

**T.R.**  
**INONU UNIVERSITY**  
**GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCE**

**COMPARISON OF EXPRESSION LEVELS OF *MMP-9* AND *TIMP-1* GENES  
IN PATIENTS DIAGNOSED WITH UNIPOLAR AND BIPOLAR DEPRESSION**



**MASTER THESIS**

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**Department of Molecular Biology and Genetics**

**Supervisor: Prof. Dr. Ceren ACAR**

**DECEMBER 2024**

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## WORD OF HONOR

I hereby declare that this study titled "Comparison of Expression Levels of *MMP-9* and *TIMP-1* Genes in Patients Diagnosed With Unipolar and Bipolar Depression", 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.

Yunus EKEN



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## **SYMBOLS AND ABBREVIATIONS**

|                                |                                                                         |
|--------------------------------|-------------------------------------------------------------------------|
| <b>5-HT</b>                    | : Serotonin                                                             |
| <b>APA</b>                     | : American Psychiatric Association                                      |
| <b>BBB</b>                     | : Blood Brain Barrier                                                   |
| <b>BD</b>                      | : Bipolar Disorder                                                      |
| <b>BD-1</b>                    | : Bipolar Disorder 1                                                    |
| <b>BD-2</b>                    | : Bipolar Disorder 2                                                    |
| <b>BDNF</b>                    | : Brain Derived Neurotrophic Factor                                     |
| <b>BMI</b>                     | : Body Mass Index                                                       |
| <b>DSM</b>                     | : Diagnostic and Statistical Manual of Mental Disorders                 |
| <b>DSM-5-TR</b>                | : Diagnostic and Statistical Manual of Mental Disorders-5-Text Revision |
| <b>ECM</b>                     | : Extracellular Matrix                                                  |
| <b>ECT</b>                     | : Electroconvulsive Therapy                                             |
| <b>GWAS</b>                    | : Genome-wide association studies                                       |
| <b>ICD</b>                     | : International Classification of Diseases                              |
| <b>LTP</b>                     | : Long Term Potentiation                                                |
| <b>MDD</b>                     | : Major Depressive Disorder                                             |
| <b>MMP</b>                     | : Matrix Metalloproteinases                                             |
| <b>MRI</b>                     | : Magnetic Resonance Imaging                                            |
| <b>mRNA</b>                    | : Messenger RNA                                                         |
| <b>NF-<math>\kappa</math>B</b> | : Nuclear Factor- $\kappa$ B                                            |
| <b>PET</b>                     | : Positron Emission Tomography                                          |
| <b>qPCR</b>                    | : Quantitative Polymerase Chain Reaction                                |
| <b>SNP</b>                     | : Single Nucleotide Polymorphisms                                       |
| <b><i>TIMP</i></b>             | : Tissue Inhibitor of Matrix Metalloproteinases                         |
| <b>UD</b>                      | : Unipolar Disorder                                                     |
| <b>WHO</b>                     | : World Health Organization                                             |

## ABSTRACT

Master Thesis

### COMPARISON OF EXPRESSION LEVELS OF *MMP-9* AND *TIMP-1* GENES IN PATIENTS DIAGNOSED WITH UNIPOLAR AND BIPOLAR DEPRESSION

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Psychiatric disorders, especially bipolar and unipolar depression, are very common diseases affecting a significant portion of the global population. Despite the fact that a significant number of people are affected by these disorders, our understanding of the disorders is still somewhat limited. The exact genetic origin and cause of this condition have not yet been determined. Although several candidate genes have been identified in previous studies, a definitive candidate gene analysis has not been performed due to the significant influence of environmental variables and living conditions. Modulation and degradation of the extracellular matrix (ECM) and its components trigger mental illnesses and may influence the progression of the condition. Matrix metalloproteinases (*MMPs*) and tissue inhibitors of matrix metalloproteinases (*TIMPs*) have been commonly identified in various psychiatric disorders. Changes in their expression levels affect the extracellular matrix and initiate these degradations. Previous studies have shown that the expression levels of the *MMP-9* and *TIMP-1* genes are altered in individuals with bipolar depression (BD) and unipolar depression (UD). To evaluate the expression level of *MMP-9* and *TIMP-1* genes in Turkish population, gene expressions were compared among 16 BD, 20 UD and 19 HC cases. Statistical analysis revealed that the expression level of *MMP-9* gene was higher in healthy controls compared to unipolar and bipolar depression patients, but no statistically significant change was found in the expression level of *TIMP-1* gene.

**Keywords:** Bipolar depression, Unipolar depression, Gene Expression, Extracellular matrix, *MMP-9*, *TIMP-1*

## ÖZET

Yüksek Lisans Tezi

### UNİPOLAR VE BİPOLAR DEPRESYON TANISI ALMIŞ HASTALARDA *MMP-9* ve *TIMP-1* GENLERİNİN İFADE DÜZEYLERİNİN KARŞILAŞTIRILMASI

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Psikiyatrik hastalıklar, özellikle bipolar ve unipolar depresyon, küresel nüfusun önemli bir bölümünü etkileyen çok yaygın hastalıklardır. Oldukça fazla insanın bu bozukluklardan etkilenmesine rağmen henüz hastalıklara ilişkin anlayışımız bir şekilde sınırlıdır. Bu durumun kesin genetik kökeni ve nedeni henüz kesin olarak belirlenmemiştir. Önceki araştırmalarda birkaç aday genin tanımlanmasına rağmen, çevresel değişkenlerin ve yaşam koşullarının önemli etkisi nedeniyle kesin bir aday gen analizi yapılmamıştır. Hücre dışı matrisin (ECM) ve bileşenlerinin modülasyonu ve bozulması, zihinsel hastalıkları tetikler ve durumun ilerlemesini etkileyebilir. Matriks metalloproteinazlar (*MMP'ler*) ve doku inhibitörleri (*TIMP'ler*), çeşitli psikiyatrik bozukluklarda yaygın olarak tanımlanmıştır. İfade seviyelerindeki değişiklikler hücre dışı matrisi etkiler ve bu bozulmaları başlatır. Önceki araştırmalar, *MMP-9* ve *TIMP-1* genlerinin ifade seviyelerinin bipolar depresyon (BD) ve unipolar depresyon (UD) olan kişilerde değiştiğini göstermiştir. Türk popülasyonunda *MMP-9* ve *TIMP-1* genlerinin ifade düzeyini değerlendirmek için 16 BD, 20 UD ve 19 SK vakası arasında gen ifadeleri karşılaştırıldı. İstatistiksel analiz, *MMP-9* geninin ifade düzeyinin sağlıklı kontrollerde unipolar ve bipolar depresyon hastalarına kıyasla daha yüksek olduğunu ortaya koydu, fakat *TIMP-1* geninin ifade düzeyinde istatistiksel olarak anlamlı bir değişiklik bulunamadı.

**Anahtar Kelimeler:** Bipolar depresyon, Unipolar depresyon, Gen İfadesi, Ekstrasellüler matrix, *MMP-9*, *TIMP-1*

# 1. INTRODUCTION

## 1.1. Mood Disorders

Various psychiatric problems have impacted a significant number of individuals due to evolving and challenging living circumstances, environmental influences, and socio-economic constraints. More than 1.2 billion people worldwide suffer from psychiatric disorders, according to a report by the World Health Organization (WHO) published in 2023. These disorders have a profound impact on the cognitive processes, emotional experiences, and actions of those afflicted, significantly reducing their ability to function and their overall quality of life (Stevenson-Hoare et al., 2024).

Bipolar depression (BD) is the predominant form of depression within the realm of mental diseases. Bipolar disorder has two distinct phases: manic and depressed episodes. Manic episodes are characterized by heightened emotional intensity, whereas depressed episodes are marked by prevalent negative thoughts, melancholy, insufficient energy, and pessimism (Bryde Christensen et al., 2024).

Unipolar depression (UD), or major depressive disorder (MDD), is characterized by a lack of interest, enjoyment, energy, sleep, and several other symptoms in affected subjects. Individuals' functionality and overall quality of life are negatively affected by emotional instability and loss (McWhirter et al., 2024).

- Approximately 280 million people worldwide have bipolar disorder and it is the 6th most common illness in the general population.
- The prevalence of bipolar depression in the 15-25 age group is higher than in other age groups.
- The incidence of bipolar depression is higher in women than in men.
- Unipolar disorder affects approximately 300 million people worldwide and is the 1st most common disorder. It can occur with bipolar disorder at any age, but it most commonly starts between the ages of 25-35.
- The incidence of unipolar depression is higher in women than in men.

Although the causes and pathogenesis of both diseases are not fully known;

- Genetic factors
- Environmental factors
- Unexpected changes in brain chemistry, often imbalances in neurotransmitters and hormones (serotonin, dopamine, norepinephrine, etc.) are thought to play a role. (Gergel et al., 2024).

In the first part, we review what is known in the literature about bipolar and unipolar depression, look at general information and psychosocial outcomes.

## **1.2. Bipolar Depression**

Bipolar depression is a psychiatric condition characterized by mood cycles that oscillate between two distinct extremes. This condition encompasses many mood episodes, including states of mania, hypomania, and depression, and results in alterations in emotions, energy levels, and cognitive functions. It presents itself in everyday life through variations in energy levels and alterations in the consistency of behavior (Cullen et al., 2016). Broadly stated, bipolar depression is classified as an emotional disease, namely a mood disorder that may occur either as a part of the psychotic process or as a chronic progression from major depressive disorder to bipolar depression (F. J. Goodwin, K., 2007). Although mania episodes are characterized by symptoms such as hyperactivity, overconfidence, reduced need for sleep, and increased mood and behavior, hypomanic periods are also marked by negative thoughts and circumstances such as reduced energy, melancholy, social retreat, and social isolation. The incidence of psychosis during manic periods is comparatively lower in depressed episodes. Although manic episodes are characterized by intense feelings, hypomanic periods are consistently more steady and less severe. Bipolar depression, marked by elevated or irritated mood and the presence of excessive energy and activity, impacts an estimated 60 million individuals globally. The heritability of the trait varies, with estimates ranging from 4-6% in extensive genome-wide association studies (GWAS). The clinical diagnosis of bipolar disorder is established by clinical interviews, without the use of objective tests such as gene expression or biomarkers. However, when bipolar disorder is in its first phases and other subtypes are also present, the process of diagnosis becomes exceedingly challenging (Göteson et al., 2022).

### **1.2.1. Bipolar depression diagnostic criteria**

The pioneering work of Emil Kraepelin established the fundamental principles of the systematic approach to the categorization of psychiatric diseases (Kraepelin, 1883). The dichotomous model suggested by Kraepelin categorizes psychiatric diseases into two primary groups. This paradigm encompasses both disorders characterized by periodic mood swings and diseases that usually start in early adulthood and continue throughout life. These diseases are distinguished by abnormal cognitive processes, belief systems, and experiences, known as psychotic symptomatology. Disorders known as manic-depressive psychosis in Kraepelin's original language and now included in the diagnostic framework of mood disorders (such as bipolar disorder) represent one extreme of this categorization. At the opposite extreme, there are conditions that Kraepelin classified as "dementia praecox" (early dementia) that are now classified in contemporary psychiatry as psychotic diseases, such as schizophrenia. In 1957, the term bipolar was first introduced in literature to refer to conditions characterized by both manic and depressed symptoms. The word has been used to denote a more precise subset of mood disorders (Ebert & Bär, 2010).

In the 1980 version of the Diagnostic and Statistical Manual of Mental Disorders (DSM), released by the American Psychiatric Association (APA), there was a substantial adjustment in the nomenclature for affective disorders. More precisely, the phrase manic depression was replaced with bipolar depression (Mihoub et al., 2024). Since its first publication in 1952, the DSM has undergone several revisions. The most recent iteration, DSM-5-TR (DSM-5-Text Revision), was published in 2022. Significant revisions were made to the diagnostic criteria for bipolar depression in this most recent version. Furthermore, bipolar depression and its related diseases were assigned a separate category division, effectively distinguishing them from other mental disorders (Arbanas, 2015). An important modification in this iteration of the diagnostic criteria for bipolar depression is the incorporation of changes in mood, activity levels, and energy states, which were not included in previous versions. Furthermore, the criteria for mixed mood episodes were revised; a diagnosis of a mixed episode during a severe depressive episode now requires the presence of more than three manic symptoms (First, 2009; Regier et al., 2013).

The etiology of bipolar depression is complex and has not yet been fully understood. Genomic studies in psychiatry are trying to uncover the relationship between psychiatric disorders through applied biomarker studies (de Jesus et al., 2017; Iavarone et al., 2014; Smirnova et al., 2019). In some blood-based proteomic studies, some abnormalities in

oxidative stress markers, neurotrophins and many inflammatory markers have been identified. In the light of these data, it is aimed to differentiate between psychiatric disorders or to facilitate the classification of mood disorders (Preece et al., 2018; Rhee et al., 2020). The globally accepted DSM-5 has created an additional category for 'Bipolar and Related Disorders', which does not allow bipolar disorders to be included among psychotic disorders or under affective disorders (APA, 2013a) (Table 1.2.1.). According to the tenth version of the International Classification of Diseases (ICD-10), bipolar depression is classified as a subgroup of mood disorders. The proposed categorization system suggests that in order to establish a conclusive diagnosis of bipolar depression, the clinical manifestation should include at least two distinct mood episodes. It is required that at least one of these episodes must exhibit either manic or hypomanic symptoms.

Conversely, the Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV), implements a more intricate method for categorizing bipolar disorders. This manual categorizes various specific subtypes of bipolar disorder, including bipolar I disorder, bipolar II disorder, cyclothymia, and atypical presentations that do not fit into the previously named categories.

The disparity in diagnostic criteria between the ICD-10 and DSM-IV highlights the intricate nature of the causes of bipolar disorder and indicates continuous attempts to improve the categorization of emotional disorders in psychiatric treatment (Table 1.2.2.). A comprehensive evaluation that considers both the severity and length of manic, hypomanic, and depressed episodes is the basis for the subcategorization of bipolar disorders. A more exact delineation of the many symptoms within the bipolar spectrum is made possible by this nuanced approach to categorization, which reflects the variability of clinical presentations found in afflicted persons (Phillips & Kupfer, 2013; Vieta et al., 2018).

**Table 1.2.1.** Diagnostic criteria for bipolar disorder and each subtype in DSM-5.

| <b>Disorder Subtype</b>                                              | <b>DSM-5 Criteria</b>                                                                                                                                                                                                                                                                                                                                            |
|----------------------------------------------------------------------|------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Bipolar I Disorder</b>                                            | It requires the occurrence of at least one manic episode in the patient's clinical history. This manic episode may temporally fall within a broader pattern of mood disorders, potentially preceding or following episodes of hypomania or major depression..                                                                                                    |
| <b>Bipolar II Disorder</b>                                           | A minimum of one hypomanic episode and one major depressive episode is required. The manic episode must never have occurred.                                                                                                                                                                                                                                     |
| <b>Cyclothymic Disorder</b>                                          | Over a minimum duration of two years (or at least one year in children and adolescents), there have been multiple intervals characterized by hypomanic symptoms that fall short of the diagnostic criteria for a hypomanic episode, as well as numerous intervals marked by depressive symptoms that do not fulfill the criteria for a major depressive episode. |
| <b>Substance/Medication-Induced Bipolar and Related Disorder</b>     | A significant and ongoing mood disruption that is central to the clinical presentation, characterized by an elevated, expansive, or irritable mood, with or without periods of depression, or a noticeable decrease in interest or pleasure in nearly all activities.                                                                                            |
| <b>Bipolar and Related Disorder Due to Another Medical Condition</b> | A significant and enduring mood disturbance that dominates the clinical picture, characterized by an elevated, expansive, or irritable mood, with or without periods of depression, or a substantial reduction in interest or pleasure in almost all activities.                                                                                                 |
| <b>Other Specified Bipolar and Related Disorder</b>                  | Symptoms indicative of a bipolar and related disorder that lead to clinically significant distress or impairment in social, occupational, or other crucial areas of functioning are predominant, yet they do not fully satisfy the criteria for any specific disorder within the bipolar and related disorders diagnostic category.                              |
| <b>Unspecified Bipolar and Related Disorder</b>                      | Symptoms typical of bipolar and related disorders that result in clinically significant distress or impairment in social, occupational, or other important areas of functioning are predominant, yet they do not fully meet the criteria for any specific disorder within the bipolar and related disorders diagnostic category.                                 |

Bipolar disorders (I and II) have many subtypes. These include cyclothymic disorder and unspecified and unspecified bipolar disorders that occur due to different medical conditions caused by substances or medication. Each of these subtypes has its own characteristic clinical symptoms and attack situations.

**Table 1.2.2.** Diagnostic criteria for bipolar disorder and its different subtypes according to DSM-IV and ICD-10.

| Disorder Subtype                     | DSM-IV Criteria                                                                                                                                                          | ICD-10 Criteria                                                                                                                                                                                                                                                                                                                                                                                 |
|--------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| <b>Bipolar disorder type I</b>       | Includes at least one instance of full-blown mania or a mixed episode (both manic and depressive symptoms). Typically, there is also at least one depressive episode.    | <ul style="list-style-type: none"> <li>• The ICD-10 does not distinguish between bipolar disorder types I and II.</li> <li>• ICD-10 requires two discrete mood episodes, at least one of which must be manic, for a bipolar disorder diagnosis. In DSM-IV, one episode of mania (or mixed), or one episode of hypomania plus a major depressive episode, suffice for a BD diagnosis.</li> </ul> |
| <b>Bipolar disorder type II</b>      | Several protracted depressive episodes and at least one hypomanic episode, but no manic episodes.                                                                        |                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Cyclothymic disorder</b>          | Multiple episodes of hypomanic and depressive symptoms occur. The depressive symptoms do not fulfill the criteria for depressive episodes.                               |                                                                                                                                                                                                                                                                                                                                                                                                 |
| <b>Bipolar disorder, unspecified</b> | Symptoms and episodes resembling depressive and hypomanic states may alternate rapidly but do not fully meet the diagnostic criteria for any of the mentioned disorders. |                                                                                                                                                                                                                                                                                                                                                                                                 |

Accurately diagnosing bipolar disorder types I and II at an early stage based on clinical features is very challenging. As a result, only 20% of patients with bipolar disorder who experience a depressive episode are diagnosed with the condition within the first year of onset (Hirschfeld et al., 2003). On average, the time between the onset of the illness and its diagnosis ranges from 5 to 10 years (Baldessarini et al., 2007). The primary challenge in diagnosis and the prolonged diagnostic process stems from the difficulty in distinguishing between bipolar disorder type I or II and major depressive disorder (Figure 1.1.). This occurs particularly in patients presenting during a depressive episode without a clear history of mania or hypomania (F. K. Goodwin, and Jamison, K.R., 2007; Hirschfeld et al., 2003).

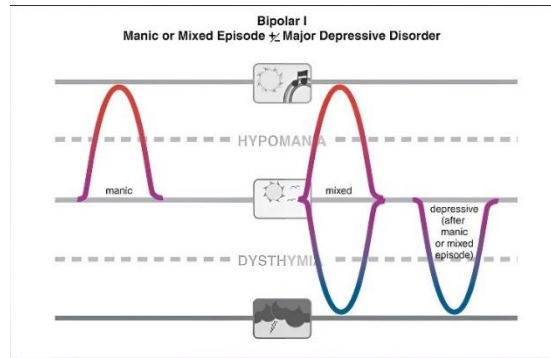


**Figure 1.1.** Bipolar disorder-I mood episodes. (Stahl, 2013).

Major depressive disorder is the most frequently misdiagnosed condition in patients with bipolar disorder, particularly type II, because individuals with this disorder never experience a manic episode by definition. Another reason for the challenge in distinguishing between bipolar disorder type I or II and major depressive disorder is that depressive symptoms are more prevalent throughout the course of bipolar disorder type I or II compared to hypomanic or manic symptoms.

