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

INVESTIGATION OF THE RELATION OF METHYLATION STATUS OF SYNAPSIN 2 -A GENE ASSOCIATED WITH ABNORMAL PRESYNAPTIC FUNCTION- WITH SCHIZOPHRENIA

MASTER THESIS

İbrahim FETTAHOĞLU

Department of Molecular Biology and Genetics

Supervisor: Assoc. Prof. Dr. Ceren ACAR

JUNE 2022

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

INVESTIGATION OF THE RELATION OF METHYLATION STATUS OF SYNAPSIN 2 -A GENE ASSOCIATED WITH ABNORMAL PRESYNAPTIC FUNCTION- WITH SCHIZOPHRENIA

MASTER THESIS

İbrahim FETTAHOĞLU
36203623001

Department of Molecular Biology and Genetics

Supervisor: Assoc. Prof. Dr. Ceren ACAR

JUNE 2022

ACKNOWLEDGMENTS

I would like to thank;

To my dear supervisor, Assoc. Prof. Dr. Ceren ACAR, for all her support to me in every step of my academic journey. I would like to express my deepest gratitude to her as an admirer of her for her advice and ideas that enlighten my way,

To Assoc. Prof. Dr. Mustafa Mert SÖZEN for his experiences, ideas and advice from my undergraduate to now. He is one of the most important people who made me love the field of genetics,

To Prof. Dr. Şükrü KARTALCI from psychiatry department for his helps to me in collecting the schizophrenia patients and his experience and advice help to me at the point of improve my studies,

To Inonu University Scientific Research Project Coordination Unit (BAP) for their moral and financial support with project number #FYL-2021/2719 during the experimental steps of the studies,

To my teammate, master's student Ezgi KARAASLAN for her important advice during all of my graduate studies,

To my dear family and Dilara BAKMAZ for all their endless support in every step of my life,

Finally and especially to my Dad, Kemal FETTAHOĞLU, for his all support and belief to me and his respect to my opinions at every important fork in my road.

Sevgili Babam'a, Saygılarımla…

WORD OF HONOR

I declare that this study titled "Investigation of The Relation of Methylation Status of Synapsin 2 -a Gene Associated with Abnormal Presynaptic Function- With Schizophrenia", which I submitted as my master's thesis, was written by me without resorting to any help that would against scientific traditions, and all the sources that I used were indicated both in the text and in the references, I proudly confirm this.

İbrahim FETTAHOĞLU

TABLE OF CONTENTS

INDEX OF TABLES

INDEX OF FIGURES

SYMBOLS AND ABBREVIATIONS

DNA : Deoxyribonucleic acid
% : Percent
£ : Pound
RNA : Ribonucleic acid
ncRNA : Non-coding ribonucleic acid
dNTPs : Deoxynucleotide triphosphates
BC : Before Christ.
WHO : World Health Organisation
CCD : Charge-coupled device
GABA : γ-Aminobutyric acid
GWAS : Genome-wide association study
SNP : Single-nucleotide polymorphism
CNV : Copy number variation
DSM-5 : The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
TRS : Treatment-resistant schizophrenia
ECT : Electroconvulsive therapy
HATs : Histone acetyltransferases
HDACs : Histone deacetylases
ATP : Adenosine triphosphate
lncRNA : Long non-coding ribonucleic acid
piRNA : piwi-interacting ribonucleic acid
UTR : Untranslated region
miRNA : Micro ribonucleic acid
mRNA : Messenger ribonucleic acid
5mC : 5-Methylcytosine
DNMTs : DNA methyltransferases
SAM : S-Adenosyl methionine
MBP : Methyl binding proteins
PCR : Polymerase chain reaction
pH : Potential of hydrogen
MDD : Major depressive disorder
ASD : Autism spectrum disorder

ABSTRACT

Master Thesis

INVESTIGATION OF THE RELATION OF METHYLATION STATUS OF SYNAPSIN 2 -A GENE ASSOCIATED WITH ABNORMAL PRESYNAPTIC FUNCTION- WITH SCHIZOPHRENIA

İbrahim FETTAHOĞLU

Inonu University
Graduate School of Natural and Applied Sciences
Department of Molecular Biology and Genetics

