaluated with the 17-item Hamilton Depression Rating Scale, Young Mania Rating Scale and Childhood Trauma Questionnaire.

Hamilton Depression Rating Scale-17 (HDRS-17): The Hamilton Depression Rating Scale (HDRS), also known as the Hamilton Rating Scale for Depression (HRSD), was developed by British psychiatrist Max Hamilton in 1960 (Akdemir, et. al., 1996). A validity and reliability study of the HDRS form was conducted in Turkish (Akdemir, et. al., 1996).

The HDRS is commonly used in clinical and research settings to evaluate the severity of depressive symptoms and track changes in symptoms over time. The scale comprises questions and observations that evaluate different aspects of depression, including mood, feelings of guilt, suicidal thoughts, and physical symptoms. Its purpose is to provide a standardised and quantitative measure of depression, enabling consistent assessment and monitoring of individuals with depressive disorders. The 17-item version is widely used.

Young Mania Rating Scale (YMRS): The Young Mania Rating Scale (YMRS) is a clinical tool used to evaluate the severity of manic episodes in patients with bipolar disorder. Developed by K. R. Young, M. W. Biggs, V. S. Ziegler, and R. A. Meyer in 1978, the YMRS assesses symptoms such as excessive energy, decreased need for sleep, and increased speech rate. A validity and reliability study of the HDRS form was conducted in Turkish (Karadağ, et. al., 2001). The scale consists of 11 items and is administered by a clinician to measure the severity of mania.

Childhood Trauma Questionnaire (CTQ): The Childhood Trauma Questionnaire (CTQ), which was originally developed by David P. Bernstein in 1995, was adapted into Turkish by Prof. Dr. Vedat Şar in 1996 with the permission of the author (Bernstein & Fink, 1998; Şar, et. al., 2020). The CTQ asks individuals about various traumatic experiences, including emotional, physical, and sexual abuse, as well as emotional and physical neglect. The CTQ is a self-report questionnaire designed to measure the severity of childhood trauma and its potential impact on mental health. The questionnaire consists of a series of questions about different types of traumatic events, and individuals rate their experiences according to the provided scale.

2.6. Whole Blood RNA purification

RNA purification is the process of separating RNA molecules from other nucleic acids and cellular components in a sample. Its purpose in gene expression studies is to obtain pure RNA, which allows for accurate measurement of gene expression levels. By analyzing the amount of specific messenger RNA (mRNA) molecules, researchers can determine which genes are actively transcribed. RNA purification enables the study of gene expression patterns and profiles. By isolating mRNA, researchers can focus on RNA molecules that encode proteins and better understand gene function. Researchers compare gene expression patterns found in different conditions or experimental groups by analyzing purified RNA from different samples. This enables the identification of genes that are up-regulated or down-regulated under certain conditions. RNAs were isolated from blood samples obtained from BD patients and healthy controls using the QIAamp RNA Blood Mini Kit (Cat No./ID:52304, Qiagen, Germany) according to the manufacturer's protocol.

The following procedure was followed for whole blood RNA isolation:

1. 50-500 µL of blood is centrifuged at 400 × g (~2,000 rpm) for 5 min at 4°C. The supernatant was discarded.

2. The resulting pellet was resuspended in 600 µL Lysis Buffer, then mixed thoroughly by vortexing.

3. 450 µL ethanol (96-100%) was added and mixed by pipetting or vortexing.

4. Half of the prepared lysate was transferred to a column placed in a collection tube. The column was centrifuged at 12,000 × g (~11,000 rpm) for 1 min. The fluid solution was discarded and the column and collection tube were recombined. The remaining lysate was transferred to the column and centrifuged as before. The collection tube containing the flow-through solution was discarded. The column was placed in a new 2 mL collection tube.

5. 700 µL Wash Buffer WB 1 (ethanol added) was added. Centrifuged at 12,000 × g (~11,000 rpm) for 1 min. The flow-through was discarded and the purification column was placed back into the collection tube.

6. 500 µL Wash Buffer 2 (ethanol added) was added to the purification column. Centrifuged at 12,000 × g (~11,000 rpm) for 1 min.

7. 500 µL Wash Buffer 2 (ethanol added) was added to the purification column. Centrifuged at 12,000 × g (~11,000 rpm) for 2 min.
8. 50 µL of nuclease-free water was added to the centre of the purification column membrane and centrifuged at 12,000 × g (~11,000 rpm) for 1 min.
9. The purification column was discarded. The purified RNA is used immediately in downstream applications or stored at -20°C until use.
RNA was kept on ice after extraction and while working with it.