Typically, these disorders begin with a depressive episode. Individuals with type II bipolar disorder spend a significant portion of their lives in a depressive state and experience more time in this phase than in hypomanic or manic phases (Figure 1.2.) (Ghaemi et al., 2002; Hirschfeld et al., 2003; Manning, 2003). For instance, in a study, individuals with bipolar disorder type I exhibited hypomanic or manic symptoms for only 9% of the follow-up period, whereas those with bipolar disorder type II experienced hypomanic symptoms for only 1% of the follow-up period (Judd et al., 2003; Judd et al., 2002; Marneros, 2001).

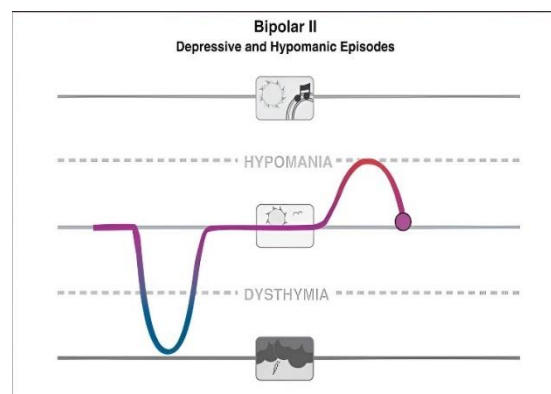


**Figure 1.2.** Bipolar disorder-2 mood episodes. (Stahl, 2013)

Moreover, many patients diagnosed with unipolar depression are believed to potentially have a misdiagnosed subtype of bipolar disorder. For instance, among patients initially diagnosed with unipolar depression, the conversion rate to bipolar disorder type II over 11 years is 9.19%, and the rate of experiencing a manic or hypomanic episode over 5 years is 20% (Goldberg et al., 2001; Rush et al., 2006; Trivedi et al., 2006). These findings indicate that the distinction between bipolar disorder, particularly type II, and unipolar depression is not well-defined, suggesting that many individuals diagnosed with unipolar depression may actually have undiagnosed bipolar disorder (Figure 1.3.) (Correa et al., 2010; Valentí et al., 2012). However, achieving an accurate diagnosis during the early stages of bipolar disorder, prior to the first episode of hypomania or mania, or in the absence of a clear history of these episodes, can help prevent the long-term negative consequences of misdiagnosis (APA, 2013b; Goodwin, 2012; Sadock, 2015).

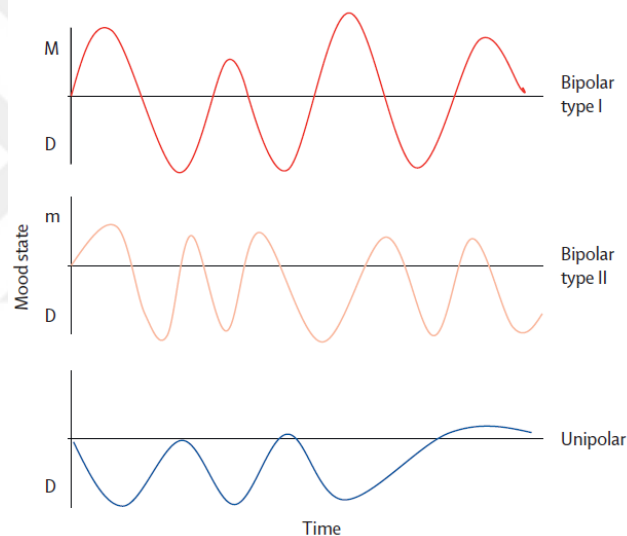


**Figure 1.3.** Mood changes over time in bipolar disorder type I, bipolar disorder type II, and recurrent unipolar depressive disorder. M: mania m: hypomania D: depression. (Scott, 2022)

### 1.2.2. Epidemiology

Bipolar disorder impacts more than 1% of the world's population (Merikangas et al., 2011). The lifetime prevalence of bipolar I disorder is 0.6% and for bipolar II disorder it is 0.4%. According to DSM-5 criteria, the global annual prevalence of bipolar I disorder is reported as 1.5%, and the lifetime prevalence is 2.1% (Blanco et al., 2017). Studies indicate that the onset of bipolar disorders occurs independently of ethnicity, nationality, and socioeconomic status.

The incidence of bipolar I disorder is comparable between men and women, whereas bipolar II disorder is more prevalent in women (Nivoli et al., 2011). Bipolar disorder begins to appear at an early age, and the average age of onset is 20 (F. J. Goodwin, K., 2007). Onset states seen at earlier ages are associated with depression. Since the disease progresses in the early stages, diagnosis and treatment usually begin in young adulthood. However, this is not always the case. Because, although some studies have shown that diagnosis is delayed by 5 years from the onset of symptoms, varying data have been reported in different cases (Dagani et al., 2017). However, patients with comorbidities and depressive onset polarity experience significantly longer times to diagnosis. Extended treatment durations influence prognosis and correlate with the duration of untreated illness, a higher incidence of suicide attempts, and longer disease duration (Altamura et al., 2010). Bipolar disorders rank 17th globally among diseases, following depression, anxiety disorders, schizophrenia, and dysthymia (Vigo et al., 2016). Bipolar disorders, which are chronic and recurrent conditions, frequently lead to functional impairment and reduced quality of life. Moreover, because they affect individuals in their economically productive years, bipolar disorders impose substantial societal costs in terms of healthcare and disability expenses (De Dios et al., 2012; Macneil et al., 2011).

### **1.2.3. Comorbidities**

When bipolar disorders co-occur with other psychiatric conditions, diagnosis and treatment can become challenging, with higher rates of comorbidity associated with poorer outcomes (Merikangas et al., 2007). On-psychiatric comorbidities are also prevalent among individuals with bipolar disorder and may include diabetes, osteoporosis, metabolic syndrome, fibromyalgia, as well as endocrine and cardiovascular disorders (Correll et al., 2017). The use of antipsychotic medications in managing metabolic syndrome can contribute to unhealthy lifestyles. The presence of comorbid conditions increases the risk of premature death in bipolar disorder patients compared to the general population (Dols et al., 2014; McElroy et al., 2016). Due to the long-term impact of mental health disorders on daily life quality, the unpredictable financial burden on affected individuals and their families due to treatment costs, and the strain on healthcare and social support systems further exacerbate the challenges associated with these disorders.

#### **1.2.4. Suicide**

Bipolar disorders exhibit the highest suicide rate among affective disorders, being 20 times higher than in the general population (Gonda et al., 2012). Approximately half of individuals with bipolar disorder attempt suicide at least once in their lives, and 15-20% of these attempts result in death. Risk factors for suicide attempts include younger age of onset, gender, depressive episodes, anxiety, substance abuse, and personality disorders (Schaffer et al., 2015). The risk of suicide is higher in untreated patients compared to those treated with antiepileptic drugs (Gibbons et al., 2009).

#### **1.2.5. Mechanisms/pathophysiology**

Bipolar disorder is a condition characterized by multifactorial causes and genetic complexity. Both common and rare genetic variants contribute to the development of the disorder in affected individuals (Lichtenstein et al., 2009; Mühleisen et al., 2014). It is estimated that the hereditary transmission of bipolar disorder is around 85% (F. J. Goodwin, K., 2007). This rate represents one of the highest estimates among psychiatric disorders to date, with a multifactorial gene-environment interaction model believed to best explain this disorder (Figure 1.4). Initial studies targeted specific genes in bipolar disorder, but genome-wide association studies have examined numerous common variants single nucleotide polymorphisms (SNPs) across the entire genome for associations with the disease. To date, significant genetic associations have been identified at 18 regions across the genome, many of which have been replicated. However, due to the modest effects of associated alleles, large case-control samples are required to validate these findings. To achieve this, consortiums can increase the number of samples, leading to more robust results (Forstner et al., 2015).

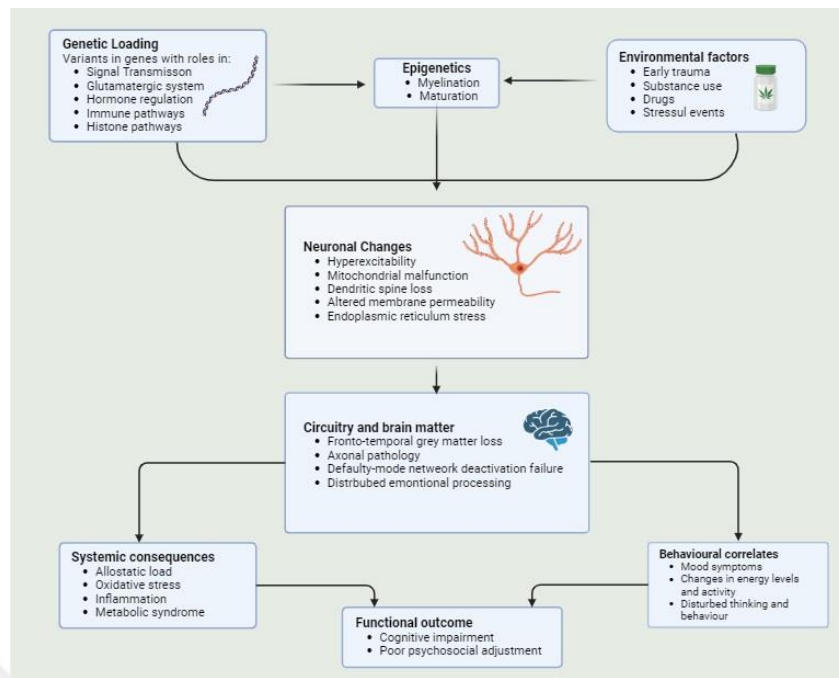


**Figure 1.4.** Causes of bipolar disorder (Created with BioRender.com)

Bipolar disorders result from a combination of genetic and environmental factors, yet they remain among the most heritable psychiatric disorders. Both genetic and environmental influences can lead to neuronal changes in the brain, which affect the body systemically and behaviorally, resulting in cognitive and psychosocial impairments. In neurobiological studies focusing on the molecular basis of bipolar depression, the monoaminergic neurotransmitter systems, particularly the serotonergic and dopaminergic systems, have been prominently discussed. These systems are widely distributed across neuronal circuits in the brain and are implicated in mood disorders. While there is evidence suggesting their potential involvement in bipolar depression, no definitive dysfunction has been conclusively identified to date. According to various hypotheses and speculations, bipolar depression is considered a disorder involving altered synapses and circuits rather than simply imbalances in individual neurotransmitters.

### 1.3. Unipolar Depression

Unipolar depression is synonymous with major depressive disorder. The term 'unipolar' indicates that this type of depression does not alternate with other mental states, such as mania. Unipolar depression is characterized as a psychiatric condition lasting at least two weeks, marked by vegetative symptoms such as depressed mood, loss of interest, inability to experience pleasure from usual activities, pessimism, lack of energy, and

resulting in decreased functionality (American Psychiatric Association & Association, 2013). Unipolar depression is a common mental illness that continues with relapses, affects the societies in which it is found, and causes deterioration in the quality of life. In 2008, the WHO ranked unipolar depression as the third leading cause of disease burden globally and predicted it would become the leading cause by 2030. While the one-year prevalence rate of unipolar depression is 6.6%, its lifetime prevalence varies between 15-18% in studies (Malhi & Mann, 2018; Otte et al., 2016). When looked at by gender; While the lifetime prevalence in men is 8-12% on average, this rate has been reported as 20-26% in women. The common conclusion reached in depression epidemiology studies is; Depression is twice as prevalent among women compared to men. It is thought that this difference may be due to hormonal factors such as genetic characteristics and menstruation, as well as neglect and abuse in childhood, being raised in a submissive style in an oppressive family environment, the roles that society imposes on women, and low education and income levels (Noble, 2005; Sadock et al., 2000). In a study conducted in our country, the one-year prevalence of major depressive disorder was found to be 8.2%, consistent with findings from other studies (Topuzoğlu et al., 2015).

Although unipolar depression is a common psychiatric disorder, its etiology and pathophysiology still remain unclear. Studies conducted to elucidate the pathogenesis of the disease have focused on the monoamine hypothesis, but since this explanation alone is insufficient, studies have continued to change direction. In studies conducted in the last 20 years, the neuronal plasticity hypothesis has been put forward, highlighting the incompatibilities in neuronal structures in UD (Sarisoy et al., 2014).

### **1.3.1. History**

Depression, which has the oldest definition among mental illnesses, derives its origin from the Latin word *depressus*, which means to suppress, to be low. Although observations of depressive disorders have occurred in different ages and societies throughout human history, this definition was first made systematically in B.C. He became Hippocrates in the 400s. Hippocrates first established a connection between human mood and body fluids, used the term "melancholia" meaning "black bile", and defined melancholy as a disease characterized by apathy, reluctance, pessimism, anxiety, insomnia, inadequacy and thoughts of death resulting from disorders in the bile ducts defined (Otte et al., 2016; Ventriglio et al., 2020). Major depressive disorder; It is the emergence and recurrence of situations such as feeling sad, depressed, pessimistic in the affective field, loss of interest in things you like to

do, lack of pleasure and decrease in functional activity, thoughts of worthlessness, sexual reluctance. When depression is experienced at a level that disrupts a person's daily life and level of functionality, it causes significant disability as a result of work and social losses. Until today, observations regarding depression have been defined and classified according to each society, religion and age from period to period. Studies on classifying and naming mental illnesses began with Hippocrates. During the Renaissance, a new perspective on mental disorders developed with the beginning of anatomical dissections, and then Kraepelin in 1896 and Leonhard in 1957 defined and classified mood disorders in detail according to the characteristics of the disease period (unipolar, bipolar) (Cuesta et al., 2024; Omrani et al., 2012).

Kraepelin saw depression not just as a symptom, but as a distinct category of own. He emphasized that the primary issue is in mood depression, together with physical and mental deceleration, which form a group of clinical markers. Throughout the time that started in this manner and extended into the twentieth century, numerous definitions were attempted to explicate depression, encompassing spiritual, psychological, and biological possibilities. Depression was initially incorporated into diagnostic systems by the American Psychiatric Association in 1952 with the introduction of the DSM (Pajević et al., 2021). Within the DSM-I, depression is categorized into two distinct types: organic and reactive. Depression in DSM-II, which was officially adopted in 1968, was categorized as both psychotic and neurotic. Subsequently, a method was implemented to provide specific criteria for diagnosing depression, and the DSM-III, released in 1980, explicitly specified the symptoms and duration criteria of depression. DSM-III categorizes depression into three distinct subtypes: melancholic, non-melancholic, and psychotic. The criteria for the number and duration of symptoms necessary for diagnosis were set in DSM-IV, which was released in 1994. Depressive disorders were classified as distinct categories in the DSM-5, which was released in 2013. Both the DSM and ICD diagnostic systems are extensively used in contemporary times, whereby depressive illnesses are categorized as 'Mental and Behavioral illnesses' within these systems (Agbir et al., 2010).

Unipolar depression is a highly prevalent and severe mood disorder. Individuals who suffer from this type of depression often experience ongoing feelings of sadness or a lack of interest in engaging with their surroundings. A study conducted in the United States (USA) found that about 7% of adults have experienced an episode of unipolar depression at some point in their lives. Unlike bipolar depression, which involves mood swings between depression and mania, unipolar depression is characterized solely by negative emotions and

feelings. It is termed unipolar because it does not fluctuate between two mood states, such as mania and depression. Also known as major depression or clinical depression, this condition impacts numerous critical areas of a person's life. It influences mood, behavior, and various physical functions (such as sleep and appetite), making it difficult for affected individuals to live healthy and fulfilling lives.

One of the most common characteristics among people with unipolar depression is a loss of interest in activities and hobbies they once enjoyed, as well as difficulty performing daily tasks. In extreme cases, these feelings can even lead to suicidal thoughts (Kim & Lee, 2021).

### **1.3.2. Epidemiology of unipolar depression**

Unipolar depression was identified by the WHO as the third cause of disease burden worldwide in 2008 and suggested that it would rise to the first place in the burden of disease by 2030. Even though different results are obtained from different studies, all opinions are common that the incidence rates are high. Although the annual prevalence rate of unipolar depression is around 6%, its lifetime prevalence has been found to be between 15-18% and has been reported to have a high prevalence rate among psychiatric disorders (Bromet et al., 2011; Malhi & Mann, 2018).

When examining gender differences, the lifetime incidence of depression is nearly double in women compared to men, with the average age of onset being approximately 25 years old for both sexes (Bromet et al., 2011; Otte et al., 2016; Salk et al., 2017).

Important data on the prevalence of depression was obtained with the Turkey Mental Health Profile research conducted by the Ministry of Health in Turkey. Within the scope of this research, the ICD was used with 7479 participants, and at the end of the study, the prevalence of depressive episodes was determined to be 4%, with gender-specific rates reported as 5.4% for women and 2.3% for men (Erol et al., 1998; Topuzoğlu et al., 2015). In the Turkey Burden of Disease Study, the prevalence of unipolar depression according to gender was reported as 16.0% in men and 26.3% in women, and 21.1% in total (Unuvar, 2004). There are results reporting that the risk of unipolar depression is higher in single, separated, widowed and divorced individuals than in married individuals. Accordingly, in our country, it has been observed that unipolar depression has the highest rate in separated, divorced and widowed individuals, and has low rates in married individuals (Celik & Hocaoglu, 2016). There are many studies highlighting a negative correlation between education level and depression (Elovainio et al., 2012; Ibrahim et al., 2013; Kim & Lee, 2021). According to the Turkey Mental Health Profile Report conducted in our country in

1998, it was determined that the rate of depression diagnosis decreased as the education level of patients increased (Erol et al., 1998). Numerous studies have explored the connection between unemployment and depression, identifying unemployment and low socioeconomic status as risk factors for depression. A comprehensive meta-analysis conducted in 2021 found that 24% of the unemployed population exhibited depressive symptoms, with the prevalence of unipolar depression being 16%. In the realm of economic factors, research has demonstrated that a reduction in income is linked to a higher risk of depression, with the prevalence of depression being greater among low-income individuals (Amiri, 2022).

### **1.3.3. Etiology of unipolar depression**

Although unipolar depression is a common mental illness in society, uncertainties about its etiology and pathophysiology continue. So far, no single model or mechanism can best explain all aspects of the disease (Malhi & Mann, 2018).

Multiple factors contribute to the development of the disorder, and as such, the etiology of depression is typically categorized into three main groups: genetic, psychosocial, and biological factors (Delgado & Moreno, 2000).

- i. Genetic factors
- ii. Psychosocial Factors
- iii. We can classify it as biological/neuroanatomical factors. It is known that environmental factors also contribute to these factors.

#### **1.3.3.1. Genetic factors**

Etiology of unipolar depression is significantly influenced by genetic factors and hereditary processes. Twin and adoption studies, which were among the initial investigations undertaken in this particular field, demonstrated that unipolar depression had a moderate degree of heritability. A further study revealed that the likelihood of acquiring the condition in immediate family members of those with unipolar depression was thrice greater (Flint & Kendler, 2014). Twin studies have determined a heritability rate that falls between 31% to 42%. Previous research has proposed that there may be a more pronounced genetic link between recurring episodes and early-onset depression. Numerous genome-wide association studies have been undertaken to examine single nucleotide polymorphisms, nevertheless, no definitive evidence has yet been acquired about the susceptibility to depression (Ebmeier et al., 2006).

In the context of the monoamine theory of unipolar depression, numerous candidate genes have been studied, particularly receptor genes for dopamine (DRD3, DRD4) and serotonin (HTR1A, HTR2A, HTR1B, HTR2C), as well as genes for noradrenaline (SLC6A2) and dopamine (SLC6A3). Genes such as monoamine oxidase A (MAO), tyrosine hydroxylase (TH), tryptophan hydroxylase 1 (TPH1), and catechol-o-methyl transferase (COMT1) have been focused on, identifying polymorphic variants associated with point mutations or repetitive polymorphisms. While these variants may contribute to the risk of developing depression, the impact is generally considered modest (Shadrina et al., 2018).