36+IX page

2022

Supervisor: Assoc. Prof. Dr. Ceren ACAR

Although schizophrenia is one of the public health problems, it is a neuropsychiatric disease whose etiology is not exactly understood. With the development of genetics, some factors that are effective in inheritance of diseases but not caused by changes in DNA sequence were discovered and these factors were called epigenetic modifications. DNA methylation is an important phenomenon that has an effect on rising of abnormal phenotypes in many cases. The most common type of DNA methylation is addition of a methyl group to the 5th position of the cytosine nucleotide of CG dinucleotides found in CpG islands. Investigation of the association between DNA methylation and schizophrenia is important for elucidating the hereditary basis of schizophrenia. Like other neuropsychiatric disorders, synapse-related genes are important candidate genes for schizophrenia. The results of previous association studies between the SYN2 gene and schizophrenia, confirm that the SYN2 gene is a strong candidate gene for schizophrenia. In the light of this scientific information, methylation patterns of 3 different CpG regions in promoter of SYN2 gene were compared between healthy controls and schizophrenia patients. 33 healthy controls and 39 schizophrenia patients were included in this study. The study was performed using pyrosequencing method. As a result of the statistical analysis, it was confirmed that there is a significant relationship between the methylation pattern of the SYN2 gene and schizophrenia. In addition to detecting significant DNA methylation differences in the 2nd and 3rd CpG regions, significant differences were also found in the average methylation ratio.

Keywords: Schizophrenia, DNA methylation, SYN2, Epigenetics.

ÖZET

Yüksek Lisans Tezi

ANORMAL PRESİNAPTİK FONKSİYON İLE İLİŞKİLİ GENLERDEN SİNAPSİN 2'NİN METİLASYON DURUMUNUN ŞİZOFRENİ İLE BAĞLANTISININ ARAŞTIRILMASI

İbrahim FETTAHOĞLU

İnönü Üniversitesi
Fen Bilimleri Enstitüsü
Moleküler Biyoloji ve Genetik Anabilim Dalı

36+IX sayfa

2022

Danışman: Doç. Dr. Ceren ACAR

Şizofreni dünya üzerindeki başlıca halk sağlığı problemlerinden birisi olmakla birlikte etiyolojisi henüz tam olarak anlaşılamamış nöropsikiyatrik bir hastalıktır. Genetik biliminin gelişmesinin akabinde hastalıkların kalıtımında etkili olan ancak DNA dizisi üzerindeki değişikliklerden kaynaklanmayan bazı etkenler keşfedilmiş ve bu etkenlere epigenetik modifikasyonlar adı verilmiştir. Başlıca epigenetik modifikasyonlardan birisi olan DNA metilasyonu, birçok durumda anormal fenotipin görülmesinde etkisi olan fenomenlerden birisidir. DNA metilasyonunun en sık görülen türü, DNA üzerindeki CpG adacıklarında bulunan CG dinükleotidlerinin sitozin nükleotidine 5. pozisyonundan metil grubunun kovalent olarak eklenmesi işlemidir. DNA metilasyonu ve şizofreni arasındaki bağlantının incelenmesi, şizofreninin kalıtsal temellerinin aydınlatılması ve moleküler patolojisinin tam olarak anlaşılabilmesi açısından önemlidir. Birçok diğer nöropsikiyatrik hastalıkta olduğu gibi, sinaps ilişkili genler şizofreni için önemli aday genlerdir. Sinaptik veziküllerin salınımından sorumlu fosfoproteinlerden birisi olan Sinapsin 2 proteinini kodlayan SYN2 geni ile şizofreni arasında daha önce yapılan bağlantı çalışmalarının sonuçları, SYN2 geninin kuvvetli bir şizofreni aday geni olduğunu doğrulamaktadır. Tüm bu bilimsel veriler ışığında, SYN2 geninin promotör bölgesinde bulunan 3 farklı CpG bölgesinin metilasyon paternleri sağlıklı kontroller ve şizofreni hastaları arasında karşılaştırılmıştır. Bisülfit dönüşümü ve pirosekanslama yöntemi kullanılarak yürütülen bu çalışmaya 33 sağlıklı kontrol ve 39 şizofreni hastası dahil edilmekle birlikte elde edilen sonuçlar istatistiki olarak incelenmiştir. Analizler sonucunda SYN2 geninin metilasyon paterni ile şizofreni hastalığı arasında anlamlı bir ilişki olduğu doğrulanmıştır. 2. ve 3. CpG bölgelerinde anlamlı DNA metilasyon farkı tespit edilmesinin yanı sıra ortalama metilasyon oranında da anlamlı farklılıklar bulunmuştur.