2.7. cDNA synthesis

cDNA synthesis is the process of creating a DNA molecule that corresponds to the RNA, typically mRNA, of an organism or cell. It is a crucial step in gene expression studies, providing a valuable tool for manipulating and analyzing genetic information. cDNA synthesis was performed using the cDNA Synthesis Using the RT 2 First Strand Kit (Cat No./ID:330404, Qiagen, Germany) according to the manufacturer's protocol.
The following procedure was followed for cDNA synthesis:
1. 5 µg total RNA was added to a sterile, nuclease-free tube.
2. 20 pmol of gene specific primers were added and the total volume was made up to 12 µl with water.
3. 4 µl of 5X reaction buffer was added.
4. 1 µl of RiboLock RNase Inhibitor (20 U/µL) was added.
5. 2 µl of 10 mMdNTP mixture was added followed by 1 µl of Reverse-Transcriptase enzyme.
6. The tube was gently mixed and incubated at 42°C for 1 hour.
7. At the end of the incubation period, the cDNAs kept at 70°C for 5 minutes can be stored at -20°C for short-term use and at -70°C for long-term storage. The cDNAs obtained were prepared for use in qPCR application.

2.8. qPCR

Quantitative Polymerase Chain Reaction (qPCR) is a laboratory technique in molecular biology that enables the amplification and measurement of a specific DNA or RNA fragment for quantitative analysis. The main objective of qPCR in this study was to determine the level of activity or dominance of the identified genes. This information is used to understand the effectiveness of particular genes in an organism's specific situation.

The following procedure was followed for qPCR:

1. In a 1.5 ml sterile microcentrifuge tube, respectively: 1 µl of 20✕ TaqMan® Gene Expression Assay, 10 µl of 2✕ TaqMan® Gene Expression Master Mix, 4 µl of cDNA sample, 5 µl of Rnase-free water were added into a 1.5 ml sterile microcentrifuge tube.

2. The tube was capped, mixed several times and gently centrifuged.

3. The reaction mixture was added to 96-well platinum wells.

4. The gently centrifuged plate was placed in the thermal cycler and the cycle was started.

Table 2.8. PCR reaction mix components.

PCR reaction mix component (MMP-9)	Single reaction	PCR reaction mix component (MMP-2)	Single reaction
2X TaqMan Fast Advanced Master Mix	10.0 µL	**2X TaqMan Fast Advanced Master Mix**	10.0 µL
20X TaqMan Gene Expression Assay/Probe	1.0 µL	**20X TaqMan Gene Expression Assay/Probe**	1.0 µL
cDNA Template (1 to 100 ng)	5.0 µL	**cDNA Template (1 to 100 ng)**	9.0 µL
Rnase-free water	4.0 µL	**Rnase-free water**	0 µL

Table 2.8. qPCR cycle reaction.

Bio-Rad CFX96 System	***UNG Activation***	***Polymerase Activation***	***PCR (40 cycles)***	
	Hold	***Hold***	***Denature***	***Anneal/extend***
Temperature	50°C	95°C	95°C	60°C
Time (min:sec)	02:00	00:20	00:03	00:30

2.9. Statistical evaluation

- Quantitative analysis of MMP-2 and MMP-9 gene expression between healthy and patient groups
- Correlation of quantitative analysis of MMP-2 and MMP-9 gene expression with clinical characteristics
- Evaluation of the quantitative analysis of MMP-2 and MMP-9 gene expressions with childhood traumatic experiences

Descriptive statistics were calculated to summarize demographic and clinical characteristics for the 2 different groups mentioned above. Gene expression data were analyzed using appropriate statistical methods, including t-tests or Mann-Whitney U tests for group comparisons and correlation analyses to explore potential associations between gene expression levels and clinical variables.

3. RESULTS

In this study, data from 20 bipolar patients who had been euthymic for at least 4 months and 19 healthy controls were analysed. All participants were between 18-60 years old and underwent a SCID-V interview, which asked about the DSM-V criteria. Individiuals who have a decompensated systemic medical condition, morbid obesity, diabetes mellitus, rheumatologic disease, active infection, or a serious neurological disease that could affect metabolic parameters, using antioxidant containing treatments or supportive products, have a serious abnormality in routine laboratory findings, have a lifelong intellectual disability or conditions affecting cognitive function (such as delirium, dementia, epilepsy, etc.), have a diagnosis of alcohol or substance abuse or addiction at any point of life, are in pregnancy, lactation period or going through menopause excluded from the study. In addition, individuals in the healthy control group who have a history of or currently had any psychiatric diagnosis excluded from the study. Individuals with BD diagnosis have a euthymic state for a minimum of 4 months, as confirmed by scores below 7 on the Hamilton Depression Scale-17 (HAMD-17) and Young Mania Rating Scale (YMRS). Childhood traumatic experiences evaluated with Childhood Trauma Questionnaire (CTQ).