Epigenetic changes that occur during some moments of stress have been associated with unipolar depression, with epigenetic changes in glucocorticoid signaling, serotonergic signaling and neurotrophins genes coming to the fore. Among the candidate genes investigated in unipolar depression, the serotonin transporter gene (SLC6A4) is one of the most extensively studied. It has been observed that in the presence of a short allele (S) in the polymorphism of this gene, the individual may be more prone to depression, and if there are negative experiences in early childhood, the risk of depression and suicide increases in adulthood (Caspi et al., 2003; Celik & Hocaoglu, 2016). It has been shown that BDNF release is impaired by the Val66Met polymorphism observed in the BDNF gene and that this condition is associated with depression (Ribeiro et al., 2007).

#### **1.3.3.2. Psychosocial causes**

##### **Psychoanalytic theory**

According to psychoanalytic theory, depression is associated with the loss of a real or imaginary love object. Individuals use an introjection defense mechanism to cope with the feeling of loss that arises from these losses, thus a part of the ego becomes an object. The feeling of loss also arouses hatred (ambivalence). The feeling of hatred towards the lost object is directed to the ego by the rigid superego. As a result of all this, self-esteem is lost and thoughts of worthlessness, guilt and death occur. When these situations occur for the person, life becomes meaningless and psychological depression occurs (Öztürk & Uluşahin, 2015)

##### **Cognitive theory**

According to Beck's cognitive theory, depression is explained through concepts such as the cognitive triad, schemas, and cognitive errors. The cognitive triad highlights negative attitudes towards oneself, the future, and the environment as central in depression. According to this concept, experiences in childhood lead to the formation of some basic thoughts and

beliefs through learning, and these are called schema. These schemas affect how a person perceives his life and his attitudes. Life events may lead to the activation of silent schemas, the emergence of 'negative automatic thoughts' and, as a result, the formation of unpleasant emotions such as anger, anxiety, guilt and sadness. Depression occurs as a result of these negative thoughts and schemas (Arkar, 1992; Başoğlu & Buldukoğlu, 2015).

#### Behaviorist theory

Behavioral theory posits that specific environmental shifts and avoidance actions hinder individuals from obtaining rewards and reinforcement, potentially leading to the onset and persistence of depressive symptoms. Thoughts and observable behaviors that facilitate avoidance are believed to be crucial factors that diminish reward and positive reinforcement, thereby making individuals more susceptible to depression and the emergence of depressive symptoms (Carvalho & Hopko, 2011). Another approach in the behaviorist view is the learned helplessness model defined by Seligman, who tried to define this theory on animal models. This model is based on the observation that the helplessness and escape behaviors of experimental animals are reduced by exposing them to repeated electric currents and preventing them from escaping (Maier & Seligman, 1976; Öztürk & Uluşahin, 2015).

#### Attachment theory

Attachment theory, initially defined by John Bowlby, serves as a key framework for comprehending how close relationships influence social and emotional development (Holmes, 2014). A fundamental premise of attachment theory is that early caregiving experiences shape individuals' perceptions of themselves and their interpersonal relationships (Khan et al., 2020). When caregivers in early childhood respond sensitively to an infant's attempts to seek closeness, the infant is likely to develop a secure attachment style and view these caregivers as a safe haven during times of distress. Secure attachment acts as a protective mechanism against the development of psychopathology. Conversely, insecure attachment can increase the risk of various psychopathologies, particularly depression. Research in this field points out that repeated experiences of the absence or unresponsiveness of the attachment figure lead to dysfunctional and negative cognitions about self and competence, which in turn increases depressive symptoms (Sprit et al., 2020).

### **1.3.3.3. Biological causes**

#### Brain imaging studies in unipolar depression

Although the neural circuits involved in the pathophysiology of unipolar depression remain unclear, human brain imaging and post-mortem studies suggest that multiple brain

regions, including the amygdala, thalamus, striatum, hippocampus, prefrontal cortex, and cingulate cortex, may contribute to the manifestation of depressive symptoms (Yang et al., 2015). Multiple studies on patients with unipolar depression have found reductions in gray matter volume and glial density in the prefrontal cortex and hippocampus. These brain regions are believed to mediate the cognitive aspects of depression, including feelings of worthlessness and guilt (Krishnan & Nestler, 2008; Pizzagalli & Roberts, 2022). Brain MRI (Magnetic Resonance Imaging) studies have reported that there is a decrease in hippocampal brain volumes in people with depression, which may be related to the duration of the disease (Campbell et al., 2004). Additionally, in prospective studies conducted with patients diagnosed with unipolar depression, it was found that the hippocampal brain volume in the patient group who achieved remission with treatment increased compared to before treatment (MacQueen et al., 2008).

#### Unipolar depression and neuroendocrine factors

A consensus has emerged from clinical and preclinical studies indicating that dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis plays a significant role in the pathophysiology of unipolar depression (Paslakis et al., 2011). Research in this area has identified elevated levels of plasma and salivary cortisol, as well as increased urinary free cortisol excretion, in patients with depression (Bao et al., 2008; Scott & Dinan, 1998). Furthermore, numerous studies have demonstrated that in patients with depression, there is an increase in the number of corticotropin-releasing hormone (CRH)-secreting neurons in limbic brain regions, a reduction in CRH binding sites in the frontal cortex due to elevated CRH levels, decreased corticosteroid receptor function, and consequently, impaired results on the dexamethasone suppression test (Holsboer, 2000).

Another neuroendocrine factor is the hypothalamus-pituitary-thyroid gland (HPT) system. Although its pathophysiology has not yet been fully elucidated, there are many studies showing changes in this pathway in depression patients (Duval et al., 2021). Research in this field has shown that the thyroid stimulating hormone (TSH) response to thyrotropin-releasing hormone (TRH) is reduced in patients with depression, even though they are chemically euthyroid (Bartalena et al., 1990; Roelfsema & Veldhuis, 2013).

#### Unipolar depression and biogenic amines

Functional disturbances related to neurotransmitters in the brain, particularly within the serotonergic and noradrenergic systems, are believed to play a crucial role in the development of unipolar depression. The earliest theory addressing the role of biogenic amines in this context is the Monoamine Hypothesis, which emerged in the 1970s.

According to the monoamine hypothesis, the primary cause of unipolar depression was thought to be a deficiency of monoamine neurotransmitters such as norepinephrine (NE), serotonin (5-HT), and dopamine (DA) (Kotan et al., 2009). This theory was proposed based on observations that antihypertensive drugs like reserpine can induce severe depression by inhibiting proteins such as the vesicular monoamine transporter (VMAT), which leads to decreased synaptic levels of monoamine neurotransmitters (Bristow et al., 2021). Based on this hypothesis, various classes of antidepressant drugs have been developed to elevate monoamine levels in the synaptic cleft by inhibiting the breakdown or reuptake of these neurotransmitters (Hirschfeld, 2000).

Later studies and post-mortem research have revealed that in depression, there is a reduction in serotonin activity in the brain and a decrease in the level of 5-hydroxyindoleacetic acid (5-HIAA), which is the primary metabolite of serotonin in the brain (Drevets et al., 1999). New data from positron emission tomography (PET) imaging studies have demonstrated that serotonin transporter (SERT) binding sites are reduced in the brainstem and amygdala of drug-free depressed patients. Additionally, these studies have shown that both presynaptic (in the brainstem) and postsynaptic (in the mesotemporal cortex) levels of serotonin (5-HT) are decreased, along with a reduction in the density of various 5-HT receptor subtypes (Albayrak & Ceylan, 2004). Dysfunction of dopamine, another monoamine, has been consistently associated with symptoms seen in depression, especially anhedonia and amotivation, as a result of many studies (Grace, 2016). Post-mortem tissue studies and PET imaging research have revealed decreased dopamine transporter (DA transporter) binding sites and increased postsynaptic D2/D3 receptor density in depression. These findings suggest a reduction in the synaptic availability of dopamine in depressive states (Saveanu & Nemeroff, 2012). In clinical practice, although the number of neurotransmitters in the synaptic gap increases within a few hours after antidepressant drugs are given, clinical improvement occurs after 1-4 weeks, agents that are not effective in monoamine reuptake, such as mianserin, have antidepressant properties, and agents that inhibit monoamine reuptake, such as amphetamine, do not have antidepressant properties. Situations such as this have caused the monoamine hypothesis to be inadequate in understanding the neurobiology of unipolar depression and have led to the need to develop this hypothesis (Kotan et al., 2009). As a result, although many safe and effective antidepressant drugs have been developed based on the monoamine hypothesis, recent scientific and clinical evidence has revealed that the original hypothesis is insufficient for fully explaining the complexities of depression (Hirschfeld, 2000).

In addition to serotonin (5-HT), norepinephrine (NE), and dopamine (DA), neurotransmitters such as acetylcholine, gamma-aminobutyric acid (GABA), and glutamate also play a role in the pathogenesis of unipolar depression (Kotan et al., 2009). Evidence has been presented that people with depression have abnormal glutamate levels and abnormal NMDA signaling in post-mortem tissue studies (Deschwenden et al., 2011). Glutamate, the primary excitatory neurotransmitter in the brain, is involved in nearly all key functions affected in depressive states. Post-mortem studies support a link between structural deteriorations in glial cells responsible for the removal of synaptic glutamate and the pathophysiology of mood disorders (Kugaya & Sanacora, 2005). Additionally, it has been noted that glutamine and glutamate levels in the cortex are reportedly lower in those with depressive disorders (Yildiz-Yesiloglu & Ankerst, 2006).

#### **1.3.3.4. Neuroplasticity**

Another theory that has been put forward in recent years and has gained an important place is the neuronal plasticity theory. Neuroplasticity is the ability of the brain to undergo neurobiological changes in response to external stimuli, especially early life challenges, and internal stimuli such as chronic stress or genetic/epigenetic influences (Wang et al., 2022). Plasticity occurs at various levels, from neurogenesis, the formation of newborn neurons in proliferative zones, to the adjustment of synaptic weights, modulating both the structure and function of neuronal networks (Castrén & Hen, 2013). The neuronal plasticity hypothesis suggests that depression might arise from harmful changes in the neuronal areas involved in regulating mood (Duman et al., 2016; Jia et al., 2021). While the cortex, septum, amygdala, and hippocampus are the primary brain regions where neuroplasticity is seen, research on the hippocampal region has primarily addressed depression (Gürpınar et al., 2007). Numerous clinical and imaging studies have shown that the hippocampus region in UD has fewer proliferative cells and less neuroplasticity; antidepressant treatment increases neurotrophic factors and neuroplasticity simultaneously (Malberg & Schechter, 2005). Intracellular components known as neurotrophins have been shown to play a significant role in the control of neuronal plasticity by impacting nearly every neuroplasticity process, including neuronal survival, differentiation, and proliferation (Levy et al., 2018).

In the central nervous system, neurotrophins play crucial roles in the regulation and realization of programmed cell death, or apoptosis. Although other neurotrophins have been found, BDNF NGF (Nerve Growth Factor), NT-3 (Neurotrophin-3), and NT-4 (Neurotrophin-4) are considered to be the most significant (Gürpınar et al., 2007). BDNF is

a protein that belongs to the neurotrophin family and is essential for sustaining the survival, development, and differentiation of neurons. Research has also indicated that it plays a significant role in controlling neurogenesis and synaptic plasticity (Levy et al., 2018). Studies have found significantly lower serum BDNF levels in patients with depression (Bocchio-Chiavetto et al., 2010). Studies have also shown that BDNF gene expression increases with antidepressant medication and that BDNF levels drop in depressed patients, hence enhancing neurogenesis and neuroplasticity—which is decreased by stress (Martinowich & Lu, 2008).

#### **1.3.4. Unipolar depression diagnosis and classification**

The DSM-5 is the most commonly used diagnosis and categorization system for unipolar depression worldwide. The DSM-5 has developed the following diagnostic standards for unipolar depression:

- A. At least five of the specified symptoms must be evident within a two-week period. One of these symptoms must include either a depressed mood (APA, 2013b) or a loss of interest or pleasure (Malhi & Mann, 2018).
  - 1. Depressed mood is present most of the day, almost every day, and is either self-reported (e.g., feels sad, empty, or hopeless) or observed by others (e.g., appears tearful).
  - 2. A noticeable decline in interest or enjoyment in all or most of the activities that take up the majority of the day occurs almost daily.
  - 3. Not trying to lose weight but yet experiencing significant weight loss or gain (e.g., a shift in weight of more than 5% in a month), or a change in your appetite to eat nearly every day.
  - 4. Experiencing insomnia or excessive sleep nearly every day.
  - 5. Psychomotor agitation or retardation almost daily (not just restlessness or a sense of slowing down; it has to be visible to others).
  - 6. Almost daily fatigue or low energy.
  - 7. Nearly daily feelings (not simply self-condemnation and guilt about being sick) of worthlessness, excessive, or inappropriate guilt (may be delusional).
  - 8. Being indecisive nearly all the time, or having trouble concentrating or thinking clearly (as reported or seen by others).

9. Recurring thoughts of suicide or death (not just dread of dying), attempts at suicide or intentions to take specific actions leading up to suicide, or recurring thoughts of death (not simply fear of death).
- B. These symptoms hinder social, vocational, or other critical domains of functioning or produce clinically severe distress.
- C. These symptoms are not caused by basic medical conditions like hypothyroidism or the direct physiological repercussions of substance usage (such as abusing drugs or medications).
- D. Schizoaffective disorder, schizophrenia, schizophreniform disorder, delusional disorder, various identified and nonspecific schizophrenia spectrum disorders, and other psychotic diseases are unable to account for the incidence of unipolar depression.
- E. Never experienced a manic or hypomanic episode.

When diagnosing according to DSM-5, it was requested to also specify whether unipolar depression had a single episode/recurrent episode feature. The severity and degree of remission of unipolar depression are also defined in DSM-5. In addition, diagnostic criteria and determinants are also listed in DSM-5, as well as features of the clinical condition. These include the following subtypes: depressive, distressed, with mixed features, with melancholic features, with atypical features, with mood-congruent psychotic features, with mood-incongruent psychotic features, with catatonia, onset in the peripartum period, and with a seasonal pattern.

#### **1.3.4.1. Clinical features and prognosis of unipolar depression**

The clinical appearance of unipolar depression ranges from affective fluctuations such as unhappiness and reluctance to psychotic symptoms such as the inability to distinguish reality. More than 90% of patients have a depressive mood characterized by feelings of grief, helplessness, unhappiness, hopelessness, sadness and worthlessness, and this is often accompanied by anhedonia and loss of interest (Namlı & Karaytuğ, 2024). Although its course throughout life varies, it is unpredictable due to the nature of the disease, and therefore the duration of complaints, the number of recurrences throughout life, and the way they occur vary (Malhi & Mann, 2018). Often of slow onset and largely episodic, patients may feel well between acute depressive episodes (Segal et al., 2003; Verduijn et al., 2017).

Upon examining the course of the illness, it was discovered that roughly 50% of individuals experiencing one depressed episode would go on to experience at least one more depressive episode; for those experiencing two or more depressive episodes, this percentage exceeded 80% (Segal et al., 2003).

Based on its prognosis, it is likely to become chronic in patients with frequent relapses and is associated with other important conditions such as more severe loss of functionality, decrease in quality of life, and suicide than non-chronic depression (Schramm et al., 2020). Accompanying suicidal thoughts may also be observed in individuals diagnosed with unipolar depression.

Higher suicide rates have been reported in people with severe disease severity, but although different rates appear in different studies, it is generally accepted that the risk of suicide increases with increasing depression severity (Thomson, 2012; Wulsin et al., 1999). Gender-based studies on suicide rates reveal that the incidence of suicide among men is about three times greater compared to that among women (Olfiffe et al., 2019).

#### **1.3.5. Unipolar depression treatment**

As the frequency of unipolar depression episodes increases, the risk of recurrence also rises, making the probability of future episodes quite high. Consequently, the goal of the treatment process extends beyond alleviating acute phase symptoms to also include the prevention of both short-term and long-term recurrences. After the acute phase of depressive symptoms has resolved, it is crucial to prevent relapse, which refers to the return of symptoms during the recovery phase. Long-term maintenance treatments are essential for preventing the onset of new depressive episodes in patients who have experienced symptom resolution, thereby addressing the risk of recurrence over time (Möller, 2008; Yüksel et al., 2010). Response to treatment is accepted as a reduction in symptom severity of more than 50% (Qaseem et al., 2016). The preferred treatment options for each patient may vary and can include pharmacotherapy, psychotherapy, a combination of pharmacotherapy and psychotherapy, and electroconvulsive therapy (ECT) (APA, 2013b). The choice of antidepressant for treating unipolar depression should be tailored to the individual characteristics of the patient. This decision should be based on factors such as the patient's history of previous medications, coexisting medical conditions that could be exacerbated by antidepressants, potential drug interactions, the short- and long-term side effects of the medication, the type and subtype of depressive symptoms, and the overall cost of treatment. Second-generation antidepressants, including selective serotonin reuptake inhibitors (SSRIs)

such as sertraline, fluoxetine, escitalopram, citalopram, paroxetine, fluvoxamine, and serotonin-norepinephrine reuptake inhibitors (SNRIs) like duloxetine and venlafaxine, are among the most frequently prescribed treatments for unipolar depression (Kennedy et al., 2016).

#### **1.4. Extracellular Matrix**

The extracellular matrix (ECM) is an intricate and multifaceted network composed of proteins, carbohydrates, and additional molecules that encase and support cells across various tissues and organs. It not only provides essential structural support but also regulates cell growth and differentiation and facilitates communication between cells. The ECM is primarily made up of three categories of proteins: collagen, non-collagenous proteins, and proteoglycans. Collagen, the predominant protein in the ECM, forms the main structural scaffold for tissues. Non-collagenous proteins, including fibronectin, laminin, and elastin, function to anchor cells to the ECM and maintain cellular stability. Proteoglycans are substantial molecules featuring a protein core adorned with glycosaminoglycans (GAGs), which are lengthy sugar chains that endow the ECM with its ability to retain water. Additionally, the ECM acts as a reservoir for growth factors and signaling molecules vital for processes such as neuronal progenitor proliferation, dendritic and axonal development, cellular migration, cortical organization, and synaptic plasticity throughout developmental stages (Long & Huttner, 2021). An essential part of the nervous system's development is the ECM. Aging plays a significant role in the development and progression of most neurodegenerative diseases (Barros et al., 2011). Aging can contribute to neurodegenerative diseases through the deterioration of the extracellular matrix over time. This deterioration results in an imbalance between extracellular matrix degradation and accumulation, and the breakdown of ECM generates signaling molecules like elastin-derived peptides (EDPs). Under aging and pathological conditions, elastin degradation may become irreversible or challenging to repair, even though elastin is normally resistant to proteolysis and can be broken down by proteinases such as *MMPs* (Soles et al., 2023). Furthermore, the ECM conveys signaling information through its biochemical makeup and the localized distribution of its components, which are crucial for gene regulation, and acts as both a reservoir and a source of signaling molecules such as cytokines (Figure 1.4.1). The ECM plays a role in guiding or regulating various developmental and cellular biological processes as detailed below (Dzamba & DeSimone, 2018; Reddig & Juliano, 2005; Smith et al., 2018);

- Cell directionality through contact guides,
- The morphogenetic processes involved in gastrulation and the formation of organs
- Stem cell differentiation
- Anchorage dependent growth
- Viability of cells

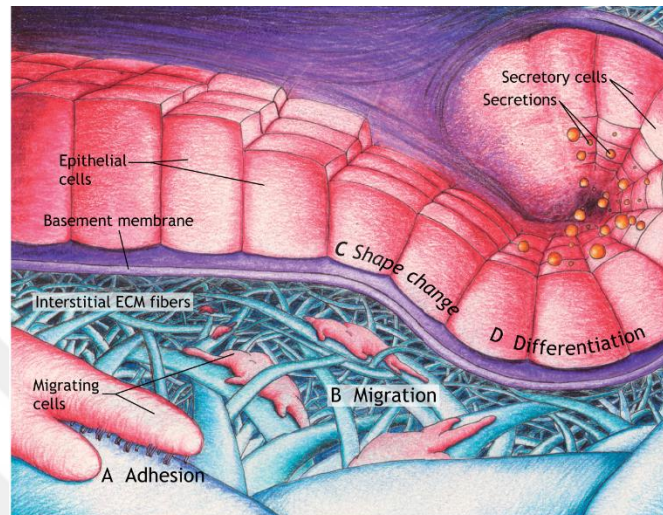


**Figure 1.5.** A summary of the regulation of developmental processes by the extracellular matrix. (Walma & Yamada, 2020)

The ECM primarily consists of basement membranes and interstitial matrices. These ECM types play crucial roles in guiding cell and tissue morphology during developmental morphogenesis by affecting processes such as cell adhesion (A), migration (B), morphology (C), and differentiation (D). (A) Integrins mediate cell-ECM adhesion through cell adhesion complexes that link cells to fibril substrates. (B) Migrating cells use directed protrusions and adhesion complexes to traverse interstitial ECM fibers. (C) Changes in epithelial cell shape, including transitions from columnar to cuboidal forms. (D) The differentiation of epithelial cells into secretory cell types (Walma & Yamada, 2020).