Anahtar Kelimeler: Şizofreni, DNA metilasyonu, SYN2, Epigenetik.

1. INTRODUCTION

Psychiatric disorders (also known as mental illness or mental disorders) are health problems that are specialized in emotion, thinking or behaviour. These problems can also be seen as a combination in most mental illnesses. Schizophrenia is a neuropsychiatric disorder with a heterogeneous genetic and neurobiological background that influence early brain development [1]. Symptoms of schizophrenia can be categorised into three main groups as positive, negative and cognitive symptoms. Hallucinations and delusions are common symptoms that are found in the positive symptom groups. Common negative symptoms can order as anhedonia (pleasure deficiency), avolition (lack of motivation), alogia (poverty of speech) and blunted effect. Poor memory is one of the frequent symptoms in schizophrenia and it is grouped on the cognitive symptoms. Other cognitive symptoms can be listed as speech and communication problems, inattention and difficulties in planning. Although the ratio changes from region to region, generally, schizophrenia affects nearly 1% of the world population [2]. The incidence is about 1.5 per 10,000 people [3]. Because of the high prevalence of the disorder, schizophrenia costs a high amount of money in developed countries. In England, schizophrenia costs £11,8 billion per year [4]. In recent years, psychotic disorders have been associated with some epigenetic modifications like DNA methylation.

Epigenetics is a field of genetics that is interested in mechanisms that do not involve changes in the genotype but regulate the gene expression [5]. These mechanisms can be categorized into three main groups as histone modifications, ncRNAs and DNA methylation. Histone modifications can be defined as adding or removing of some special chemical groups like methyl, acetyl or etc. to histone proteins. They control the gene expression by remodelling chromatin structure. The second group of epigenetic modifications is ncRNAs' mechanisms. ncRNAs control gene expression in different ways and at different levels. They interact with DNA, RNAs and different proteins to regulate them [6]. DNA methylation is just adding of a methyl group (CH_3) to bases of DNA. Most studied and well-known epigenetic modification type is also DNA methylation.

Synapses are important elements that help the transmission of information and molecules between neurons or from neurons to another target cell. The first evidence of relationship

between schizophrenia and synapse is not clear but today we know both neurological and neuropsychiatric disorders have relationships with synapses [7]. Presynaptic terminals are considerable regions to understand the relationship between synapse and mood disorders. Synapsins are special phosphoproteins, they have critical roles in formation and controlling of the synaptic vesicles in the presynaptic region. There are three different forms of Synapsin. Synapsin I, Synapsin II and Synapsin III, respectively, and SYN1, SYN2 and SYN3 are genes that encode the synapsin proteins.

1.1 Schizophrenia

Schizophrenia is a chronic neuropsychiatric illness with different symptoms and it has complex background. First symptoms of schizophrenia are generally emerging in late adolescence and early adulthood. Effect of the psychosis can show variety from person to person. Psychosis refers to loss of contact with reality. The person who is diagnosed as schizophrenic cannot distinguish the difference between hallucinations and reality. Due to both its high prevalence and cost to society, schizophrenia is one of the major problems in public health.

1.1.1 History of schizophrenia

The etymologic roots of the word “schizophrenia” is coming from ancient Greek. “Schizein” and “phrene” words are meaning “split” and “mind”, respectively, therefore the meaning of “schizophrenia” is splitting of mind in ancient Greek. Actually, psychiatric and mood disorders are admitted as old as human race and history of schizophrenia is back to prehistoric civilizations. The patients were recorded who have the symptoms today known as symptoms of schizophrenia in ancient Greek, Egypt and Indian civilizations. The earliest records about the psychoses are found in the Vedas (religious texts originating in ancient India) of ancient Hindus (1400 BC). Like all other religions sources, people with psychosis are depicted as their souls are seized by fictitious and mythological characters like devils or etc. in The Vedas. Similar texts have also been found on tablets in Mesopotamia, today's the Middle East. In the west, prehistoric Greek doctors' records have revealed clinical cases with similar symptoms.