Peripheral blood samples were collected from both groups and total RNA was extracted for gene expression analysis. MMP-9 and MMP-2 mRNA levels were measured using quantitative polymerase chain reaction (q-PCR). Gene expression data were analysed using appropriate statistical methods, including correlation analyses, to investigate potential associations between gene expression levels and clinical variables.

3.1. Comparison of MMP-9 Gene Expression Levels of Participants

To investigate potential differences in MMP-9 gene expression levels, which were analysed in a sample of 20 bipolar patients and 19 healthy individuals. As a result of the analysis, the *p* value between the two groups was found to be 0.901. Within the parameters of this study, a *p* value of 0.901 indicates that the observed differences in MMP-9 expression levels between the two groups are not statistically significant. This means that any variation in MMP-9 gene expression is probably due to 90.1% chance rather than a real difference between the groups. The relationship between the MMP-9 gene expression levels of the study groups is given in Table 3.1.

Table 3.1. Comparison of MMP-9 gene expression levels of participants

Group	N	Median	Minimum	Maximum	*p*
Healthy Control	19	0.7682	0.11	7.91	**0.901**
Bipolar Disorder	20	1.4341	0.06	3.77	

3.2. The Relationship Between Clinical Features of Participants with MMP-9 Gene Expression Levels

There are various parameters in the analysis of the relationship between the clinical features of the participants and MMP-9 gene expression levels. These are gender, smoking/alcohol use, body mass index (BMI) and age. The relationship between gender parameter and MMP-9 gene expression level was analysed in two ways. In the first analysis, the relationship between gender and MMP-9 gene expression was analysed in only 25 females and 14 males, regardless of patient-healthy individuals. As a result of the analysis, the *p*-value was found to be 0.118. This *p*-value is above the commonly used threshold for statistical significance and means that there is an 11.8% chance that the difference in MMP-9 gene expression levels between the genders is due to random chance. The second analysis examined the relationship between MMP-9 gene expression levels and gender in the patient and healthy groups. When the relationship between 12 healthy women and 13 patient women groups and MMP-9 gene expression levels was analysed, the *p*-value was 0.538, while the *p*-value was 0.383 for the relationship between 7 healthy men and 7 patient men groups and MMP-9 gene expression levels. The *p*-values obtained in the second analysis indicate that the study does not provide evidence of a significant relationship between gender and MMP-9 gene expression levels in the patient and healthy groups. Another clinical parameter is smoking and alcohol use. In these parameters, we focussed only on the relationship between smoking or alcohol use and MMP-9 gene expression levels without making any distinction between patients and healthy individuals. The *p* value of the association of 17 non-smokers and 22 smokers with MMP-9 gene expression was 0.790, while the *p* value of the association of 17 non-alcohol and 22 alcohol users with MMP-9 gene expression was 0.769. These high *p*-values, expressed as 79.0% and 76.9% respectively, indicate that there is no statistically significant relationship between these lifestyle factors (smoking and alcohol use) and MMP-9 gene expression levels in the analysed sample. Table 3.2.4 summarises the relationship between BMI and age and MMP-9 gene expression levels using Spearman's rho correlation coefficients. According to the data in this table, weak negative correlations are observed between both BMI and age parameters and MMP-9 gene expression levels. However, *p*=0.288 for BMI and *p*=0.349 for age indicate that both correlations are not significant.

Data analysing biochemical parameters in terms of the relationship between clinical features and MMP-9 gene expression are given in Table 3.2.5. The parameters analysed included general treatment status, mood stabiliser, lithium, valproate, antipsychotic and antidepressant use. In 20 participants who did not receive any psychotic treatment and in 19 participants who received treatment, no significant difference was found between the group and MMP-9 gene with a *p*-value of 0.879. When the relationship with MMP-9 was analysed depending on whether the participants used mood stabilisers, lithium, valproate and antipsychotic drugs or not, the *p*-values were found to be 0.989, 0.941, 0.824 and 0.832, respectively. These values are far from the commonly used threshold for statistical significance (0.05) and are not significant. Finally, the *p*-value for the association of antidepressant use with MMP-9 gene expression was 0.052, indicating a 5.2% chance that the observed differences were random. This *p*-value is close to the commonly used threshold for statistical significance and implies that there may be a borderline significant association between antidepressant use and low MMP-9 expression levels.