The space outside neurons and glial cells is made up of the extracellular matrix (Zimmermann & Dours-Zimmermann, 2008). During the last phase of neuronal circuit maturation, a distinct and densely packed form of ECM develops. These extracellular macromolecular clusters, known as perineuronal networks, create intricate network-like structures surrounding specific groups of neurons (Yamaguchi, 2000). In addition to being synthesized by glial cells and neurons, which secrete ECM components into the extracellular space, ECM levels are also controlled locally through proteolytic cleavage by proteases. The composition and stability of the ECM play a crucial role in determining neurotransmission

rates and neural plasticity (Gundelfinger et al., 2010). In the neurobiological foundation of psychiatric disorders, processes related to neurodevelopment and neural plasticity—such as cell migration, synaptogenesis, synaptic pruning, and long-term potentiation (LTP)—seem to be disrupted (Bambico & Belzung, 2013; Russo et al., 2010; Woo, 2014). It is believed that abnormalities in ECM composition contribute to the development of psychiatric disorders. ECM-mediated neural mechanisms, influenced by environmental factors and specific gene variants within the ECM, play a role in the pathology of these disorders (Lubbers et al., 2014).

The concept of biphasic dysregulation of the local balance between extracellular proteases and the ECM has emerged in the context of psychiatric diseases. Initially, the ECM is degraded due to elevated expression and activity of proteases such as *MMPs*. In the chronic phase, there is increased expression of ECM molecules or heightened expression/activity of tissue inhibitors of metalloproteinases (*TIMPs*). While the initial downregulation of ECM creates a window of pathophysiological plasticity to remodel neural networks, the subsequent upregulation of ECM during the chronic phase impairs neuroplasticity and contributes to the establishment of the diseased state. The expression of various *MMPs* in the nervous system alters in response to injury and neurological diseases (Yong et al., 2001). One of the most important factors regulating the activity of *MMPs* is how long the enzymes are present on or near the cell surface in active or inactive states (Ethell & Ethell, 2007).

#### **1.4.1. Cellular and extracellular effects of ECM mutations**

The body's primary connective tissues, including skin, tendons, ligaments, cartilage, and bone, form the structural and informational foundation essential for development. The extensive extracellular matrix within these tissues is a complex, interactive network of proteins, glycoproteins, and proteoglycans, creating a dynamic and crucial three-dimensional environment that supports cell maintenance, growth, and differentiation. Beyond providing an organized framework for cells, the extracellular matrix mediates signals to and from cells, playing a role in cell differentiation, migration, and repair processes during development. The extracellular matrix diversity and functional importance are evident from the emergence of numerous genetic or acquired connective tissue disorders. Mutations in individual extracellular matrix genes result in conditions such as osteogenesis imperfecta (OI), multiple chondrodysplasias, Ehlers-Danlos syndrome, and Marfan syndrome. Although these conditions are individually rare, they collectively represent a significant health burden (Bateman et al., 2009). The extracellular matrix is highly diverse,

consisting of precisely arranged molecular components that confer tissue-specific properties. These range from the rigid structure of bone to the elasticity of ligaments and skin, and from the durability of adult articular cartilage to the temporary nature of growth plate cartilage (Kadler et al., 2007). Extracellular matrix components can be generally categorized into several groups: structural components; matricellular proteins that modulate cell-ECM interactions, growth factor, and protease activity; cell surface receptors and accessory proteins that interact with the extracellular matrix, serving signaling and structural roles; and proteins that maintain extracellular matrix homeostasis and remodeling, such as proteinases and their inhibitors. Major ECM components include collagens, proteoglycans, and various non-collagenous glycoproteins and proteins (Iozzo, 1999; Svensson et al., 2001). A crucial first step in facilitating the multivalent extracellular contacts that generate the architecturally accurate matrix required for appropriate function is the formation of multimers within the cell by a number of ECM structural components. Cells are integrated into functional assemblies through the interaction of the extracellular matrix, which offers bidirectional pathways for mechanotransduction and signaling (Lamoureux et al., 2007; Whitelock et al., 2008).

#### **1.4.2. Molecular Pathology of ECM gene mutations**

Numerous alterations in the ECM's structural elements and the enzymes responsible for their folding and post-translational processing have been identified. The nature of the mutation, tissue distribution, and the function of the gene product determine the molecular basis by which these mutations cause a variety of connective tissue illnesses (Aszódi et al., 2006).

##### **1.4.2.1. Loss-of-function mutations**

Mutations resulting in the insertion of premature termination codons (PTCs) are the most common genetic causes of reduced synthesis of gene products. The presence of a PTC triggers mRNA surveillance and nonsense-mediated decay (NMD), leading to the rapid degradation of abnormal mRNAs, separating them from normal mRNAs (Frischmeyer & Dietz, 1999; Schell et al., 2002). Perineuronal networks (PNNs) and the extracellular matrix that form or interact with PNNs are known to play a role in the pathophysiology of various psychiatric disorders. Research on schizophrenia, autism spectrum disorders, mood disorders, Alzheimer's disease, and epilepsy indicates the involvement of ECM molecules such as chondroitin sulfate proteoglycans, Reelin, and matrix metalloproteases, along with

their cell surface receptors (Berretta, 2012; Folsom & Fatemi, 2013). The perspective that psychiatric disorders are purely neuronal has been challenged in recent years by evidence indicating that the extracellular matrix, glial cells, and their interactions also play significant roles (Yamamuro et al., 2015). Synaptic regulation is just one of the several crucial functions performed by the ECM during both pre- and postnatal brain development, as well as throughout adulthood (Berretta et al., 2015; Kato et al., 2013). Thus, beyond synaptic dysregulation, abnormalities in brain ECM can have complex and extensive consequences. These can include disruptions in axonal guidance, neuronal differentiation, and migration during early brain development, as well as issues with circuit consolidation and the closure of critical periods in postnatal development. In the adult brain, ECM abnormalities can affect axonal signaling and the regulation of the blood-brain barrier (Pantazopoulos & Berretta, 2016; Williams & Umemori, 2014).

#### **1.4.3. ECM pathology in mood disorders**

Mood disorders, encompassing bipolar disorder and unipolar depression, are a category of psychiatric conditions marked by persistently fluctuating emotional states. The involvement of ECM molecules and the presence of synaptic pathology in these disorders have been extensively documented (Guidotti et al., 2000; Pantazopoulos et al., 2015). For instance, studies have reported reduced Reelin expression in the blood, prefrontal cortex, hippocampus, and cerebellum of individuals with bipolar disorder or unipolar depression (Fatemi et al., 2001). Postmortem studies in bipolar disorder have revealed significant reductions in CS-6(3B3)-immunolabeled perineuronal nets in various nuclei within the amygdala. Additionally, more moderate reductions in aggrecan-immunolabeled PNNs were observed specifically in the accessory basal nucleus of the amygdala (Pantazopoulos et al., 2015). Similarly to findings in schizophrenia and autism, increased levels of *MMP-9* have been reported in blood samples from individuals with unipolar depression and young individuals with bipolar disorder experiencing a depressive state (Rybakowski et al., 2013). In bipolar disorder, genome-wide association studies have identified a genetic variant of NCAN, which encodes the chondroitin sulfate proteoglycan neuronal 1 (CSPG1), as a risk factor for this disorder (Cichon et al., 2011). Consistently, variants of the NCAN gene have been associated with manic symptoms in human subjects, and studies involving NCAN knockout mice have shown that these animals exhibit manic-like behavior (Miró et al., 2012). Interestingly, some of the most effective treatments for mood disorders affect ECM molecules and PNN composition. For instance, chronic treatment with the selective

serotonin reuptake inhibitor fluoxetine, which is effective in treating depression and anxiety, leads to a reduction in the number of WFA-labeled perineuronal nets (PNNs) in the hippocampus and medial prefrontal cortex of mice. This is accompanied by markers of immature neurons and an increase in dendritic spine density in interneurons (Ohira et al., 2013). Lithium, one of the most effective treatments for bipolar disorder, has been shown to contribute to the digestion of CSPG (chondroitin sulfate proteoglycan), which is a component of the extracellular matrix. This digestion process may play a role in lithium's therapeutic effects in bipolar disorder (Frederick et al., 2008). Consistently, the number of glial clusters labeled with a CS-6-specific antibody (CS56), which has been observed to be reduced in the amygdala of individuals with bipolar disorder, was positively correlated with lifetime lithium exposure. It has been found that chronic lithium exposure does not affect CSPG sulfation patterns in bipolar disorder. These findings suggest a potential therapeutic effect of lithium related to the maintenance or modulation of glial clusters in the amygdala (Irwin et al., 2000; Pantazopoulos & Berretta, 2016).

#### **1.4.3.1.Schizophrenia**

Schizophrenia is a chronic and severe brain disorder characterized by psychotic symptoms and disturbances in emotions and behavior. Increasing evidence suggests that abnormalities in the extracellular matrix are integral to the underlying pathophysiology of schizophrenia. Recent findings from human genetic and postmortem studies have implicated genes encoding several crucial ECM molecules, including chondroitin sulfate proteoglycans (CSPGs), Reelin, semaphorin 3A, integrins, and remodeling enzymes. These studies also highlight dysregulated expression of these molecules in glial cells and disruption of organized ECM structures such as perineuronal nets. These abnormalities underscore genetic vulnerabilities and molecular dysregulations within the ECM that contribute to the pathogenesis of schizophrenia (Cabungcal et al., 2013; Takahashi et al., 2011).

#### **1.4.3.2.Autism spectrum disorders**

There is substantial evidence implicating ECM abnormalities in autism spectrum disorders (ASD), a diverse group of neurodevelopmental conditions characterized by persistent deficits in social communication and interaction, along with restricted and repetitive behaviors. Genetic studies have identified several ECM-related molecules as potential contributors to the etiology of autism. Analysis of multiple GWAS on autism has highlighted genes such as the ECM remodeling enzymes ADAMTS3, ADAMTS5, and

ADAMTS14, ECM molecules like RELN, SEM3A, SEM4D, the hyaluronan surface receptor CD44, and OTX2, a transcription factor involved in PNN formation. These findings underscore the involvement of ECM components and PNNs in the regulatory mechanisms relevant to ASD (Spatazza et al., 2013; Weiss et al., 2009). The most compelling evidence implicating the ECM in the pathophysiology of autism comes from research focused on Reelin. Multiple GWAS and several association studies investigating Reelin's role in autism have consistently suggested that Reelin is a susceptibility gene for autism. Additionally, meta-analyses of these studies further support the association of Reelin with autism spectrum disorder. This underscores the significant role of Reelin and potentially other ECM-related factors in the vulnerability to autism (Persico et al., 2001; Poelmans et al., 2013).

#### **1.4.3.3.Fragile-X syndrome**

Fragile X syndrome is a monogenic disorder characterized by inherited intellectual disability, often accompanied by prominent autistic symptoms (Gillberg and Billstedt, 2000). The role of *MMP-9* in the pathophysiology of Fragile X syndrome illustrates the interactions between ECM molecules and synaptic pathology in psychiatric disorders. Fragile X syndrome results from transcriptional silencing of the *Fmr1* gene, which encodes the mRNA-binding protein Fragile X Mental Retardation Protein (FMRP) (O'Donnell & Warren, 2002). FMRP regulates mRNAs that encode various proteins involved in both pre- and postsynaptic functions, including scaffolding proteins, neurotransmitter receptors, and signaling molecules, in an activity-dependent manner (Antar et al., 2004; Darnell et al., 2011). Decreased expression of FMRP leads to increased protein synthesis at the synapse, loss of regulation by neuronal activity, heightened neuronal excitability, and abnormal spine morphology characterized by immaturity (Irwin et al., 2000; Rudelli et al., 1985). Based on available data, it appears that FMRP interacts with matrix metalloproteinase-9 which is known to have an activity-dependent role in dendritic spine remodeling (Dityatev et al., 2010; Michaluk et al., 2007), may be critical for these synaptic abnormalities. According to recent research, FMRP controls the translation and transport of *MMP-9* mRNA within the synapse; higher levels of FMRP translate into higher levels of *MMP-9* (Janusz et al., 2013).

### 1.5. Matrix Metalloproteinase

Matrix metalloproteinases (*MMPs*) form a significant group of zinc-dependent endopeptidases that can break down protein components of the extracellular matrix, cell adhesion molecules, cytokines, and growth factors (Rybakowski et al., 2013). *MMPs* are also capable of activating or inactivating various signaling molecules, such as adhesion molecules, receptors, and growth factors (Li et al., 2022). The *MMP* family consists of 21 enzymes, subdivided according to their structure and categorized according to enzyme groups. These include collagenases, stromelysins, gelatinases, and membrane-type metalloproteinases. *MMPs* degrade a diverse array of extracellular matrix proteins and are constitutively expressed in various cell types and tissues. *MMPs* typically exist in an inactive form and must be activated to function. The active and inactive states of *MMPs* are regulated by endogenous inhibitors, known as tissue inhibitors of *MMPs*, and the endogenous stimulator, extracellular matrix metalloproteinase inducer (EMPRIN) (Bajor & Kaczmarek, 2013; Muir et al., 2002; Porter et al., 2005). Furthermore, *MMPs* can be activated by plasmin to break down various extracellular matrix components. Reactive oxygen species (ROS) have the ability to activate *MMPs* or induce the translation of their mRNA through NF- $\kappa$ B signaling. *MMPs* that are activated are involved in numerous processes, including angiogenesis, signaling, cell motility, and survival (Egeblad & Werb, 2002; Lattanzi et al., 2020). By altering death molecules, rupturing myelin, and sustaining neuroinflammation, it can harm brain cells (Lattanzi et al., 2020; Yong et al., 2001). *MMPs* are synthesized as proenzymes. One of the activation pathways involves the action of transmembrane *MMPs*, which require tissue inhibitors of *MMPs* for activation. *TIMPs* (*TIMP-1*, -2, -3, -4) are endogenous proteins that inhibit *MMPs*. In pathological conditions with increased *MMP* activity, *TIMP* levels, which are directly responsible for *MMP* activation, decrease. *TIMPs* inhibit all known *MMPs* and also have other functions: promoting cell growth (*TIMP-1*, -2), inhibiting the growth of endothelial cells activated by fibroblast growth factors (*TIMP-2*), stabilizing the structure of cell receptors for pro-apoptosis and TNF- $\alpha$  (*TIMP-3*), and having anti-apoptotic effects (*TIMP-1*, -2).

*MMPs* are secreted by various connective tissue and pro-inflammatory cells, including fibroblasts, osteoblasts, endothelial cells, macrophages, neutrophils, and lymphocytes. Under normal physiological conditions, the proteolytic activity of *MMPs* is mainly regulated through three known steps: transcription, activation of zymogens, and inhibition of active forms by various *TIMPs* (Ross, 1996). *MMPs* are secreted as latent

enzymes and need to be activated. They are made as zymogens that, when activated, require the removal of a pro-peptide region and signal sequence. Tight regulation of their proteolysis guards against tissue injury (Pietersen et al., 2014).

*MMP-9* is one of the most well-characterized *MMPs* in the central nervous system. It is expressed by neurons and glial cells and is released in response to increased neuronal activity under both physiological and pathological conditions (Chaillan et al., 2006). It is involved in synaptic pruning, myelin development, consolidation of long-term potentiation, pre- and post-synaptic receptor dynamics, and dendritic spine remodeling and stabilization (Michaluk et al., 2011). Additionally, *MMP-9* is involved in axon regeneration, pathfinding, and sprouting (Magnowska et al., 2016; Reinhard et al., 2015). *MMP-9* expression is increased in many cells, including endothelial cells and neutrophils, in some brain injury conditions (Romanic et al., 1998). Its induction is brief, with activity observed within minutes of neuronal stimulation. This activity fades within the next 10–15 minutes due to the influence of endogenous inhibitors like *TIMP-1* (Michaluk et al., 2007). At the subcellular level in neurons, *MMP-9* is located in dendritic spines, where most of the brain's excitatory synapses are found. Notably, *MMP-9* mRNA is locally translated in dendrites and synapses in response to neuronal stimulation by glutamate, a process regulated by FMRP (Wilczynski et al., 2008). Specifically, the *MMP-9* gene is found in chromosome region 20q11–13, which has been the subject of much research on psychiatric illnesses and linked to schizophrenia (St-Jean, 2009). Additionally, certain potential proteins linked to schizophrenia have functional relationships with *MMP-9* or *MMP-9*-interacting proteins, including N-methyl-D-aspartate (NMDA) receptors and brain-derived neurotrophic factor (Gurling et al., 2001; Purcell et al., 2009). While the impact of *MMPs* on neurological disorders are well established, little is known regarding the part *MMPs* play in depression (Yong et al., 2001). Recent research has conclusively shown that *MMP-9* and the symptomatology of depression are positively correlated (Beroun et al., 2019; Vafadari et al., 2016).

#### **1.5.1. Neural function of *MMP***

*MMPs* can be secreted or membrane-bound, and they both contribute to pericellular proteolysis (Baker et al., 2002). Through the remodeling or proteolytic destruction of extracellular matrix proteins, *MMPs* impact the migration, proliferation, survival, and differentiation of cells while concurrently activating cell surface receptor ligands. Because of all these benefits, *MMPs* play crucial roles in the restoration and healing of wounds

(Watanabe & Ikeda, 2004). In the central nervous system, the ECM is composed of three primary components: the interstitial matrix that connects nerve cells, perineuronal networks that surround certain groups of neurons, and the basement membrane that lines blood vessels (Lau et al., 2013). Through the breakdown of extracellular matrix and basement membrane components, *MMPs* can assess the integrity of the basement membrane and, consequently, the blood brain barrier (BBB) (Ulbrich et al., 2021). An indication of brain trauma or stress injury is disruption of the BBB, which increases the permeability of injured endothelial cells and allows inflammatory chemicals to enter the brain. Bipolar depression patients' brain abnormalities and cognitive deterioration in both white and gray matter are hypothesized to be influenced by increased neuroinflammation (Patel & Frey, 2015). A physical structure known as the perineuronal networks envelops the cell soma and proximal processes of many neurons, particularly parvalbumin-expressing GABAergic neurons (Deepa et al., 2006; Soleman et al., 2013). The perineuronal network is assumed to be the storage location for many long-term memories, including fear conditioning. Neurological dysfunctions like epilepsy, Alzheimer's disease, and stroke are brought on by abnormal perineuronal signaling (Miao et al., 2014).