Although modern schizophrenia studies were beginning with German psychiatrist Emil Wilhelm Magnus Kraepelin, some important studies also have been done by other scientists before him. In the first half of the 19th century, Philippe Pinel, John Haslam, George Man Burrows and Wilhelm Griesinger were European scientists, who try to understand and

classify schizophrenia and they use “dementia” term to describe schizophrenia [8]. Emil Kraepelin uses the Latin originated term “*dementia praecox*” in literature to describe schizophrenia. Dementia praecox meaning is early dementia and Kraepelin believe patients with “dementia praecox” have problem with their brain. According to Kraepelin, their brain had begun to deteriorate prematurely. Kraepelin’s studies about age of onset and family history of schizophrenia are important milestones to understanding the difference between schizophrenia and major depressive disorders. He specially emphasizes the family history in the “*dementia praecox*”. His studies on family history of schizophrenia also are precursor for genetic studies of mental illness.

The word “schizophrenia” was coined to literature by the Swiss psychiatrist Paul Eugen Bleuler in a scientific meeting of German Association for Psychiatry in 1908. He classified symptoms of schizophrenia into two groups and he called them as positive and negative symptoms. According to Bleuler, schizophrenia cannot be cured completely. Unlike Kraepelin, he knows dementia is not one of the first reasons of schizophrenia. Bleuler also studied about the basic and accessory symptoms of schizophrenia and he categorised symptoms according to frequency in patients. Basic symptoms are seen in all of the patients while accessory symptoms are not. Bleuler associates the basic symptoms with neurobiological processes [9, 10].

Post-Bleuler studies of schizophrenia was especially performed by scientists in USA. Adolf Meyer who was a Swiss-born American scientist tried to threat schizophrenic patients with modern and humanistic methods. Our understanding about schizophrenia is improved over a hundred years after Bleuler. Psychiatrists try to find new and effective treatment methods against schizophrenia. First neuroleptics (antipsychotics) were used in 1950s. Today, schizophrenia studies are going on all around the world with the technology of the 21st century [11].

1.1.2 Symptoms of schizophrenia

Patients with schizophrenia can show a variety of symptoms and some of these symptoms can change from person to person. Today, symptoms of schizophrenia fall into three main groups. These are positive, negative and cognitive symptoms of schizophrenia. Positive symptoms mainly include hallucinations, such as hearing or seeing the things that doesn’t exist, formal thought disorder, paranoia and inflated perceptions or beliefs [12]. These positive symptoms are generally can be taken under control with antipsychotic drugs. Negative symptoms generally specialized in social life of the people. There is a huge

decrease or completely loss in the ability to initiate plans, speak, express emotion or find pleasure. Negative symptoms specially effect the social life of patients. The third and last group of symptoms are cognitive symptoms and are involved in confused thinking and speech, troubles with logic and inflated behavior or movements. Deficit of attention and troubles in short-term memory are also important parts of the cognitive symptoms of schizophrenia [13].

1.1.3 Epidemiology of schizophrenia

Epidemiological data have a critical role to analyse and understand disorders. Since schizophrenia research have been started, epidemiologists are focused on the epidemiological part of these studies. There are confirmed and strong factors associated with the onset of schizophrenia. These factors can mainly be listed as; age and gender, addiction, ethnicity, family history and social class of the person. Except those, there are comorbidity between schizophrenia and some other disorders like cardiovascular disorders and obesity [14]. Besides, different ethnic groups have different ratio of schizophrenia. For instance, Hispanic Americans and Black Americans under higher risk of schizophrenia compared with the White Americans. In Europe, African immigrants have more incidence of schizophrenia than White Europeans. Another interesting ethnic group based on schizophrenia incidence is Caribbeans. Caribbean based people have higher risk of schizophrenia [15]. Other interesting factors that have an effect on the onset of schizophrenia are season of birth, individuals with relatively young and old parents (older than the 40s and younger than 20s) and place of birth. Individuals born in late winter or early spring have a higher risk than others based on schizophrenia. The people who were born and raised in large cities under higher risk of schizophrenia compared with the people who were born and raised in villages [16-18].

According to WHO (World Health Organisation) data in 2022, schizophrenia affects approximately 24 million people worldwide. The distribution of disorder is not equal among all of age and gender. Schizophrenia rate in adults is 1 in 222 people and its equal to 0.45% according to WHO data. Age of onset is often during late adolescence and the twenties, and it tends to exist earlier among men than among women.