Table 3.2.1. The relationship of clinical characteristics of the participants with MMP-9 gene expression levels

	Gender	N	Median	Minimum	Maximum	*p*
General comparison between genders	**Female**	25	1.5363	0.08	7.91	**0.118**
	Male	14	0.8147	0.06	2.99	
HC-BD comparison within genders	**Female**	12 (HC)	2.4317	0.15	7.91	**0.538**
		13 (BD)	1.3894	0.08	3.77	
	Male	7 (HC)	0.5546	0.11	2.38	**0.383**
		7 (BD)	1.4788	0.06	2.99	

Table 3.2.2. The relationship of clinical characteristics of the participants with MMP-9 gene expression levels

Cigarette	N	Median	Minimum	Maximum	*p*
No	17	1.3894	0.06	3.77	**0.790**
Yes	22	1.0087	0.08	7.91	

Table 3.2.3. The relationship of clinical characteristics of the participants with MMP-9 gene expression levels

Alcohol	N	Median	Minimum	Maximum	*p*
No	17	1.1929	0.15	3.63	**0.769**
Yes	22	1.1988	0.06	7.91	

Table 3.2.4. The relationship of clinical characteristics of the participants with MMP-9 gene expression levels

			BMI	Age
Spearman's rho	**MMP-9**	**Correlation Coefficient**	-0.175	-0.154
		Sig. (2-tailed) (*p*)	**0.288**	**0.349**
		N	39	39

Table 3.2.5. The relationship of clinical characteristics of the participants with MMP-9 gene expression levels

	N	Median	Minimum	Maximum	*p*
Treatment					
No	20	0.8147	0.11	7.91	**0.879**
Yes	19	1.3894	0.06	3.77	
Mood stabilizers					
No	21	0.7682	0.11	7.91	**0.989**
Yes	18	1.4341	0.06	3.77	
Lithium					
No	9	1.5363	0.26	2.99	**0.941**
Yes	11	1.3894	0.06	3.77	
Valproate					
No	9	1.3894	0.06	3.77	**0.824**
Yes	11	1.4788	0.26	2.99	
AP					
No	23	0.8612	0.11	7.91	**0.832**
Yes	16	1.4341	0.06	3.42	
Antidepresan					
No	35	1.4788	0.11	7.91	**0.052**
Yes	4	0.3994	0.06	1.19	

3.3. The Relationship Between Childhood Traumatic Experiences of Participants with MMP-9 Gene Expression Levels

Table 3.3. shows the strength of the relationship between 5 different childhood traumatic experiences and MMP-9 gene expression levels with Spearman's rho correlation coefficients and *p*-values. Although there are very weak positive correlations between emotional abuse and emotional neglect and MMP-9 gene expression levels, the *p*-values of

0.914 and 0.428, respectively, indicate that there was no significant relationship. Very weak or negligible negative correlations were observed between physical abuse, sexual abuse and physical neglect and MMP-9 gene expression level. At the same time, *p*=0.973 for physical abuse, *p*=0.513 for sexual abuse and *p*=0.843 for physical neglect proves that there was no relationship with MMP-9 gene expression.

Table 3.3. The relationship of childhood traumatic experiences of the participants with MMP-9 gene expression levels

<table>
<tr><td rowspan="15">Spearman's rho</td><td rowspan="15">MMP-9</td><td rowspan="3">Emotional abuse</td><td>Correlation coefficient</td><td>0.018</td></tr>
<tr><td>Sig. (2-tailed) p</td><td>0.914</td></tr>
<tr><td>N</td><td>39</td></tr>
<tr><td rowspan="3">Physical abuse</td><td>Correlation coefficient</td><td>-0.006</td></tr>
<tr><td>Sig. (2-tailed) p</td><td>0.973</td></tr>
<tr><td>N</td><td>39</td></tr>
<tr><td rowspan="3">Sexual abuse</td><td>Correlation coefficient</td><td>-0.108</td></tr>
<tr><td>Sig. (2-tailed) p</td><td>0.513</td></tr>
<tr><td>N</td><td>39</td></tr>
<tr><td rowspan="3">Emotional neglect</td><td>Correlation coefficient</td><td>0.131</td></tr>
<tr><td>Sig. (2-tailed) p</td><td>0.428</td></tr>
<tr><td>N</td><td>39</td></tr>
<tr><td rowspan="3">Physical neglect</td><td>Correlation coefficient</td><td>-0.033</td></tr>
<tr><td>Sig. (2-tailed) p</td><td>0.843</td></tr>
<tr><td>N</td><td>39</td></tr>
</table>

3.4. Comparison of MMP-2 Gene Expression Levels of Participants

In our study, it was also aimed to analyse the expression level of MMP-2, another candidate gene, between the patient and healthy groups. When Table 3.1. and Table 3.4. are compared, both MMP-2 and MMP-9 expression levels show a slightly higher median in the bipolar disorder group compared to the healthy control group, but the difference is minimal and not statistically significant. At the same time, the range of expression levels for both MMP-2 and MMP-9 varied over a wider range in the healthy control group than in the bipolar disorder group. Despite these minimal differences, when the gene expression levels of MMP-2 were analysed between the patient and healthy groups, a *p* value of 0.550 was found and it was concluded that it was not statistically significant.