Interestingly, extracellular matrix components in perineuronal networks are targeted by some of the most successful treatments for mood disorders (Pantazopoulos & Berretta, 2016). Given that *MMP-9* activity was co-localized with synaptic markers and perineuronal networks in mice between 4 and 8 weeks postnatally, it was determined that *MMP-9* influences cerebellar synaptic plasticity (Stamenkovic et al., 2017). In mice with a persistent stress disorder brought on by social defeat, the perineuronal net density surrounding parvalbumin-expressing hippocampal interneurons was enhanced; this was eliminated after three weeks of antidepressant treatment (Riga et al., 2017). It is yet unclear, though, if *MMP-9* activity plays a role in the pathophysiology of depression by altering the perineuronal network. Numerous studies have demonstrated the involvement of *MMPs* in demyelination; demyelination has also been linked to neuropsychiatric conditions like autism and depression (Li et al., 2022). Matrix Metalloproteinase-9, also termed metzincin, is a member of the matrix metalloproteinase family (84.92 kDa), a protease that primarily functions outside of cells to participate in a variety of physiological and pathological events. It is also referred to as gelatinase B or type IV collagenase. It is a member of a broad family of enzymes. The *MMP-9* gene has 12 introns and 13 exons, and it is found on chromosome 20. *MMP-9* belongs to a family of extracellular and extrasynaptically active proteases that play a critical role in the plasticity of excitatory synapses and are notably activated in response to

environmental movements. It is therefore believed to be beneficial in the genesis of numerous mental diseases. Studies on high *MMP-9* levels in schizophrenia abound, yet there is a dearth of information about bipolar disorder. Nonetheless, prior research has indicated that the *MMP-9* gene polymorphism raises the likelihood of bipolar disorder (Hannocks et al., 2019; Vandenbroucke & Libert, 2014). Certain inhibitory proteins, such as *TIMP-1*, a tissue inhibitor of metalloproteinase-1 that actively inhibits *MMP-9*, can suppress *MMPs* within minutes of their enzymatic activity. This method refers to the modulation of *MMP* as a *hic et nunc* event. Local protein inhibitors like *TIMP-1* prevent synaptically released *MMP-9* from carrying out tightly controlled enzymatic activities within a restricted time frame in response to physiological circumstances. This scenario states that *MMP-9*'s primary function is to transform small spines into long, filopodia-like spines, which, when *MMP-9* is inhibited, take on the structure of a mature mushroom (Chaturvedi & Kaczmarek, 2014).

At first, it was believed that *MMP-9* primarily functioned in the breakdown of extracellular matrix components. According to this viewpoint, *MMP-9* has been linked to a number of significant developmental processes in humans, including osteoarthritis and tumor metastasis. *MMP-9* was dubbed a regulator and enhancer of immune functions (Opdenakker et al., 2001). Sorokin and colleagues (Hannocks et al., 2019) after looking at how *MMP-9* affected neuroinflammation, researchers found that while gelatinases can break down specific ECM components, this is not how they work primarily when neuroinflammation is present. Gelatinases instead primarily function by increasing the effectiveness of inflammatory process events, such as chemokine activation, inactivation/elimination, and adjustment of local proinflammatory cytokine levels (Opdenakker et al., 2022). The disruption of the blood-brain barrier integrity caused by *MMP-9* is another significant function of the protein in the brain. This leads to barrier leakage, which in turn causes inflammatory molecule infiltration, immune cell extravasation, and brain penetration (Rempe et al., 2016). All of these findings point to the possibility that different brain cell types, such as neurons and astro-hypertrophic cells, can manufacture *MMP-9*. Nothing in-depth has been studied regarding *MMP-9*'s function in neural plasticity thus far. However, in the light of the data obtained, it has been observed that *MMP-9* is released depending on the activity occurring in the postsynaptic compartment of excitatory synapses. After activation, *MMP-9* degrades synaptic CAMs (cell adhesion molecules) and modifies the synapse's NMDA and AMPAR mobility and function. Additionally, pro-BDNF is changed into mBDNF by *MMP-9*. By applying an electrical or chemical stimulus, long-term potentiation (LTP)-induced plasticity was shown to produce an extension of the

dendritic spine that is dependent on *MMP-9*, an accumulation of AMPAR at the synapse, and an increase in the number of spikes in neurons. The preservation of late phase, NMDA-dependent LTP in different brain areas depends on *MMP-9*'s transitory function (Kaczmarek et al., 2023). Research on experimental animals has shown that elevated *MMP-9* levels in different learning paradigms would have deleterious effects on memory maintenance. Furthermore, it should be mentioned that high *MMP-9* activity interferes with normal memory, synaptic plasticity, and learning.

Despite having all of these properties concentrated in the hippocampus, *MMP-9* is also present in the cortical, striatal, and amygdala, where it contributes to synaptic plasticity. The technique of controlling *MMP-9* expression is quite intricate. The nuclear factor- $\kappa$ B (NF- $\kappa$ B) and activator protein-1 (AP-1) are two transcription factors with binding sites in the *MMP-9* gene promoter region. Both transcription factors must be activated for *MMP-9* production to occur (Rosenberg, 2002; Vandooren et al., 2013; Wang et al., 2013). According to clinical research, patients with unipolar depression have reduced cortical synaptic LTP-like plasticity, which increases during remission. It has been suggested that long-term structural remodeling of synaptic connections is what causes LTP. Persistent LTP is linked to the development of new dendritic spines, an increase in density or the creation of numerous synaptic collars, and the enlargement of spine heads (Kuhn et al., 2016). It has been demonstrated that activated *MMP-9* localizes to synapses and uses integrin receptors to modify hippocampal synapses. In this case, *MMP-9*-mediated augmentation of synaptic signal intensity is prevented by blocking integrin activity (Nagy et al., 2006; Wiera & Mozrzymas, 2021). These results strongly imply a potential relationship between depression and *MMP-9*-associated long-term potentiation (Figure 1.6.).

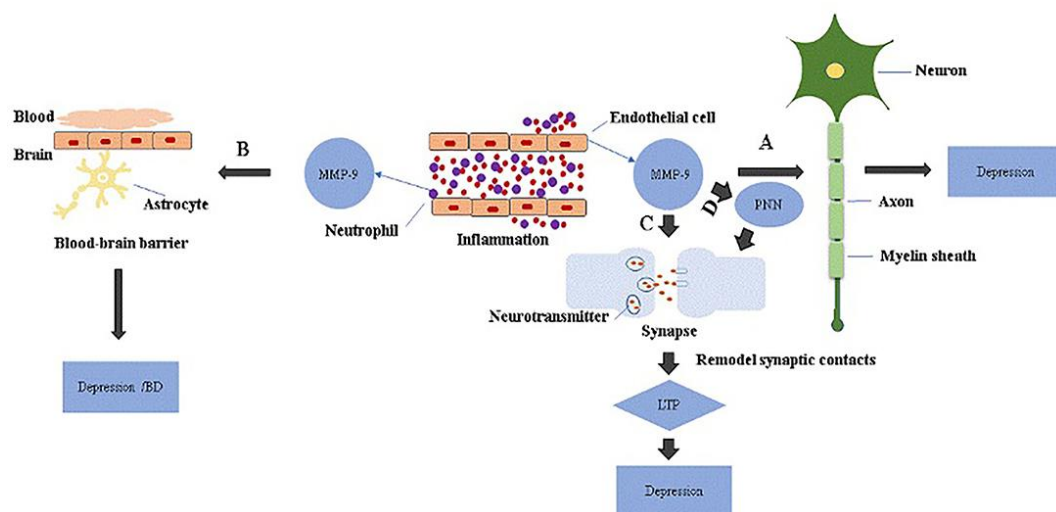


**Figure 1.6.** The role of *MMP-9* in depression. (Li et al., 2022)

During inflammation, endothelial cells and neutrophils have higher levels of *MMP-9*. Proof that overexpression of *MMP-9* is useful in treating depression-related demyelination. The BBB is disturbed by *MMP-9*, which raises neuroinflammation and may lead to cognitive impairment and depression or bipolar illness. Activated *MMP-9* partially localizes to synapses and contributes to the reduction of cortical synaptic-like plasticity and synaptic pruning, which are necessary for long-term potentiation. It is believed that these illnesses are all related to depression. The perineuronal networks that restrict synaptic plasticity are reconstituted by *MMP-9*. Atypical perineuronal net signaling can lead to depression (Li et al., 2022).

### **1.5.2. *MMP-9* gene polymorphisms and mental illness**

Functional gene polymorphisms in *MMP-9* alter the protein's concentration and activity, and these variations have a significant impact on the gene's level of expression. The *MMP-9* gene has three main polymorphisms in its untranslated, coding, and promoter regions. It is found on chromosome 20q12.2-13.1. The T-1562 allele has a stronger promoter activity in driving gene expression than the C-1562 allele due to the C-1562T (rs 3918242) polymorphism in the promoter region (Buraczynska et al., 2015).

The T allele of the -1562 C/T polymorphism in *MMP-9* was associated with the C allele in patients with bipolar mood disorder compared to healthy control subjects, according to Rybakowski et al.'s analysis of the functional-1562 C/T polymorphism genotype in a cohort of patients with bipolar disorder and healthy controls. It was discovered to have a greater dominance over (Rybakowski, 2009). In a different study, Bobińska et al. found that the T-1702A polymorphism for *MMP-9* raised the risk of recurrent depression for the T allele and T/T genotype, but decreased this risk for the A allele and A/A genotype. The study involved depression patients and healthy controls. The *MMP-9* gene is a pathogenic tool in patients with bipolar illness and depression, according to all of the available data (Bobińska et al., 2016). *MMP-9* has been linked many times with evidence obtained from both animal models and humans and from materials collected from them to investigate mental states and their effects on them (Beroun et al., 2019).

A quick summary of these data by illness is provided below, followed by a description of the human data that implicate *MMP-9*. Specifically, there is a wealth of information regarding *MMP-9* single nucleotide gene polymorphisms (SNPs) and blood (plasma or serum) protein levels. Additionally, it has been shown that the peripheral *MMP-9* level does not express itself in the same way as the brain. It is impossible to rule out the

possibility that, for instance, increased blood levels of *MMP-9* could indicate brain inflammation; as a result, there will undoubtedly be a correlation between brain and blood *MMP-9* levels (Pabian-Jewuła & Rylski, 2023).

#### **1.5.2.1.Schizophrenia**

Studies on the *MMP-9* protein level in people with schizophrenia revealed that the patients' levels were higher than those of healthy people (Beroun et al., 2019; Schoretsanitis et al., 2021). Additionally, it is known that in patients' peripheral blood cells, *MMP-9* is increased at the mRNA level (Kumarasinghe et al., 2013). Furthermore, Shibasaki et al. observed that following electroconvulsive therapy, blood levels of *MMP-9* in persons with schizophrenia considerably dropped. The *MMP-9* gene's polymorphisms and altered epigenetic regulation have also been linked to schizophrenia and have been demonstrated to have an impact on the pathophysiology of the illness in this regard (Beroun et al., 2019; Shibasaki et al., 2016).

#### **1.5.2.2.Bipolar disorder**

Serum *MMP-9* levels are frequently elevated in bipolar disorder patients. Lithium and other mood stabilizers are frequently used to treat bipolar disorder. GSK-3 is one of the enzymes that lithium salts are known to inhibit; when GSK-3 interacts with *MMP-9*, dendritic spines are altered (Parikh et al., 2016). Studies on the *MMP-9* gene polymorphism in patients' cognitive impairment and lithium responses have provided evidence linking the disease to bipolar disorder (Beroun et al., 2019; Reininghaus et al., 2016; Rybakowski et al., 2013).

#### **1.5.3. *MMP-9* as a Biomarker for Depression**

Studies have reported that there are changes in *MMP-9* expression level as well as gene polymorphisms in patients with depression (Table 1.5.1).

**Table 1.5.1.** *MMP-9* as a potential biomarker for depression- results from clinical studies. (Li et al., 2022).

| References              | Study Design                                                                                                    | Findings                                                                                                                                                                                                                                                                   |
|-------------------------|-----------------------------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| (Domenici et al., 2010) | Plasma levels of <i>MMP-9</i>                                                                                   | <i>MMP-9</i> was more in depressed individuals than in the control group.                                                                                                                                                                                                  |
| (Yoshida et al., 2012)  | Serum levels of <i>MMP-9</i>                                                                                    | Serum <i>MMP-9</i> levels are correlated with depression severity                                                                                                                                                                                                          |
| (Chiarani et al., 2013) | PCR to measure the blood cells' <i>MMP</i> mRNA levels                                                          | Blood <i>MMP-9</i> and <i>MMP-2</i> expressions did not differ between bipolar disorder patients and the control group.                                                                                                                                                    |
| (Bobińska et al., 2016) | Blood's <i>MMP-9</i> gene expression at the mRNA level                                                          | Patients with depression had higher levels of <i>MMP-9</i> mRNA than the control group.                                                                                                                                                                                    |
| (Bobińska et al., 2016) | Expression of the <i>MMP-2</i> , <i>MMP-9</i> , and <i>TIMP-2</i> genes in blood at the protein and mRNA levels | Compared to the control group, depression patients had reduced levels of <i>MMP-9</i> , <i>MMP-2</i> , and <i>TIMP-2</i> expression on both a protein and mRNA level. Increased <i>MMP-9</i> , <i>MMP-2</i> , and <i>TIMP-2</i> expression enhances cognitive performance. |

In contrast to the aforementioned research and data, certain investigations have not discovered a connection between depression and the *MMP-9* level. Real-time quantitative polymerase chain reaction was used to measure the *MMP* level in the blood of 20 bipolar disorder patients and 20 healthy controls in a study by Chiarani et al. There was no discernible difference between the bipolar disorder patients' and the control group's *MMP-9* expression pattern (Chiarani et al., 2013).

## 1.6. Tissue Inhibitors of Metalloproteinases (*TIMPs*)

Tissue inhibitor of matrix metalloproteinases-1, is a 28.5 kDa glycoprotein that was originally isolated from rabbits. All activated collagenases are inhibited by it. *MMP* types other than *Mt-1-MMP* and *MMP-2* are inhibited by *TIMP-1* (Nelson et al., 2000). Numerous triggers, including phorbol esters, interleukin-1, platelet-derived growth factor, and fibroblast growth factor, enhance the expression of *TIMP-1*. Pro-*MMP-9*, a member of the gelatinase class, binds to *TIMP-1*, forming the pro*MMP-9/TIMP-1/MMP* complex (Curran

& Murray, 1999). For multicellular animals to organize their cells and tissues and to create an environment that is conducive to cellular processes, the various components of the extracellular matrix must be assembled correctly. Changes in the composition of the extracellular matrix (ECM) are crucial for processes like morphogenesis, tissue remodeling, and repair during embryonic development. The primary enzymes involved in ECM breakdown include membrane-type *MMPs* and ADAMTS metalloproteinases (a disintegrin and metalloproteinase with thrombospondin motifs), which consist of collagenases, gelatinases, stromelysins, matrilysins, and other related proteinases (Murphy & Nagase, 2008; Porter et al., 2005). Diseases such as arthritis, cardiovascular conditions, cancer, lung disease, nephritis, neurological disorders, and tissue ulceration are associated with uncontrolled ECM turnover, inflammation, cell proliferation, and migration, often triggered by an imbalance between the production and inhibition of active enzymes. *TIMPs*, the natural inhibitors of these metalloproteinases, play a key role in regulating extracellular matrix turnover, tissue remodeling, and cellular processes (Brew & Nagase, 2010b).

*TIMP-1* was shown to have collagenase inhibitory action when it was combined with human serum, bovine cartilage, and cultured human skin fibroblasts (Bauer et al., 1975; Kuettnner et al., 1976; Woolley et al., 1975). Because they inhibit gelatinase and proteoglycans (*MMP-3*) in addition to collagenase, they are known as tissue inhibitor of metalloproteinases, or *TIMP* (Cawston et al., 1981). There are four paralogous *TIMP* genes (*TIMP-1* to -4) in the human genome. With their inhibitor-protease dual affinities with *MMPs*, all *TIMPs* in the human genome can inhibit all *MMPs*. *TIMP-3*, which has the broadest range of inhibition, can also inhibit several variants of the ADAM and ADAMTS families (Nagase & Murphy, 2008). It is distinct from others in addition to being firmly attached to the extracellular matrix. It possesses several biological activities, including pro- and anti-apoptotic and synaptic plasticity activities. In addition to their metalloproteinase activities, *TIMPs* also have pro- and anti-apoptotic effects and on synaptic plasticity. The majority of these activities are unaffected by metalloproteinase inhibition (Pavloff et al., 1992).

Tissue *MMP* inhibitors, or *TIMPs*, endogenously suppress *MMPs*. *TIMPs* are crucial modulators of *MMP* function. In humans, four different kinds of *TIMPs* have been seen. The 184–194 amino acids that make up the human *TIMPs* comprise an N-domain and a C-subdomain that are supported by six disulfide links. Of these, hemopexin of the *MMP-9* and *MMP-2* proenzymes is bound by the C-terminal domains of *TIMP-1* and *TIMP-2*, respectively (Shibasaki et al., 2016). This implies that the primary inhibitors of *MMP-9* and

*MMP-2* on an individual basis are *TIMP-1* and *TIMP-2*. *TIMPs* share overlapping properties and are around 40% equivalent in their ability to inhibit individual *MMPs*. *MMP* activity is inhibited when *TIMPs* bind to their particular *MMPs*. The inhibitory domain of *MMPs* is an amino-terminal region, which binds to the active site (Goldberg et al., 1992).

#### **1.6.1. Genes and protein structures of *TIMPs***

The structure, activity, and biological role of the four *TIMPs* encoded by the human genome have been most thoroughly studied. Although *TIMPs* have several homologs in both vertebrates and invertebrates, little is known about *TIMPs* in animals other than mammals (Stetefeld et al., 2001). Every mammalian *TIMP* has two unique domains. Three disulfide bonds within each domain stabilize it: the N-terminal domain has around 125 amino acid sequences, whereas the C-terminal domain has about 65 amino acid sequences (Lambert et al., 2004; Williamson et al., 1990). The sequence similarity between human *TIMPs* is about 40%; *TIMP-2* and *TIMP-4* have a 50% similarity rate. Just 37–41% of *TIMP-1* is the same as other *TIMPs*. N-terminal domains of *TIMPs* are stable native structures that are completely powerful inhibitors of matrix metalloproteinases and certain disintegrin-metalloproteinases (ADAMs and ADAMTSs). These forms of the N-terminal domains of *TIMPs* are expressed in heterologous systems (Huang et al., 1996; Murphy et al., 1991).

#### **1.6.2. Functional specialization for metalloproteinase inhibition**

Although *TIMPs* are highly similar to one another, they can either bind to extracellular matrix components (*TIMP-3*) or be released outside the cell in soluble form (*TIMP-1*, *TIMP-2*, and *TIMP-4*). It has been demonstrated that *TIMPs* bind highly selectively to the pro-enzyme forms of *MMP-9* and *MMP-2*. By inhibiting activation, this interaction adds another degree of control. Pro-*MMP-9* is bound by *TIMP-1*, whereas pro-*MMP-2* is bound by *TIMP-2*. All of the *MMPs* that were examined were inhibited by *TIMP-1*, *TIMP-2*, *TIMP-3*, and *TIMP-4*, however *TIMP-1* only slightly inhibits MT1-*MMP*, MT3-*MMP*, MT5-*MMP*, and *MMP-19* (Birkedal-Hansen et al., 1993). Under normal physiological conditions, *MMP* activity is meticulously regulated at four distinct levels: transcription, zymogen precursor activation, interaction with certain ECM components, and inhibition by *TIMPs*. Compared to the other three *TIMPs*, *TIMP-1*, which is secreted by most body cells, has a narrower inhibitory range. It inhibits all forms of *MMP*, with the exception of *MMP-14*, *MMP-16*, *MMP-18*, *MMP-19*, MT1-*MMP*, and MT2-*MMP*, mostly by binding strongly to *MMP-9* and pro-*MMP-9* (Vandenbroucke & Libert, 2014). *TIMP-4* is expressed

in the heart, ovaries, kidneys, pancreas, colon, testes, brain, and adipose tissue, whereas *TIMP-3* is expressed as a matrix protein in the tissues and basement membranes of the eyes and kidneys. *TIMPs* exhibit constitutively or inducibly specific expression in some tissues, and their transcriptional level is controlled by growth factors, chemokines, and cytokines (Figure 1.7) (Brew & Nagase, 2010b). The physiological processes of neurogenesis, oligodendrogenesis, and brain plasticity are mediated by *MMPs* and *TIMPs*. *MMPs* have crucial roles in the formation and development of the central nervous system (CNS). The degeneration of neurons in various nervous system regions and the consequent decrease of function are the hallmarks of neurodegenerative disorders (Martino et al., 2015). *MMP-2* and *MMP-9* are produced by microglia, astrocytes, and neurons in the central nervous system, playing a pathological role in blood-brain barrier disruption by activating microglia and facilitating the infiltration of inflammatory cells. One potential therapeutic approach for the treatment of neurodegenerative illnesses has been the inhibition of *MMPs* (Brkic et al., 2015; Cabral-Pacheco et al., 2020).