1.1.3.1 Age and gender in schizophrenia

The age of onset and distribution in genders of schizophrenia are most investigated epidemiological studies in literature. Actually, the incidence of schizophrenia is higher in

men than women. Although the incidence of schizophrenia is higher in men than in women, if we consider the second half of the life, after 40 years old, the incidence is the opposite. While the age of onset is between 15 and 25 years old in men, the onset peak is between 20 and 35 years old in women. Besides those, another important increased age of onset is between 45 and 50 years old in women related to a significant drop in estrogen levels [19].

1.1.3.2 Addiction and schizophrenia

Epidemiological studies done in schizophrenic populations show there is a huge ratio of addiction in schizophrenia patients. There are two main hypotheses about this phenomenon. First, the possible common biological background between schizophrenia and addictions and the second is the theory of self-medication. The common biological background theory suggests some biological reasons found in schizophrenics cause the addiction while self-medication theory suggests schizophrenics try to counter the deficit linked to their disorders by using the substances they take to cope with their emotional problems. Alcohol addiction, smoking and abuse of psychostimulants are among the most frequently reported addictions in schizophrenia. The prevalence of alcoholism in schizophrenic population is 50% in the USA [20]. More interestingly, smoking is highly prevalent in schizophrenics. The prevalence of smoking is about 85% schizophrenic population in the USA [21].

1.1.3.3 Family history in schizophrenia

Genetic basis of disorders was one of the attractive topics during history. Like many other genetic disorders, family history is one of the most important risk factors for schizophrenia. Schizophrenia researchers discover the strong family history in schizophrenia patients. After the modern schizophrenia studies start, family studies indicate the rate of schizophrenia is higher in individuals in the same families. In the first half of 20^{th} century, twin studies were the spearhead for understanding of the genetic basis of schizophrenia. Monozygotic and dizygotic twins were used in these studies and results show identical twins have higher risk of schizophrenia [22]. These studies were also prototype of the genetic researches in the field of schizophrenia.

1.1.4 Etiology of schizophrenia

Although schizophrenia researches ongoing for a long time, etiology of the illness is still unclear. Most important reason of these situation is there are a lot of different factors of disorder. Main factors that have an effect on schizophrenia list as; genetic factors, neurodegenerative factors, neurochemical factors, environmental factors and infections [23].

1.1.4.1 Neurochemical hypotheses in etiology of schizophrenia

Neurotransmitters are chemical groups that are released by neurons and stimulate neighbouring neurons or muscle cells. Neuropsychiatric disorders like schizophrenia are highly multifactorial and their etiologies are confusing. One of the important hypotheses about the etiology of schizophrenia is neurochemical hypothesis. Neurochemical hypothesis suggests a relationship between neurotransmitters and schizophrenia. The neurotransmitters that have a critical role in etiology of schizophrenia are mainly dopamine, serotonin, glutamate and γ-aminobutyric acid (GABA) [24, 25].

1.1.4.1.1 Dopamine hypothesis

Dopamine is a neurochemical molecule that play an important role in cells and communication between cells. The rising of the dopamine hypothesis started with the discovery that neuroleptics are blocking the dopamine receptors in the brain in the 1960s. Researchers discover the blocking of dopamine receptors are reducing the positive symptoms of schizophrenia. The dopamine hypothesis suggests dopaminergic hyperactivation in mesolimbic pathway cause the positive symptoms of schizophrenia and negative symptoms arise as a result of decreased dopamine activity in the mesocortical pathway. Today, most antipsychotic mechanisms are based on the dopamine hypothesis and they block the dopamine receptors in the postsynaptic region to reduce positive symptoms of schizophrenia, especially dopamine receptor D2 [26].

1.1.4.1.2 Serotonin hypothesis

Serotonin is a critical molecule for living organisms and it has a role both as a neurotransmitter and hormone in the body. It plays a crucial role in mood, emotions, appetite, and digestion. As the precursor of melatonin, it helps regulate sleep cycles and the body clock. The serotonin hypothesis of schizophrenia suggests chronic stress-induced serotonergic overdrive in the cerebral cortex in schizophrenia is one of the basic causes of the disease. Blockage of the serotonergic receptors in postsynaptic regions by atypical neuroleptics (they have low affinity to dopamine receptors and more affinity to serotonin receptors) again slows down the progress of schizophrenia [27].