Table 3.4. Comparison of MMP-2 gene expression levels of participants

Group	N	Median	Minimum	Maximum	*p*
Healthy Control	19	1.0000	0.00	25.40	**0.550**
Bipolar Disorder	20	1.0500	0.00	14.10	

3.5. The Relationship Between Clinical Features of Participants with MMP-2 Gene Expression Levels

In the analysis of the relationship between the clinical features of the participants and MMP-2 gene expression levels, the same method was followed as in the subtitle "3.2. The Relationship Between Clinical Features of Participants with MMP-9 Gene Expression Levels". As given in Table 3.5.1, the relationship between the gender parameter and MMP-2 gene was analysed in two ways. In the first analysis, all participants were analysed only as gender-MMP-2 gene expression and the *p* value was found to be 0.276. This *p* value is above the threshold value and indicates that there was no significant relationship. The second analysis was for the relationship between MMP-2 gene expression levels and gender in the patient and healthy groups, and the *p* value for the relationship between MMP-2 in the patient-healthy groups in women was 0.650, and the *p* value for the relationship between MMP-2 in the patient-healthy groups in men was 0.620, indicating a non-significant relationship. This indicates that there was no difference between men and women. As seen in Table 3.5.2, it is seen that the *p* value of the participants' smoking status in relation to MMP-2 gene expression is 0.081, which is close to the statistical threshold value, but it was still not sufficient to prove the existence of a significant relationship. In addition, Table 3.5.3 shows that the *p* value of the relationship between the participants' alcohol use status and MMP-2 gene expression is 0.585. According to smoking status, it is very far from the statistical threshold value and was not significant. Table 3.5.4 shows a weak negative correlation for body mass index and an equally weak positive correlation for age parameter. Since the correlations are in the opposite direction but equally weak, the *p*-value for BMI is 0.521 and the *p*-value for age is 0.523, giving insignificant values very close to each other.

Data analysing biochemical parameters in terms of the relationship between clinical features and MMP-2 gene expression are given in Table 3.5.5. The parameters analysed included general treatment status (*p*=0.687), mood stabiliser (*p*=0.443)), lithium (*p*=0.766), valproate (*p*=0.941), antipsychotic (*p*=0.944) and antidepressant (*p*=0.433) use and no significant results were found.

Table 3.5.1. The relationship of clinical characteristics of the participants with MMP-2 gene expression levels

	Gender	N	Median	Minimum	Maximum	*p*
General comparison between genders	**Female**	25	1.2000	0.00	25.40	**0.276**
	Male	14	0.8500	0.00	14.10	
HC-BD comparison within genders	**Female**	12 (HC)	1.6500	0.00	25.40	**0.650**
		13 (BD)	1.2000	0.00	6.90	
	Male	7 (HC)	0.9000	0.10	2.80	**0.620**
		7 (BD)	0.3000	0.00	14.10	

Table 3.5.2. The relationship of clinical characteristics of the participants with MMP-2 gene expression levels

Cigarette	N	Median	Minimum	Maximum	*p*
No	17	0.8000	0.00	15.30	**0.081**
Yes	22	1.4500	0.00	25.40	

Table 3.5.3. The relationship of clinical characteristics of the participants with MMP-2 gene expression levels

Alcohol	N	Median	Minimum	Maximum	*p*
No	17	1.2000	0.00	14.10	**0.585**
Yes	22	0.9500	0.00	25.40	

Table 3.5.4. The relationship of clinical characteristics of the participants with MMP-2 gene expression levels

			BMI	Age
Spearman's rho	**MMP-2**	**Correlation Coefficient**	-0.106	0.106
		Sig. (2-tailed) (*p*)	**0.521**	**0.523**
		N	39	39

Table 3.5.5. The relationship of clinical characteristics of the participants with MMP-2 gene expression levels

	N	Median	Minimum	Maximum	*p*
Treatment					
No	20	0.9500	0.00	25.40	**0.687**
Yes	19	1.2000	0.00	14.10	
Mood stabilizers					
No	21	1.0000	0.00	25.40	**0.443**
Yes	18	1.0500	0.00	14.10	
Lithium					
No	9	0.800	0.00	14.10	**0.766**
Yes	11	1.5000	0.00	6.90	
Valproate					
No	9	0.9000	0.00	3.10	**0.941**
Yes	11	1.2000	0.00	14.10	
AP					
No	23	0.9000	0.00	25.40	**0.944**
Yes	16	1.2500	0.00	14.10	
Antidepresan					
No	35	0.9000	0.00	25.40	**0.433**
Yes	4	2.1500	0.30	3.10	