Three of the genes encoding *TIMPs* are located within the introns of genes that encode synaptic cleft proteins, indicating an intriguing genetic link between *TIMPs* and the nervous system. Specifically, the *TIMP-1* gene is found within the Synapsin-1 gene, *TIMP-3* within the Synapsin-3 gene, and *TIMP-4* within the Synapsin-2 gene (Pérez-Martínez & Jaworski, 2005). *TIMP-1* is a candidate gene that may be linked to plasticity and is highly expressed in the hippocampal region. Research indicates that the lack of *TIMP-1* causes a notable impairment in the establishment and retrieval of reward connections. Rather than *TIMP-1* having a direct impact on memory, this effect is most likely mediated via its influence on neuronal growth (Chaillan et al., 2006).

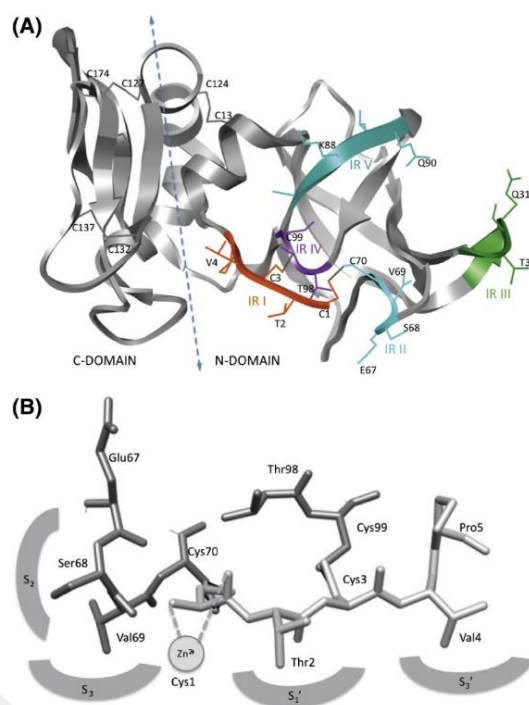


**Figure 1.7.** Structure of *TIMP-1* and its metalloproteinase interaction regions. (A) A three-dimensional structure of *TIMP-1* showing the locations of disulfide bonds and regions that interact with metzincins. (B) Structure of the core of the *TIMP* interaction domain in *MMP* active sites (Brew & Nagase, 2010a).

### 1.6.3. Relationships with *MMPs*

The contacts between the hemopexin domains of the zymogen and the C-terminal domains of *TIMP* stabilize the complexes produced by *TIMPs* with *MMPs*. The way that *TIMPs* and *MMPs* interact is rather specialized; specifically, *TIMP-2*, *TIMP-3*, or *TIMP-4* interact with pro-*MMP-2*, while *TIMP-1* or *TIMP-3* interact with *MMP-9*. These complexes can interact with a second *MMP* molecule since the N-terminal domain of *TIMP* is not involved in the interactions within them (Nagase & Murphy, 2008).

### 1.6.4. The biological roles of *TIMPs*

*TIMPs* modulate a variety of biological processes, such as apoptosis, anti-angiogenesis, cell migration and invasion, and synaptic plasticity. Although many of these functions are independent of *MMP* inhibition, it is possible that metalloproteinase inhibition has a role in them, given that the regulation of ECM catabolism might impact cellular behavior (Stetler-Stevenson, 2008). *TIMPs* have a key function in tissue remodeling, which involves inhibiting matrices through the formation of enzyme-inhibitor complexes. *TIMPs* have been demonstrated in several studies to prevent angiogenesis, metastasis, tumorigenesis, and cellular invasion (Gomez et al., 1997). These *TIMP* activities show a

range of distinct cellular functions, even though they seem to be a result of matrix inhibition. *TIMP-1*'s erythroid-potentiating action was first shown when it was cloned. Furthermore, previous research has demonstrated the effectiveness of *TIMPs* on a variety of parameters, including the reduction of tumor cell development by overexpression of *TIMP-1*, *TIMP-2*, and *TIMP-3* (Bian et al., 1996; Edwards et al., 1996).

#### **1.6.5. Synaptic plasticity**

*TIMP-1* null mice showed impaired formation of command-reward linkages after being tested for hippocampal, long-term potentiation, seizures, kindling, learning, and memory. Additionally, *TIMP-1* has been discovered to be a potential gene associated to plasticity, whose expression in the hippocampus is significantly elevated with glutamate analog stimulation (Nedivi et al., 1993). This impact is probably mediated by an influence on neuronal growth brought on by aberrant extracellular matrix turnover during the development of the central nervous system, rather than a direct effect of *TIMP-1* on memory (Chaillan et al., 2006). Long-term alterations in synaptic transmission and structure are necessary for learning possible threats and remembering such inputs. This kind of synaptic plasticity is believed to be influenced by modifications in the ECM structure of neurons. Because *TIMP-2* is abundantly expressed in the amygdala, which is engaged in synaptic plasticity linked to fear training, it therefore contributes to neuronal ECM turnover (Jaworski & Pérez-Martínez, 2006).

#### **1.6.6. Mood disorders and *TIMP***

*TIMPs* have great importance on brain health and functionality, especially by being effective on neuroinflammation and neuroplasticity. Increasing evidence shows that *MMPs* and *TIMPs* have important effects on the pathology of neurodegenerative and neuroinflammatory diseases (Bjerke et al., 2011). It has been noted that whereas *TIMP-2* expression levels are higher in those with bipolar disorder, *TIMP-1* and *TIMP-3* expression levels are often higher in those with depression (Brew et al., 2000). In addition, *TIMPs* are thought to support the development of mood disorders by increasing inflammation and oxidative stress in the regions of the brain that regulate mood. Numerous sample studies have shown that people with unipolar depression have lower *TIMP-2* levels than people in good health. *TIMP-2* levels were found to be 30% lower in unipolar depression patients than in the control group in the Zhang et al. research (Ge et al., 2020).

This thesis study was designed to complete our missing information about bipolar depression and unipolar depression, which are very common psychiatric disorders in the world and in our country. While bipolar depression is a disorder in which both manic and depressive states are seen and transitions between these two states are experienced, unipolar depression is a psychiatric disorder in which only depressive states are seen. However, due to the similar symptoms they show, it is sometimes quite difficult to distinguish these two disease groups from each other. Of course, failure to make a distinction negatively affects patients in the diagnosis and treatment processes. The etiology of BD and UD is quite complex and has not yet been sufficiently elucidated. It is known that genetic predispositions and environmental factors are potentially effective. Since enzyme groups such as matrix metalloproteinase-9 and tissue inhibitor of matrix metalloproteinases-1 are known to have effects on synaptic plasticity, neuroinflammation and extracellular matrix regulation, our study was remarkable. The relationship between the gene expression levels of *MMP-9* and *TIMP-1* in bipolar depression and unipolar depression patients and disease prognosis has not been fully explained. This thesis aims to investigate the biological mechanisms of these enzyme groups in the bipolar depression and unipolar depression patient groups we are interested in and to fill the gap in knowledge on this subject by evaluating their gene expression profiles and examining them together with the clinical status of the diseases. In this way, it is aimed to contribute to the complex etiology of BD and UD and to clarify and potentially support the use of biomarkers.

## 2. MATERIALS AND METHOD

### 2.1. Patient Selection and Sample Collection

Blood collection procedures of the participants whose samples are available in the archive were carried out at Dokuz Eylül University Central Laboratory under the supervision of the executive clinician (Dr. Deniz Ceylan Tufan Özalp) within the scope of the TÜBİTAK project numbered 216S778. Written consent was obtained from the volunteers for the use and archiving of blood samples taken in future studies. The use of samples in our study was approved by Koç University Ethics Committee with ethics committee approval number 2024.202.IRB2.088. The following inclusion and exclusion criteria were used to choose the participants.

Standards for study inclusion;

- People in the 18–45 age range
- At least a graduate of elementary school
- Able to give written consent for the study
- Hamilton Depression Scale score of 18 and/or above
- Bipolar disorder, depressive episode, Unipolar disorder/major depressive disorder, single episode, and Major depressive disorder, recurrent are among the SCID-I diagnoses that the patient group has been given.
- The individuals in the healthy control group, known as -will be of the same age and number as the patient group and will not have had any psychiatric diagnosis or treatment. They will also not have met any exclusion criteria at the conclusion of the examination. The participants in this healthy volunteer program will be those from whom the researchers have gained informed consent.

Standards for study exclusion;

- The existence of a major neurological illness, diabetes mellitus, rheumatological conditions, active infections, or a recognized decompensated systemic medical ailment,
- Applying supporting items and therapies containing antioxidants that may influence oxidative parameters,
- Severe deviations from normal test results,
- Any axis psychiatric disorder, diseases impacting cognitive functioning (such as delirium, dementia, epilepsy, etc.), and lifelong mental impairment.

- Ongoing substance and alcohol addiction diseases
- Remission lasting fewer than six months for those with a lifetime diagnosis of alcohol and/or drug abuse disorders

Criteria for leaving the study;

- Volunteer's own request
- Failure to fulfill the requirements of the work monitoring scheme, disrupting the work schedule
- The onset of a mania episode that has to be treated during follow-up, or the emergence of a systemic medical condition (diabetes mellitus, rheumatological disease, active infection, neurological disease, etc.).

## 2.2. Sample Preparation and Volunteer Clinical Information

When the effect size was anticipated to be 0.67 for comparison with 95% confidence level ( $\alpha=0.05$ ) and 80% power ( $\beta=0.20$ ), the minimum number of patients to be included in the research for the comparison of gene expression from bipolar and unipolar patients was determined to be 16. According to our study;

- 20 individuals with Bipolar depression
- 20 individuals with Unipolar depression
- 20 healthy controls

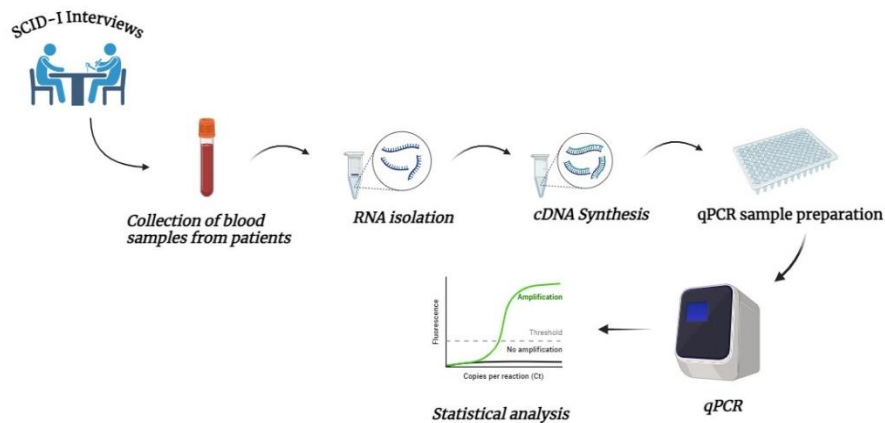


**Figure 2.1.** General Experimental steps of study.(Created by BioRender.com)

**Table 2.2.1.** Clinical information of participants

|                            | <b>Sample Code</b> | <b>Gender</b> | <b>Age</b> | <b>BMI</b> | <b>Smoking Status</b> | <b>Alcohol Status</b> |
|----------------------------|--------------------|---------------|------------|------------|-----------------------|-----------------------|
| <b>Healthy Controls</b>    | <b>HC002</b>       | Male          | 32         | 23,18      | YES                   | YES                   |
|                            | <b>HC003</b>       | Female        | 29         | 22,48      | YES                   | YES                   |
|                            | <b>HC004</b>       | Female        | 27         | 25,65      | YES                   | YES                   |
|                            | <b>HC006</b>       | Female        | 20         | 20,31      | YES                   | YES                   |
|                            | <b>HC007</b>       | Female        | 36         | 22,04      | YES                   | YES                   |
|                            | <b>HC008</b>       | Female        | 33         | 25         | NO                    | NO                    |
|                            | <b>HC010</b>       | Female        | 30         | 20,32      | NO                    | YES                   |
|                            | <b>HC011</b>       | Male          | 31         | 25,35      | YES                   | YES                   |
|                            | <b>HC012</b>       | Female        | 37         | 28,13      | YES                   | YES                   |
|                            | <b>HC015</b>       | Male          | 26         | 28,07      | YES                   | YES                   |
|                            | <b>HC016</b>       | Male          | 44         | 27,1       | YES                   | YES                   |
|                            | <b>HC018</b>       | Female        | 41         | 18,75      | NO                    | NO                    |
|                            | <b>HC026</b>       | Male          | 32         | 27,68      | NO                    | YES                   |
|                            | <b>HC027</b>       | Female        | 24         | 22,76      | NO                    | NO                    |
|                            | <b>HC030</b>       | Female        | 26         | 20,76      | NO                    | YES                   |
|                            | <b>HC032</b>       | Male          | 32         | 25,31      | YES                   | NO                    |
|                            | <b>HC034</b>       | Female        | 47         | 25,89      | YES                   | YES                   |
|                            | <b>HC035</b>       | Male          | 37         | 24,33      | YES                   | YES                   |
|                            | <b>HC043</b>       | Female        | 48         | 27,34      | YES                   | NO                    |
| <b>Unipolar Depression</b> | <b>UD011</b>       | Female        | 25         | 26,18      | NO                    | YES                   |
|                            | <b>UD013</b>       | Female        | 36         | 24,17      | NO                    | NO                    |
|                            | <b>UD018</b>       | Female        | 18         | 32,04      | YES                   | YES                   |
|                            | <b>UD019</b>       | Female        | 44         | 28,04      | YES                   | NO                    |
|                            | <b>UD022</b>       | Female        | 27         | 21,99      | YES                   | YES                   |
|                            | <b>UD025</b>       | Female        | 40         | 26,04      | NO                    | NO                    |
|                            | <b>UD026</b>       | Female        | 45         | 24,8       | YES                   | YES                   |
|                            | <b>UD027</b>       | Female        | 21         | 22,76      | YES                   | YES                   |
|                            | <b>UD028</b>       | Male          | 22         | 26,75      | YES                   | NO                    |
|                            | <b>UD036</b>       | Female        | 23         | 25,39      | NO                    | YES                   |
|                            | <b>UD037</b>       | Female        | 24         | 17,15      | NO                    | NO                    |
|                            | <b>UD038</b>       | Male          | 27         | 26,42      | NO                    | NO                    |
|                            | <b>UD045</b>       | Female        | 29         | 18,83      | YES                   | YES                   |
|                            | <b>UD047</b>       | Female        | 40         | 23,66      | YES                   | NO                    |
|                            | <b>UD053</b>       | Female        | 37         | 19         | YES                   | NO                    |

Table 2.2.1. (continue)

|                            | Sample Code  | Gender | Age | BMI   | Smoking Status | Alcohol Status |
|----------------------------|--------------|--------|-----|-------|----------------|----------------|
| <b>Unipolar Depression</b> | <b>UD058</b> | Female | 44  | 31,62 | YES            | YES            |
|                            | <b>UD060</b> | Female | 40  | 25,71 | NO             | NO             |
|                            | <b>UD063</b> | Female | 25  | 30,44 | YES            | NO             |
|                            | <b>UD067</b> | Male   | 43  | 27,78 | YES            | NO             |
|                            | <b>UD111</b> | Female | 37  | 19    | YES            | NO             |
| <b>Bipolar Depression</b>  | <b>BD004</b> | Female | 45  | 23    | NO             | YES            |
|                            | <b>BD005</b> | Male   | 25  | 28    | YES            | NO             |
|                            | <b>BD021</b> | Male   | 31  | 31    | NO             | YES            |
|                            | <b>BD024</b> | Female | 44  | 22    | NO             | YES            |
|                            | <b>BD030</b> | Female | 32  | 23    | NO             | YES            |
|                            | <b>BD032</b> | Female | 45  | 30    | NO             | NO             |
|                            | <b>BD034</b> | Male   | 38  | 27    | NO             | YES            |
|                            | <b>BD035</b> | Female | 37  | 25    | YES            | NO             |
|                            | <b>BD041</b> | Female | 19  | 17    | NO             | YES            |
|                            | <b>BD042</b> | Female | 39  | 24    | NO             | NO             |
|                            | <b>BD043</b> | Male   | 38  | 32    | NO             | NO             |
|                            | <b>BD046</b> | Male   | 33  | 23    | YES            | NO             |
|                            | <b>BD048</b> | Male   | 30  | 34    | NO             | NO             |
|                            | <b>BD050</b> | Male   | 26  | 24    | NO             | NO             |
|                            | <b>BD057</b> | Male   | 32  | 27    | NO             | YES            |
|                            | <b>BD059</b> | Female | 30  | 19    | NO             | YES            |
|                            | <b>BD044</b> | Female | 39  | 33    | YES            | NO             |

## 2.3. Method

### 2.3.1. RNA isolation from blood

RNAs were extracted using the QIAamp RNA Blood Mini Kit (Cat No./ID:52304, Qiagen, Germany) in accordance with the manufacturer's instructions from blood samples acquired from BD and UD patients as well as healthy controls. For the purpose of cDNA synthesis, samples having an A260/A280 ratio of 1.8–2.1 for RNA were utilized. RNA quantities were measured using Nanodrop (Thermo Fisher Scientific, Wilmington, DE). In RNA isolation, there are four primary steps. These procedures consist of RNA binding, washing, elution, and lysis (Chomczynski & Sacchi, 1987).

1. A volume of 50–500  $\mu\text{L}$  of blood was centrifuged for 5 minutes at  $4^{\circ}\text{C}$  at  $400 \times g$  (2,000 rpm), with the supernatant being disposed of.
2. The pellet was suspended in 600  $\mu\text{L}$  of Lysis Buffer, which was added after the supernatant was discarded, and the mixture was vigorously vortexed.
3. Using a pipette or vortex, 450  $\mu\text{L}$  of ethanol (96–100%) was added and stirred.
4. After being prepared, half of the lysate was put in a collecting tube and sent to a column. For a minute, the column was centrifuged at  $12,000 \times g$ , (or 11,000 rpm). After filling the collecting tube with the supernatant, the column was put back inside. After being carefully moved to the column, the remaining half of the lysate was centrifuged once more for one minute at  $12,000 \times g$  (or 11,000 rpm). After once more pouring off the supernatant, the column was put in a fresh 2 mL collecting tube.
5. Wash Buffer (WB 1, with added ethanol) was applied in an amount of 700  $\mu\text{L}$ . Centrifuged for one minute at  $12,000 \times g$  ( $\sim 11,000$  rpm). The column was put back into the collecting tube after the supernatant was disposed of.
6. After adding 500  $\mu\text{L}$  of Wash Buffer 2 (ethanol added) to the column, it was centrifuged for one minute at  $12,000 \times g$  (11,000 rpm).
7. The column was once again filled with 500  $\mu\text{L}$  of Wash Buffer 2 (WB2, with added ethanol). Centrifuged for two minutes at  $12,000 \times g$  ( $\sim 11,000$  rpm).
  - 7.1. The collection tube was thrown away in accordance with the manufacturer's instructions, and the column was centrifuged empty for one minute at the highest speed ( $\geq 20,000 \times g$ ,  $\geq 14,000$  rpm). After that, the column was moved to a fresh 1.5 mL microcentrifuge tube devoid of Rnase and the collection tube was disposed of.