3.6. The Relationship Between Childhood Traumatic Experiences of Participants with MMP-2 Gene Expression Levels

Table 3.6. shows the strength of the relationship between 5 different childhood traumatic experiences and MMP-2 gene expression levels with Spearman's rho correlation coefficients and *p*-values. Emotional abuse (*p*=0.935), physical abuse (*p*=0.658), emotional neglect (*p*=0.732) and physical neglect (*p*=0.474) have very weak positive correlations with MMP-2 gene expression levels and there was no statistically significant relationship in terms of *p*-values. When we look at the sexual abuse parameter, it shows a weak positive correlation with the MMP-2 gene expression levels like the other types of abuse and neglect. However, unlike the other types of trauma, there was a value of 0.053, which is closer to the statistical threshold value in terms of *p*-value. However, it is not a sufficient value to state that there was a significant difference.

Table 3.6. The relationship of childhood traumatic experiences of the participants with MMP-2 gene expression levels

Spearman's rho	**MMP-2**	**Emotional abuse**	**Correlation coefficient**	0.013
			Sig. (2-tailed) *p*	**0.935**
			N	39
		Physical abuse	**Correlation coefficient**	0.073
			Sig. (2-tailed) *p*	**0.658**
			N	39
		Sexual abuse	**Correlation coefficient**	0.312
			Sig. (2-tailed) *p*	**0.053**
			N	39
		Emotional neglect	**Correlation coefficient**	0.057
			Sig. (2-tailed) *p*	**0.732**
			N	39
		Physical neglect	**Correlation coefficient**	0.118
			Sig. (2-tailed) *p*	**0.474**
			N	39

4. DISCUSSION

"MMP-9 and MMP-2 Gene Expression Levels in Bipolar Disorder: Evaluating Relationships with Clinical Features and Childhood Traumatic Experiences" addressed several fundamental problems at the intersection of psychiatry and genetics. The purpose of this thesis study was to investigate the expression levels of MMP-9 and MMP-2 in individuals diagnosed with bipolar disorder who had been euthymic for at least 4 months and healthy controls. In addition, the relationship between MMP-9 and MMP-2 expression levels, clinical features and childhood traumatic experiences was also investigated. The data of 39 participants, including 20 patients with bipolar disorder and 19 healthy controls, were analysed in this study. To the best of our knowledge, no previous study has employed extracellular matrix markers MMP-9 and MMP-2 with a similar population group to those included in the groups formed according to the detailed inclusion and exclusion criteria presented in Table 2.3. The first analysis was to compare the expression levels of MMP-9/MMP-2 genes between bipolar patients and healthy controls. While the p value for MMP-9 was 0.901, the p value for MMP-2 was 0.550. This shows that no significant difference was found between the groups in our study in terms of both MMP-9 and MMP-2 gene expression. However, a study conducted in 2022 shows that MMP-9 levels are increased in patients with bipolar disorder, especially in relation to depressive and manic symptoms (Kothandan, et. al., 2022). Another study consistently reports that the T allele of the -1562C/T polymorphism in the MMP-9 gene has a higher frequency among patients with bipolar disorder, especially those with bipolar II subtype (Rybakowski et. al., 2011). Another study conducted in 2013 evaluated the expression pattern of MMP-2 and MMP-9 in blood collected from patients with bipolar disorder during and after acute mania in comparison with healthy controls (Chiarani, et. al., 2013a; 2013b). As a result of the evaluation, it was found that there was no significant difference between patients and controls in MMP-2 and MMP-9 mRNA expression (Chiarani, et. al., 2013a; 2013b). The difference between the findings of our study and those of other scientific studies seems to be due to many factors. The characteristics of the patient groups formed in each study differ from each other. The inclusion and exclusion criteria preferred in the formation of patient groups seem to affect the results of gene expression. At the same time, examining the frequencies of polymorphisms of targeted genes instead of whole gene expression analysis between patient-healthy groups may provide healthier data. Another arm of our study included the association of clinical characteristics such as gender, smoking/alcohol use, BMI, age, general treatment status, drug use (mood stabilisers, lithium, valproate, antipsychotic and antidepressant) with the targeted genes. The overall gender comparison between men and women showed *p*-values of 0.118 for MMP-9 and 0.276 for MMP-2, indicating that the expression status of our candidate genes did not differ significantly between the genders. When the candidate genes were compared between the patient and healthy groups according to gender, the *p*-values for MMP-9 (p=0.538 for women, p=0.383 for men) and MMP-2 (p=0.650 for women, p=0.620 for men) were not found to be statistically insignificant. According to the article published by Arnold Lesley M. in (2003), women with bipolar disorder tend to have a later age of onset compared to men (Arnold, 2003). However, the article mentions that menopause and reproductive cycles may significantly affect women with bipolar disorder and states that approximately 19% of postmenopausal women with bipolar disorder report worsening of mood symptoms, mostly depression, following menopause (Arnold, 2003). In this context, the fact that we preferred menopause/pregnancy as an exclusion criterion for our study may have obscured the differences between the sexes. At the same time, preferring genes, proteins or pathways involving gender-specific mechanisms instead of MMP-9 and MMP-2, which we prefer to examine gene expression between genders, may lead to more different gender-gene expression results. Another parameter of our study was the association of smoking/alcohol use with the expression of candidate genes. The association of MMP-9 gene expression with both smoking (p=0.790) and alcohol (p=0.769) was well above the statistical threshold value, but the association of MMP-2 gene expression with smoking was close to the statistical threshold value with a *p*-value of 0.081, while the association with alcohol yielded a *p*-value of 0.585. In our study, the relationship between MMP-2 and smoking is more likely to yield a significant statistical result. Therefore, the inclusion of more participants in the study seems more likely to ensure that the result is significant. In a study that preferred to use the Mendelian randomisation (MR) technique, the results suggested that smoking status may be a risk factor for bipolar disorder, while there was no causal relationship with BMI (He, et. al., 2023). In this context, it seems that many factors such as the techniques used, the difference in the effect of the factors preferred together in the patient group on each other cause the results obtained between the studies to be different from each other. In our study, the fact that BMI and age factors showed a very weak negative correlation with MMP-9 gene expression did not affect the statistical significance of the *p*-values. At the same time, a very weak negative correlation was observed between BMI and MMP-2 gene expression, but a very weak positive correlation was observed between age and MMP-2. Nevertheless, we also obtained insignificant statistical results for MMP-2 gene expression. The fact that the BMI factor did not yield significant results on behalf of our study may be because patients with morbid obesity were not included in our study group. At the same time, the classification of obesity in different degrees may have affected the results. The inclusion of individuals with advanced obesity in the study may contribute to finding more significant relationships with the expression levels of candidate genes. The fact that we preferred a very wide age range for the age factor seems to have affected the results again. In the context of the age factor, it may be a more effective solution to make age classification for the lifespan of individuals.

We thought that biochemical inputs to the body indirectly affecting epiegenetic changes, especially DNA methylation, may have affected the expression levels of candidate genes among the participants. In this context, we preferred to look at how the general treatment status and the use of drugs preferred by medical doctors in the treatment of bipolar disorder by the participants affected the expression of our candidate genes. No significant difference was found between MMP-9 gene expression and general treatment status (p=0.879), mood stabiliser (p=0.989), lithium (p=0.941), valproate (p=0.824) and antipsychotic (p=0.832) use, and a p-value closer to the statistical threshold of 0.052 was obtained for antidepressants. In the presence of more participants, it was more likely that we would have reached a significant result in the relationship between antidepressants and MMP-9 gene expression in our study. No significant difference was found between MMP-2 gene expression and general treatment status (p=0.687), mood stabiliser (p=0.443), lithium (p=0.766), valproate (p=0.941), antipsychotic (p=0.944) and antidepressant (p=0.433) use. In another article published in 2013, regarding pharmacological treatments, the authors state that antipsychotic drugs are effective in the treatment of acute mania, but their effectiveness in the treatment of bipolar depression is more variable (Geddes & Miklowitz, 2013). The role of antidepressants in bipolar depression remains controversial and emerging evidence points to limited efficacy (Geddes & Miklowitz, 2013). Lithium remains the best long-term treatment, reducing the risk of suicide as well as the risk of manic and depressive relapse (Geddes & Miklowitz, 2013). When evaluated in this context, the fact that we obtained the closest result to significance between MMP-9 gene expression and antidepressant in our study, while we obtained an insignificant result for lithium, can be interpreted that different medicinal drugs may be effective in different stages of bipolar disorder, but not all of them may have a positive effect in all stages of the disease at the same time. Nevertheless, we are of the opinion that in order for these parameters to provide healthier and clearer results, populations in which different types of drugs are taken as treatment in patients at different stages of bipolar disorder should be formed and re-studied again for the 2 genes we have shown as candidates. Thus, we can reach more enlightening results in which stage of bipolar disorder patients respond more favourably to each drug treatment and the possible relationships with candidate genes. At the same time, performing gene expression studies in participants who had never used any medication may have completely changed the results. This is the subject of another study. Environmental factors play an important role in the development and course of bipolar disorder. Stressful life events, childhood traumas, family dynamics, substance abuse and environmental stress factors may affect the mental health of individuals and contribute to the emergence of bipolar disorder. Numerous studies have shown that traumatic events in childhood are an important risk factor for the development of bipolar disorder. Patients with bipolar disorder report much higher rates of childhood trauma compared to healthy controls, especially for emotional, physical and sexual abuse as well as emotional neglect (Aas, et. al., 2016). In this context, it is known that environmental stress factors may affect epigenetics which mechanisms that change the way genes are expressed, but do not change the DNA sequence. In this framework, we aimed to examine possible associations with MMP-9 and MMP-2 gene expression levels by considering childhood traumatic experiences. MMP-9 gene expression was not significantly associated with emotional abuse (p=0.914), physical abuse (p=0.973), sexual abuse (p=0.513), emotional neglect (p=0.428) and physical neglect (p=0.843). At the same time, the expression of MMP-2 gene could not establish a significant relationship with any of the parameters of emotional abuse (p=0.935), physical abuse (p=0.658), emotional neglect (p=0.732) and physical neglect (p=0.474), while it gave a p-value of 0.053 for sexual abuse, but it is not a healthy enough result. We have very little data on the epidemiology of trauma types and their effects on bipolar disorder. Abuse and neglect are often underreported or difficult to detect during clinical interviews. At the same time, the timing and severity of trauma acquisition are among the factors that are effective in the negative evaluation of our results. For this reason, it seems useful to pay attention to criteria such as how long it has been since the participants experienced the trauma and how severely they continue to feel the trauma during the clinical interviews.