8. After adding 50  $\mu\text{L}$  of nuclease-free water to the membrane's center in the column, the mixture was centrifuged for one minute at  $12,000 \times g$  ( $\sim 11,000$  rpm).
9. The liquid collected in the whole tube was kept for later processing at  $-20^{\circ}\text{C}$ , and the column was disposed of.

This method was adapted and modified from Chomczynski et al. The resulting RNA molecules were stored at  $-80^{\circ}\text{C}$  in RNase-free distilled water (Chomczynski & Sacchi, 1987).

### **2.3.2. cDNA synthesis**

cDNA synthesis was performed using the RT 2 First Strand Kit (Cat No./ID:330404, Qiagen, Germany) to synthesize cDNA according to the manufacturer's instructions. Cold storage was used for cDNAs (Wiame et al., 2000). The following is the methodology for cDNA synthesis:

1. 5  $\mu\text{g}$  of total RNA was added to a sterile nuclease-free tube.
2. After adding 20 pmol of gene-specific primer, distilled water was added to bring the total amount to 12  $\mu\text{l}$ .
3. The addition of 4  $\mu\text{l}$  of 5X reaction buffer.
4. 1  $\mu\text{l}$  of 20 U/ $\mu\text{L}$  RiboLock Rnase Inhibitor was added.
5. One microliter of reverse transcriptase enzyme and two microliters of a 10 mM dNTP mixture were added.
6. After gently mixing the tube, it was incubated for an hour at  $42^{\circ}\text{C}$ .
7. cDNAs were incubated for five minutes at  $70^{\circ}\text{C}$ . For short-term usage, they were kept at  $-20^{\circ}\text{C}$ , and for long-term storage, at  $-70^{\circ}\text{C}$ . The acquired cDNAs were prepared for qPCR application.

### **2.3.3. qPCR stage**

qPCR is used to measure the amount of cDNA. It helps to quantify the expression of specific genes in this way. A real-time monitoring of DNA amplification is possible with the qPCR technology, which is a variation of PCR. The introduction of fluorescent dyes and probe technology allowed for the creation of qPCR. This makes it possible to quantify analysis and measure the quantity of DNA in each cycle.

Using particular temperature cycles to accomplish this amplification, qPCR is used to amplify a particular area of DNA and monitor this process in real time. It is often performed 30–40 times and comprises of cycles for denaturation, binding, and extension. The qPCR gadget

measures and records the signal that the fluorescent dye or probe generates during DNA amplification in each cycle. The quantity of DNA is directly correlated with the amount of fluorescence signal that results.

Utilizing the Ct (cycle threshold) value, qPCR data is examined. The number of cycles in which the fluorescent signal above a particular threshold is known as the Ct value. A low Ct value denotes a significant amount of beginning DNA since the Ct value is inversely proportional to the initial amount of DNA.  $\Delta\Delta C_t$  technique is used to compare gene expression with the acquired Ct values. This technique computes the variations in gene expression between samples and normalizes the expression of the target gene in relation to the reference gene.

The protocol used for the qPCR process is as follows:

1. To make a reaction mixture of 20  $\mu$ l, put the following ingredients into a sterile 1.5 mL microcentrifuge tube: 1.0  $\mu$ l 20X TaqMan® Gene Expression Assay, 10.0  $\mu$ l 2X TaqMan Fast Advanced Master Mix, 5  $\mu$ l cDNA (to be added later), 4  $\mu$ l RNase-free water.
2. After gently centrifuging the mixture created in the tube, the lid was closed and the mixture was stirred many times.
3. 15  $\mu$ l of the prepared reaction mixture was poured into each well of a 96-well plate. Next, 5  $\mu$ l of the cDNA sample was put into each sample's matching well.
4. The 96-well plate is gently mixed and placed in the thermal cycler and the cycle is started. RT-qPCR reactions were performed using a Biorad CFX96 instrument (Cat No./ID: 1855196, Bio-Rad Laboratories, Inc., California).

**Table 2.3.1** qPCR reaction components.

| <b>Component</b>                            | <b>Per reaction</b> |
|---------------------------------------------|---------------------|
| <b>TaqMan Fast Advanced Master Mix (2X)</b> | 10.0 $\mu$ l        |
| <b>TaqMan Assay primer/probe (20X)</b>      | 1.0 $\mu$ l         |
| <b>cDNA Template (1 to 100 ng)</b>          | 5.0 $\mu$ l         |
| <b>Nuclease-free water</b>                  | 4.0 $\mu$ l         |

And qPCR run Method;

**Table 2.3.2.** Conditions of the thermal cycler for qPCR reactions

| 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         |

### 2.3.3.1. TaqMan gene expression

TaqMan is one of the commonly used methods in real-time PCR (qPCR) experiments. Because TaqMan probes are designed with MGB (Minor Groove Binder) technology, it reduces the risk of false amplification measurements by ensuring that the probe binds more tightly to the target DNA. FAM (6-carboxyfluorescein) fluorescent dye is used to label the 5' end of TaqMan probes, whereas NFQ (non-fluorescent quencher) quencher is used to identify the 3' end. A fluorescent signal is generated during amplification when DNA polymerase cleaves the FAM-stained nucleotide at the probe's 5' end (Figure 2.2). Fluorescence spectrophotometry allows for the instantaneous measurement of this resultant radiation. The value obtained is directly proportional to the target gene's copy number. These probes' characteristics allow them to identify gene expression even at extremely low concentrations. Every sample was tested using a minimum of two duplicates.

The  $\beta$ -actin reference gene was used to normalize the results of the relative gene expression study. RT-qPCR assays were run using the Biorad CFX96 apparatus. Using  $2^{-(\Delta\Delta Ct)}$  values and the SPSS (IBM Corp., Version 24.0. Armonk, NY) software, the expression levels of all genes were computed as fold changes relative to the control sample group.

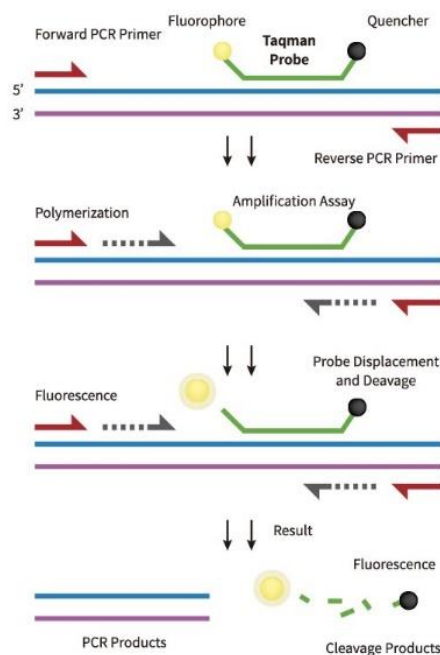


**Figure 2.2.** Schematic representation of the TaqMan gene expression assay method.(SynBioTechnologies)

The TaqMan probe, which has a reporter dye (R) and a quencher dye (Q) in it, anneals to one strand of the DNA template between the forward and reverse PCR primers only during polymerization. Taq polymerase displaces the fluorogenic probe during amplification and subsequently degrades, releasing the reporter dye. Following fragmentation, the shorter probe is detached from the template, enabling the strand's polymerization to finish. The quantity of product deposited determines the amount of fluorescence.

#### 2.3.4. Statistical analysis

Every sample was tested using a minimum of two duplicates. The  $\beta$ -actin reference gene was used to normalize the results of the relative gene expression study. Using  $2^{-(\Delta\Delta Ct)}$  values and the SPSS (IBM Corp., Version 24.0, Armonk, NY) software, the expression levels of all genes were computed as fold changes relative to the control sample group. Following a statistical analysis, the results were intended to be presented as an addition to the body of literature.

### 3. RESULT

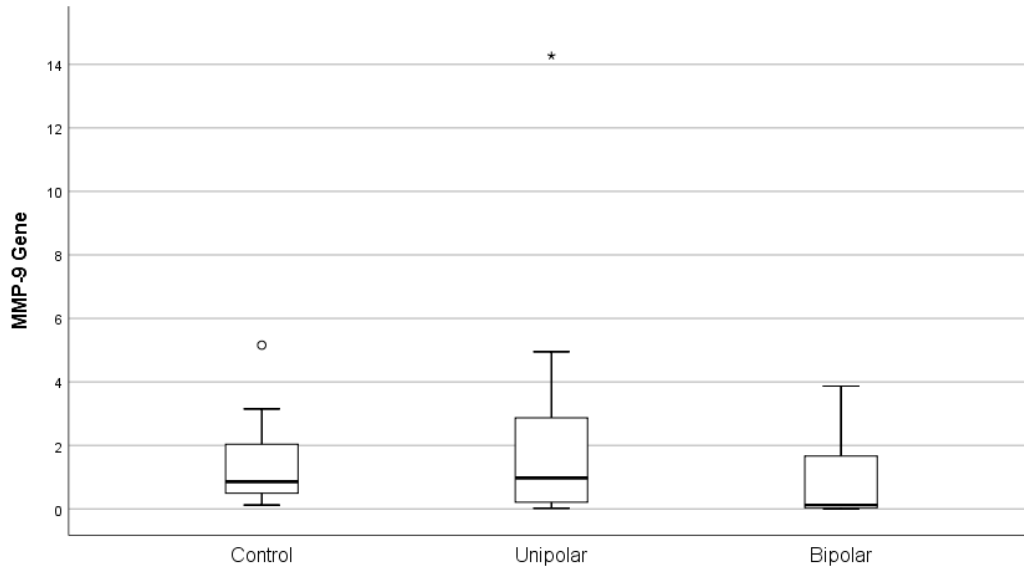
The aim of this study was to compare the expression levels of *MMP-9* and *TIMP-1* genes in bipolar disorder, unipolar disorder and healthy controls. To investigate the expression levels, RNAs were isolated from blood samples taken from 16 patients with bipolar disorder, 20 patients with unipolar disorder and 19 healthy controls. Then, cDNA synthesis and RT-qPCR were followed and  $2^{-(\Delta\Delta C_t)}$  values were calculated using Mann-Whitney U test and Spearman Correlation using SPSS software. According to the statistical findings, *MMP-9* gene was expressed at a significantly higher level ( $p < 0.05$ ) in healthy controls compared to patients with bipolar and unipolar disorders. It was revealed that there was no change for *TIMP-1* gene and there was no statistically significant result.

#### 3.1. Statistical Analysis With SPSS

Statistical analysis of the data obtained was applied by including the results obtained from 19 healthy controls, 16 bipolar and 20 unipolar disorder patients. Median, minimum and maximum values were used as descriptors. The Mann-Whitney U test and Spearman correlation analysis were used to compare the groups. For all tests, a two-way significance threshold of  $p < 0.05$  was acceptable (Vafadari et al., 2016). The statistical comparison of the *MMP-9* gene among the sample groups yielded a p-value of 0.002 ( $p < 0.05$  is considered significant), indicating statistically significant differences between the groups. Considering that the median value of the healthy control group was higher, it can be concluded that the expression level of the *MMP-9* gene is higher in this group compared to individuals with unipolar and bipolar depression. Additionally, the "a" and "b" indices accompanying the median values are used to denote differences between each group. Accordingly, groups sharing the same index exhibit no differences in expression levels, while groups with different indices show statistically significant differences in *MMP-9* gene expression levels. Significant differences in gene expression levels were observed between the Bipolar and Healthy Control groups, as well as between the Unipolar and Healthy Control groups. For the *TIMP-1* gene, the p-value of 0.469 indicates that no significant variation in *TIMP-1* gene expression was observed among the groups. The distinct differences observed in the healthy control group compared to the other groups suggest that this group may differ clinically or physiologically from the others. Although variations in the median values of *TIMP-1* among the groups were noted, these differences were not statistically significant (Table 3.1.1. and Table 3.1.2.).

**Table 3.1.1.** General results of statistical analysis

| Sample Groups       | <i>MMP-9</i> |              |       |        | <i>TIMP-1</i> |        |              |       |
|---------------------|--------------|--------------|-------|--------|---------------|--------|--------------|-------|
|                     | N            | Median       | Min   | Max    | N             | Median | Min          | Max   |
| Healthy Control     | 19           | 0.768        | 0.110 | 7.914  | 19            | 0.906  | 0.414        | 3.926 |
| Unipolar Depression | 20           | 0.144        | 0.014 | 3.654  | 20            | 1.153  | 0.584        | 2.691 |
| Bipolar Depression  | 16           | 0.241        | 0.013 | 13.590 | 17            | 1.085  | 0.445        | 2.140 |
| p value             |              | <b>0.002</b> |       |        |               |        | <b>0.469</b> |       |

**Table 3.1.2.** Visualization of the distribution of groups with significant differences in MMP-9 gene expression levels through a box plot.

The statistical analysis p values ( $p = 0.566$ ;  $p = 0.440$ ) demonstrate that there are no appreciable gender differences when it comes to the *MMP-9* and *TIMP-1* genes (Table 3.1.3).

**Table 3.1.3.** Gender differences effect on gene expression.

|               | Women |        |       |       | Men |        |       |       | p value      |
|---------------|-------|--------|-------|-------|-----|--------|-------|-------|--------------|
|               | N     | Median | Min   | Max   | N   | Median | Min   | Max   |              |
| <i>MMP-9</i>  | 37    | 0.322  | 0.014 | 7.914 | 18  | 0.395  | 0.013 | 13.59 | <b>0.566</b> |
| <i>TIMP-1</i> | 38    | 1.083  | 0.414 | 3.926 | 18  | 1.232  | 0.479 | 2.27  | <b>0.440</b> |

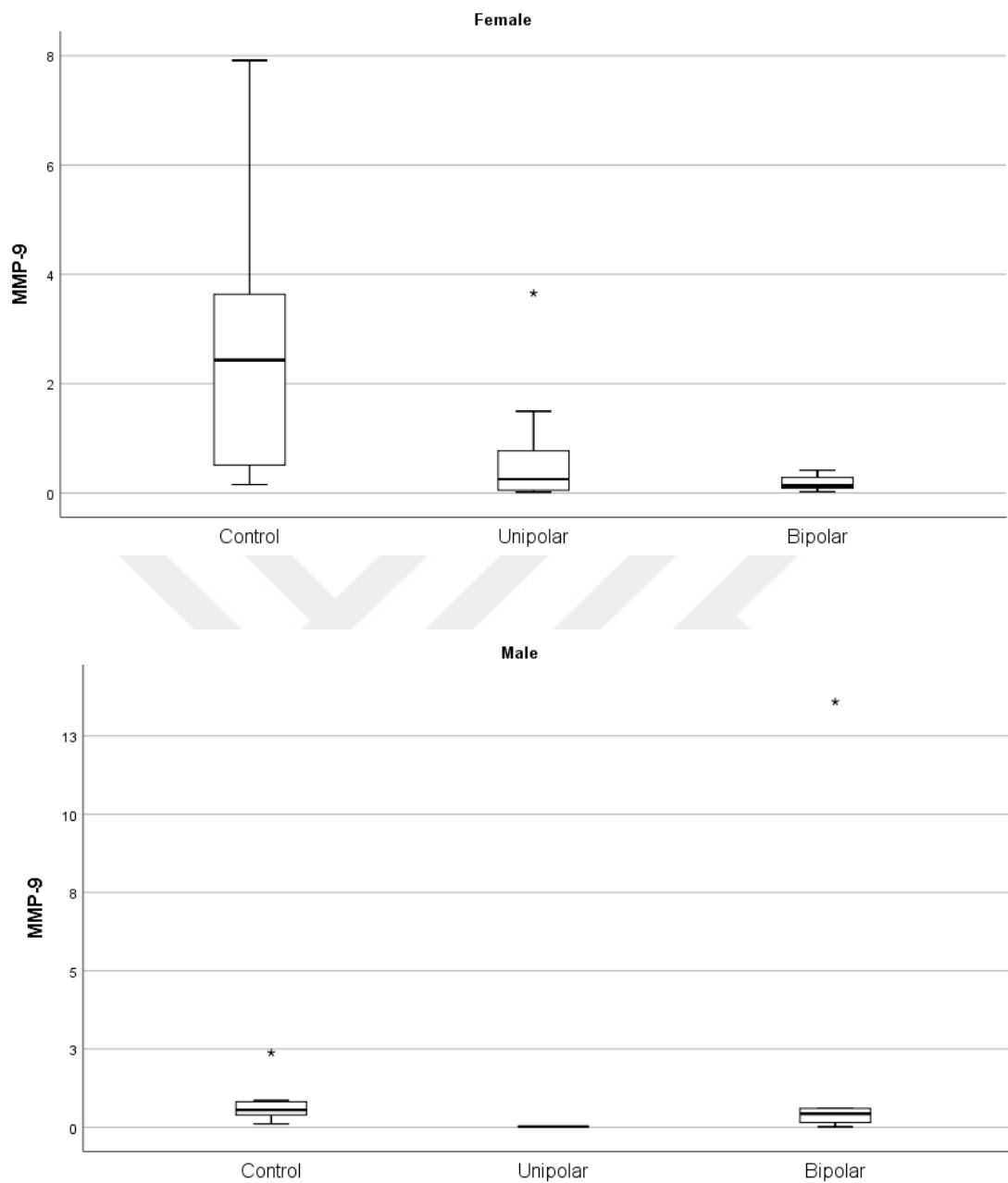
The given tables contain the median, minimum and maximum values of *MMP-9* and *TIMP-1* variables separated by gender in three different sample groups. Since the  $p=0.012$  value for the *MMP-9* gene in individuals with UD was  $<0.05$ , it showed that it was statistically significant due to gender differences in this patient group. The  $p$  values found in the light of the analyzes for other sample groups and *TIMP-1* indicate that there are no significant differences between genders (Table 3.1.4). Detailed distribution of groups with significant differences between them for the *MMP-9* gene in Table 3.1.4. on the box plot graph (Table 3.1.5). In addition to these data, a greater variation in gene expression levels is observed in the control group in both graphs, which may reflect individual differences in gene expression levels in the control group.

**Table 3.1.4.** Effect of gender on *MMP-9* and *TIMP-1* expression

|                     | <i>MMP-9</i> |    |        |       |       |              |
|---------------------|--------------|----|--------|-------|-------|--------------|
| Sample Groups       | Gender       | N  | Median | Min   | Max   | p value      |
| Healthy Controls    | Female       | 12 | 2.432  | 0.154 | 7.914 | 0.142        |
|                     | Male         | 7  | 0.555  | 0.11  | 2.378 |              |
| Unipolar Depression | Female       | 17 | 0.253  | 0.014 | 3.654 | <b>0.012</b> |
|                     | Male         | 3  | 0.022  | 0.021 | 0.023 |              |
| Bipolar Depression  | Female       | 8  | 0.131  | 0.024 | 0.416 | 0.195        |
|                     | Male         | 8  | 0.431  | 0.013 | 13.59 |              |

|                     | <i>TIMP-1</i> |    |        |       |       |         |
|---------------------|---------------|----|--------|-------|-------|---------|
| Sample Groups       | Gender        | N  | Median | Min   | Max   | p value |
| Healthy Controls    | Female        | 12 | 0.843  | 0.414 | 3.926 | 0.650   |
|                     | Male          | 7  | 1.123  | 0.479 | 2.185 |         |
| Unipolar Depression | Female        | 17 | 1.135  | 1.135 | 2.691 | 0.921   |
|                     | Male          | 3  | 1.264  | 1.264 | 2.27  |         |
| Bipolar Depression  | Female        | 9  | 0.82   | 0.445 | 2.1   | 0.277   |
|                     | Male          | 8  | 1.427  | 0.578 | 2.14  |         |

**Table 3.1.5.** Box plot representation of groups with significant differences for the *MMP-9* gene.



It has been shown that among the male and female population, there are notable variations in the *MMP-9* gene between the sick and healthy control groups. In conclusion, there were notable variations between the groups for *MMP-9* in the populations of both sexes ( $p=0.001$  for females and  $p=0.049$  for males). These results may suggest that *MMP-9* may be a biomarker that differs between groups, whereas *TIMP-1* remains more constant. In addition to the information here for the *MMP-9* gene, our a and b indices, which we show next to the

median values, show the differences between the groups. While there is no difference between groups with the same index, differences are observed between groups with different indexes (Table 3.1.6).