Several studies have reported increased activity of MMPs in the nervous system in neurological conditions such as Parkinson's disease and Alzheimer's disease (Rivera, 2019), as well as processes such as synaptic plasticity, demyelination, neuroinflammation and neurotoxicity (Ciro De Luca & Papa, M., 2017). Given the complex interaction between MMPs and neural processes, investigating the involvement of MMPs in neuropsychiatric disorders such as bipolar disorder holds significant promise. In conclusion, investigating the relationship between MMPs, the nervous system and bipolar disorder may provide valuable insights into the mechanisms underlying this disorder and potentially reveal novel therapeutic targets. In contrast to previous studies, our study is the first to examine the expression of MMP-9 and MMP-2 genes in a specific cohort of BD patients. Furthermore, our study is the first to examine the relationship between these genes and specifically defined clinical features and childhood traumatic experiences. In general terms, the lack of significant differences between the parameters we targeted in our study and MMP-9/MMP-2 gene expressions can be explained by various factors. The first factor may be that MMP-9/MMP-2 gene expression differs between tissues. Peripheral blood samples were used in our study. Gene expression may have been less in peripheral blood samples. On the contrary, the study of MMP-9/MMP-2 gene expression in brain tissue, which may be an animal study, could have affected the results. It is possible that the sample size in our study was insufficient to detect subtle differences in MMP-9 or MMP-2 gene expression between individuals with bipolar disorder and healthy controls. A larger and more homogenous cohort may be required to detect significant differences. Bipolar disorder is a complex illness with symptoms and disease course that differ significantly between individuals. It is possible that heterogeneity among the group of individuals with bipolar disorder in terms of disease characteristics and course may have influenced gene expression of MMP-9 or MMP-2, thus masking differences between groups. The nonsignificant *p*-values associated with biochemical parameters (general treatment status and various drug use) and MMP-9/MMP-2 gene expressions indicate that the candidate genes that we specifically identified may not be an important determinant of the results of mood stabilisers/lithium/valproate/antipsychotic/antidepressant treatments in bipolar disorder. The relationship between genetic factors, environmental influences such as traumatic experiences in childhood and the expression of genes such as MMP-9/MMP-2 is extremely complex. It is possible that other genetic variations or environmental factors were present in our study population and may have masked the observed association. At the same time, epigenetic studies are of great importance as they help to understand the complex interactions between genetics, environment and disease. Thus, methylation changes may occur in response to factors such as childhood traumatic experiences or therapeutic drug use.

Therefore, investigating methylation changes on target genes before performing gene expression studies can identify potential epigenetic regulators that affect the activity of target genes. Heterogeneity between individuals with bipolar disorder and healthy individuals in terms of the type and severity of traumatic experiences and other individual differences may have influenced the results. A more homogenous sample may be required to identify specific associations. The lack of a significant association between MMP-9/MMP-2 gene expression and childhood traumatic experiences in bipolar disorder in our study may be explained by a combination of factors such as complex interactions, sample heterogeneity, methodological differences, timing of trauma acquisition and interactions with other factors.

The prevalence of bipolar disorder, which is hypothesised to be the result of a complex interaction between genetic, environmental and biological factors, has been increasing in recent years. Further research with larger sample sizes, homogenous samples and different cohorts is recommended to elucidate the complex interactions between gene expression, clinical features and environmental factors in the context of bipolar disorder.

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