**Table 3.1.6.** Comparative p values of sample groups within the Female and Male population for *MMP-9* and *TIMP-1* genes.

|        |        | <i>MMP-9</i> |                    |         |         |                |
|--------|--------|--------------|--------------------|---------|---------|----------------|
| Gender | Groups | N            | Median             | Minimum | Maximum | <i>p value</i> |
| Female | HC     | 12           | 2.432 <sup>a</sup> | 0.154   | 7.914   | 0.001          |
|        | UD     | 17           | 0.253 <sup>b</sup> | 0.014   | 3.654   |                |
|        | BD     | 8            | 0.131 <sup>b</sup> | 0.024   | 0.416   |                |
| Male   | HC     | 7            | 0.555 <sup>a</sup> | 0.11    | 2.378   | 0.049          |
|        | UD     | 3            | 0.022 <sup>b</sup> | 0.021   | 0.023   |                |
|        | BD     | 8            | 0.431 <sup>a</sup> | 0.013   | 13.59   |                |

|        |        | <i>TIMP-1</i> |        |         |         |                |
|--------|--------|---------------|--------|---------|---------|----------------|
| Gender | Groups | N             | Median | Minimum | Maximum | <i>p value</i> |
| Female | HC     | 12            | 0.843  | 0.414   | 3.926   | 0.282          |
|        | UD     | 17            | 1.135  | 0.584   | 2.691   |                |
|        | BD     | 9             | 0.82   | 0.445   | 2.1     |                |
| Male   | HC     | 7             | 1.123  | 0.479   | 2.185   | 0.792          |
|        | UD     | 3             | 1.264  | 0.673   | 2.27    |                |
|        | BD     | 8             | 1.427  | 0.578   | 2.14    |                |

Although there is no significant difference between smoking statuses for *MMP-9*, the p-value shows a borderline value of 0.062. This may indicate that different results may be obtained in studies with a larger sample size. For *TIMP-1*, there is no significant difference between smoking statuses. These results may suggest that *TIMP-1* is a biomarker that does not vary according to smoking status (Table 3.1.7).

**Table 3.1.7.** Effects of smoking status on the expression levels of *MMP-9* and *TIMP-1* genes.

|                |    | <i>MMP-9</i> |         |         |                |  |
|----------------|----|--------------|---------|---------|----------------|--|
| Smoking Status | N  | Median       | Minimum | Maximum | <i>p value</i> |  |
| NO             | 26 | 0.196        | 0.013   | 13.590  | 0.062          |  |
| YES            | 29 | 0.545        | 0.021   | 7.914   |                |  |

|                |    | <i>TIMP-1</i> |         |         |                |  |
|----------------|----|---------------|---------|---------|----------------|--|
| Smoking Status | N  | Median        | Minimum | Maximum | <i>p value</i> |  |
| NO             | 26 | 1.183         | 0.414   | 2.467   | 0.599          |  |
| YES            | 30 | 1.102         | 0.479   | 3.926   |                |  |

There is no statistically significant difference between alcohol use statuses for both *MMP-9* and *TIMP-1*. These results may suggest that these biomarkers are characteristics that do not vary according to alcohol use status (Table 3.1.8).

**Table 3.1.8.** Effects of alcohol use on the expression levels of *MMP-9* and *TIMP-1* genes.

|                | <i>MMP-9</i> |        |         |         |                |
|----------------|--------------|--------|---------|---------|----------------|
| Alcohol Status | N            | Median | Minimum | Maximum | <i>p value</i> |
| NO             | 25           | 0.267  | 0.021   | 3.654   | 0.335          |
| YES            | 30           | 0.474  | 0.013   | 13.590  |                |

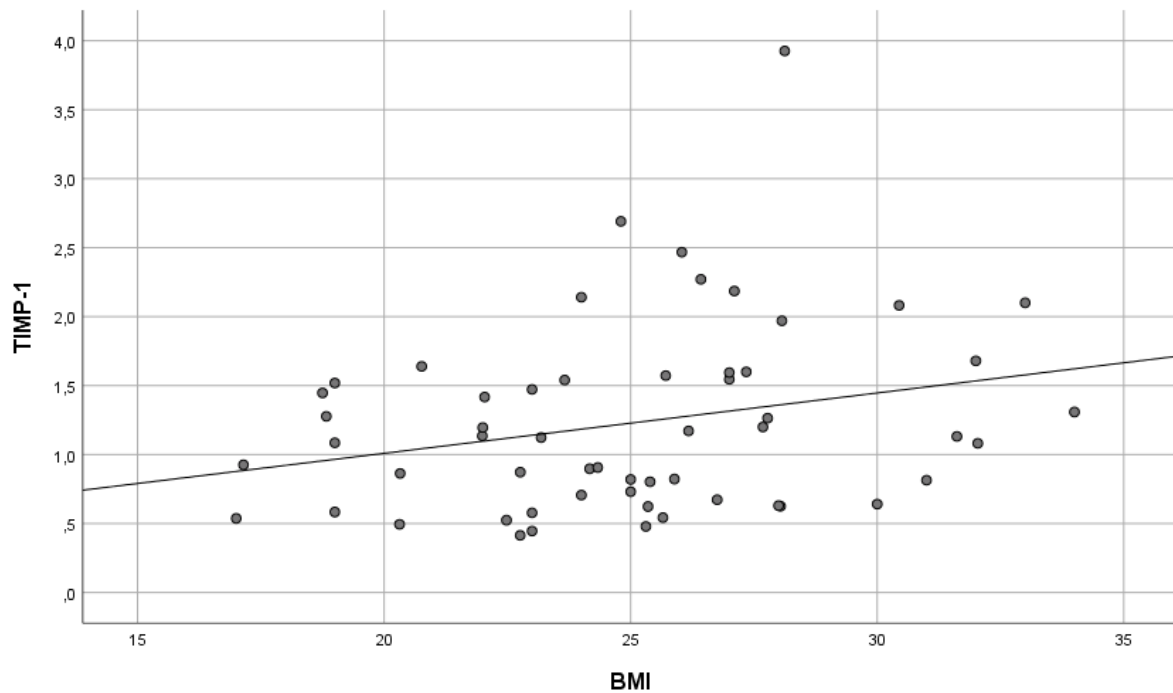
|                | <i>TIMP-1</i> |        |         |         |                |
|----------------|---------------|--------|---------|---------|----------------|
| Alcohol Status | N             | Median | Minimum | Maximum | <i>p value</i> |
| NO             | 26            | 1.095  | 0.414   | 2.467   | 0.882          |
| YES            | 30            | 1.127  | 0.445   | 3.926   |                |

The monotonic relationship between two variables is measured using Spearman's rho, also called Spearman's rank correlation coefficient. Here, the effects of age and body mass index (BMI) variables on the expression levels of *TIMP-1* and *MMP-9* genes were investigated. The results showed that the expression level of *MMP-9* gene was not affected by age or BMI variables, whereas *TIMP-1* gene showed a weak positive correlation with Age ( $p = 0.075$ ). The positive correlation between BMI and *TIMP-1* shows that as BMI increases, the *TIMP-1* value also increases, and a moderate relationship was observed ( $p = 0.034$ ) (Table 3.1.9). The display of the progression of the significant and positive correlation between them on the scatter plot clearly shows this relationship (Table 3.1.10).

**Table 3.1.9.** Effect of age and BMI ratios on gene expression.

|                       |                         | <i>MMP-9</i> | <i>TIMP-1</i> |
|-----------------------|-------------------------|--------------|---------------|
| Age                   | Correlation Coefficient | 0.003        | 0.24          |
|                       | Sig. (2-tailed) p       | 0.985        | 0.075         |
|                       | N                       | 55           | 56            |
| BMI (Body Mass Index) | Correlation Coefficient | -0.177       | <b>0.284</b>  |
|                       | Sig. (2-tailed) p       | 0.195        | <b>0.034</b>  |
|                       | N                       | 55           | 56            |

**Table 3.1.1** *TIMP-1*/BMI relationship shown on a scatter plot.



Examination of all groups separately using Spearman's rho. While the *TIMP-1* variable has stronger and significant relationships with age and BMI, such strong relationships are not observed for the *MMP-9* variable. Notably, the effect of BMI on *TIMP-1* shows a statistically significant and moderate correlation. In healthy controls, there was a weak and statistically insignificant link between age and *MMP-9* expression; however, there was a modest positive and statistically significant association ( $p=0.046$ ) between age and *TIMP-1* expression. It was shown that the expression levels of both genes were unaffected by the BMI index across all groups (Table 3.1.11).

**Table 3.1.11** Spearman's rho Analysis

| <b>Groups</b>              |     |                         | <b><i>MMP-9</i></b> | <b><i>TIMP-1</i></b> |
|----------------------------|-----|-------------------------|---------------------|----------------------|
| <b>Healthy Controls</b>    | Age | Correlation Coefficient | -0.216              | 0.463                |
|                            |     | Sig. (2-tailed)         | 0.374               | 0.046                |
|                            |     | N                       | 19                  | 19                   |
|                            | BMI | Correlation Coefficient | -0.205              | 0.363                |
|                            |     | Sig. (2-tailed)         | 0.399               | 0.126                |
|                            |     | N                       | 19                  | 19                   |
| <b>Unipolar Depression</b> | Age | Correlation Coefficient | 0.297               | 0.353                |
|                            |     | Sig. (2-tailed)         | 0.203               | 0.127                |
|                            |     | N                       | 20                  | 20                   |
|                            | BMI | Correlation Coefficient | -0.370              | 0.061                |
|                            |     | Sig. (2-tailed)         | 0.108               | 0.799                |
|                            |     | N                       | 20                  | 20                   |
| <b>Bipolar Depression</b>  | Age | Correlation Coefficient | -0.285              | -0.034               |
|                            |     | Sig. (2-tailed)         | 0.285               | 0.896                |
|                            |     | N                       | 16                  | 17                   |
|                            | BMI | Correlation Coefficient | 0.235               | 0.394                |
|                            |     | Sig. (2-tailed)         | 0.381               | 0.118                |
|                            |     | N                       | 16                  | 17                   |

The Spearman's rho correlation coefficients for the variables *MMP-9* and *TIMP-1* as well as the accompanying p values between age and BMI are shown in this table for the control and total patient groups. In the control group, there was a noteworthy positive connection seen between age and *TIMP-1*. Nonetheless, relationships between age and BMI, as well as between *MMP-9* and *TIMP-1*, were typically weak and not statistically significant throughout the whole patient sample. This might suggest that the control group and the entire patient group vary biologically (Table 3.1.12).

**Table 3.1.12.** Comparison of the total patient group and the control group.

| Groups                  |     |                         | <i><b>MMP-9</b></i> | <i><b>TIMP-1</b></i> |
|-------------------------|-----|-------------------------|---------------------|----------------------|
| <b>Healthy Controls</b> | Age | Correlation Coefficient | -0.216              | 0.463                |
|                         |     | Sig. (2-tailed)         | 0.374               | 0.046                |
|                         |     | N                       | 19                  | 19                   |
|                         | BMI | Correlation Coefficient | -0.205              | 0.363                |
|                         |     | Sig. (2-tailed)         | 0.399               | 0.126                |
|                         |     | N                       | 19                  | 19                   |
| <b>Total Patients</b>   | Age | Correlation Coefficient | 0.085               | 0.136                |
|                         |     | Sig. (2-tailed)         | 0.622               | 0.422                |
|                         |     | N                       | 36                  | 37                   |
|                         | BMI | Correlation Coefficient | -0.113              | 0.225                |
|                         |     | Sig. (2-tailed)         | 0.511               | 0.182                |
|                         |     | N                       | 36                  | 37                   |

#### 4. DISCUSSION

Our study was based on the hypothesis "What might be the differences between the expression levels of *MMP-9* and *TIMP-1* genes in individuals with bipolar and unipolar depression in the Turkish population", both between the two disease groups, and compared to healthy controls? In order to better understand the pathophysiology of the disorders, we used blood samples from patients as well as healthy controls to measure the expression levels of the *MMP-9* and *TIMP-1* genes. From there, we were able to interpret the outcomes and draw findings about the pathophysiology and course of the disease in both bipolar and unipolar depression patients. In order to do this, blood samples were drawn from 20 patients with bipolar disorder, 20 patients with unipolar depression, and 20 healthy controls. The inclusion and exclusion criteria were decided on in the Dokuz Eylül University Central Laboratory. Bipolar and unipolar depression diagnoses were established throughout the group creation process using ICD-I interviews and the DSM-5 diagnostic criteria. To date, a great deal of research and studies have been carried out to identify the pathophysiology of various kinds of diseases, to create personalized therapies, and to fill in the gaps in our understanding of the illnesses. These illnesses, which are also known as mood disorders, have a wide variety of putative genes, and their identities are still being discovered.

Within tissues and organs, cells are surrounded and supported by a complex framework called extracellular matrix, which is made up of proteins, carbohydrates, and other necessary substances. Three primary protein types make up the ECM, which enables intercellular communication: collagen, non-collagenous proteins, and proteoglycans. The ECM is crucial for cortical connection, synaptic plasticity, dendritic and axonal growth and guided movement, and the proliferation and differentiation of neuronal progenitors throughout development. Neurodegenerative illnesses associated with aging arise as a result of ECM breakdown. In neurological or pathological situations, as well as with age, elastin degeneration may become permanent or irreparable. Although elastin is often resistant to proteolysis, proteinases like *MMPs* can break it down. Countless research and experimental techniques, as well as countless instances of embryonic lethality or functional problems brought on by deficiencies or mutations in a variety of genetic illnesses, have revealed the significance of the ECM in mouse and human development (Arseni et al., 2018; Lamandé & Bateman, 2020).

Postmortem data from people with bipolar disorder changes in the number and size of different kinds of neurons have been noted in tissue investigations. Furthermore, glial-fibrillary acidic protein (GFAP), an astrocyte-specific protein that is highly expressed, showed disease-specific alterations in proteomic analyses carried out on the brain tissues of patients with bipolar and unipolar depression (Johnston-Wilson et al., 2000)

Matrix metalloproteinases are members of a broad family of multidomain zinc endopeptidases that break down growth factors, cell adhesion molecules, and extracellular matrix constituents. It is a big set of twenty-one enzymes that are categorized based on their structural makeup and are crucial to the degradation of the extracellular matrix. Transmembrane *MMP* activity is one of the activation mechanisms, and tissue inhibitor of *MMPs* (*TIMP*) is necessary for this activity. *TIMPs* (also known as *MMP* inhibitors) are endogenous proteins (*TIMP*-1, -2, -3, -4). *TIMP* levels, which are directly in charge of *MMP* activation, fall in pathological circumstances when *MMP* activity rises. *TIMPs* block every known *MMP*. Additional roles of *TIMP* include pro-apoptosis, maintaining the structure of TNF- $\alpha$  receptor-bound cells, limiting the development of endothelial cells stimulated by fibroblast growth factors (*TIMP*-2), and encouraging cell proliferation (*TIMP*-1, -2). *TIMP* is accessible. According to earlier research, people with bipolar and unipolar depression express the *MMP*-9 gene at a greater level than healthy controls. These findings corroborate the involvement of *MMP*-9 in neuroinflammation and permeability of the blood-brain barrier (BBB) (Chiarani et al., 2013).

The expression of many *MMPs* is altered by the nervous system in conditions involving injury and neurological disorders. *MMPs* have a long history of regulating nervous system function, even in the presence of diseases including neurotoxicity, neuroinflammation, and demyelination. Because of this, it has been shown that individuals with Parkinson's and Alzheimer's disease exhibit increased *MMP* activity. The association between the *MMP*-9 gene polymorphism and schizophrenia risk has been the subject of contradictory findings in several case-controlled molecular genetic research. But there isn't enough information about bipolar illness to support this. The anomalies in *MMP*-9 blood levels, which are usually seen in individuals with shorter illness durations and fewer affective episodes, may be related to the depressive episode. The Center for Epidemiological Studies Depression Questionnaire was used to measure plasma *MMP*-9 levels, which were strongly correlated with depressive symptoms in a Swedish research comprising 402 participants who were selected from the middle-aged normal population. It should be highlighted that individuals with bipolar mood

illness also have an elevated risk of cardiovascular disease and increased cancer morbidity, given the high levels of *MMP-9* that have been linked to both cancer and cardiovascular disease (BarChana et al., 2008; Ösby et al., 2001). Knowledge in the subject of *TIMPs* is being advanced by study on their structure, functions, and biological and pathological roles because of the importance of these proteins to a variety of human disorders and their involvement in maintaining ECM homeostasis. Although *TIMPs* were first identified as *MMP* inhibitors, it is now understood that they have a far wider inhibitory range. Specifically, elevated levels of *MMP-9* have been linked to BBB deterioration and brain tissue inflammation. However, there was no discernible change in the expression of the *TIMP-1* gene between the healthy controls and the BD and UD patients. Given that *TIMP-1* inhibits *MMP-9*, there are several biological explanations for this finding. For instance, it's possible that the rise in *MMP-9* levels is the result of an inflammatory reaction that gets beyond *TIMP-1* regulating systems. Nevertheless, conclusive findings are hindered by the fact that changes in *TIMP-1* levels are not yet apparent (Shibasaki et al., 2016). Saravanan et al. found that the *MMP-9* gene polymorphism raises the incidence of bipolar depression in a recent research. In order to explain the correlation between the genetic variation rs17576 of the *MMP-9* gene and the illness, it was determined that bipolar depression patients with AG and GG genotypes had considerably higher levels of *MMP-9* expression than controls (Saravanan et al., 2022). Euthymic bipolar patients have been found to have higher expression levels of *MMP-9* and soluble intercellular adhesion molecule 1 (sICAM-1) compared to healthy controls. This is because these molecules are involved in the restructuring of connective tissues, and it has been suggested that the expression of sICAM-1 increases over time as the disease progresses. Because of the proteolysis of the blood-brain barrier, which allows cytokines and neurotoxic chemicals to get through the brain parenchyma and into the central nervous system, it is believed that *MMP-9* may have a role in the pathophysiology of bipolar depression (Halaris et al., 2023).

The demographic or sample size analyzed may have produced different results when our findings were compared to those found in the literature. Our investigation revealed a substantial variation in the amount of *MMP-9* gene expression between bipolar and unipolar depression patients and healthy controls. The analyses done for the *TIMP-1* gene revealed no discernible differences. Considering that *TIMP-1* is a protein that inhibits *MMP-9* activity, it may arise that stability in *TIMP-1* levels may be indicative of an inflammatory response that overcomes the regulatory mechanisms of increased activity of *MMP-9*. There may be many different reasons why these findings are contrary to the literature. For example, conditions such

as the age at which the disease begins, environmental factors, and genetic factors can cause these differences. However, the difference in the results we obtained in our study supports the idea that such differences exist between populations in which individuals with bipolar and unipolar depression interact with the environment and where environmental factors as well as genetic factors are effective in the pathogenesis of the disease. In addition, sample size is also very effective in producing these results.

The generalizability of our findings may be restricted due to some limitations in our investigation, including the relatively small sample size and the inclusion of healthy controls and patients admitted to a single facility. Furthermore, blood samples from patients and healthy controls were used in our investigation to just assess the expression levels of the *MMP-9* and *TIMP-1* genes; changes in protein levels and their functional implications were not covered. As far as we are aware, the pathophysiology of unipolar and bipolar depression is influenced by other potential genes.

Examining *MMP-9* and *TIMP-1* levels with bigger samples that include a variety of demographic groups is crucial for future research. Longitudinal studies should be carried out to further explore the impact of these genes' expression levels on the progression of the illness. The outcomes of all these research projects might pave the way for the creation of novel therapeutic approaches.

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