1.1.4.1.3 Glutamate hypothesis

The glutamate hypothesis of schizophrenia is focused on the deficiency of the activity of glutamate in the prefrontal cortex. Dopamine inhibits glutamate release. Therefore, high levels of dopaminergic activity have similar effects to decrease levels of glutamate activity.

Interestingly, some drugs that block glutamate receptors may increase the symptoms of schizophrenia by increasing the dopamine effects and disrupting the balance between dopamine and glutamate [28].

1.1.4.2 Genetic factors in etiology of schizophrenia

Modern genetics research shows that genetic factors have a huge effect on the pathophysiology of most diseases. Since modern schizophrenia research begins, the hereditary part of the disorder is an attractive issue for scientists. The scientists who studied early genetics studies of schizophrenia discover the hereditary model of schizophrenia is not a classical Mendelian monogenic model. The studies of genetic background of the schizophrenia began with the twin and family experiments. Family studies show changes in the risk of schizophrenia between individuals according to their kinship. As previously stated, genetically identical twins are the individuals who have the most risk based on schizophrenia. The risk of schizophrenia is about 50% in monozygotic twins and these risks reduce with the low kindship relation. A graph is given in Figure 1.1 that show the risk rate of schizophrenia in different kindship. These data are enough to categorize schizophrenia as a genetic disorder [29].

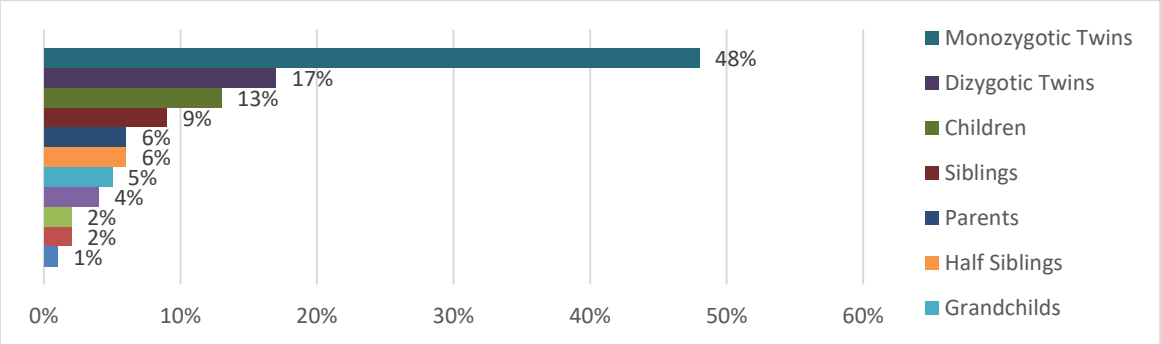

Figure 1.1 Relationship between schizophrenia and kindship [30].

Genome-wide association studies (GWAS) are changing our understanding of the genetics of schizophrenia. With the start of the Human Genome Project, schizophrenia studies entered a new age. New InDels, SNPs and CNVs that associated with schizophrenia discovered by geneticist. Beyond these, some important genes are directly associated with schizophrenia. These genes mainly include DISC1, NRG1, DTNBP1, PRODH2, COMT, 5HT2A, DRD2 and DRD3. Besides those, there are thousands of candidate genes that are still investigated by scientist. Recent times, synapse-related genes are common research subjects in schizophrenia candidate gene researches. The studies that were completed in the

last decade showed expressions of synaptic function-related genes like CALM2, SYN1, RAB3A and TUBB4 decreased in psychiatric disorders [31].

Although genetic factors are effective on schizophrenia, the effects of thousands of genes are heterogeneous and they are not sharp as in single-gene disorders genes. Modern genetics studies are still ongoing about schizophrenia and our knowledge increase day by day. The role of genetic factors in the etiology of schizophrenia is an issue that is illuminated day by day.

1.1.4.3 Environmental factors in the etiology of schizophrenia

The etiologies of multifactorial disorders like schizophrenia may be confusing because of their nature. Except for the biological and genetic background, some other factors can cause schizophrenia. These factors can be categorised into a class called environmental factors. Environmental factors do have not as sharp effect as biological factors but still, they must be taken into attention. Mainly environmental factors in the etiology of schizophrenia can be listed as; obstetric complications, season of birth, prenatal and postnatal infections, maternal malnutrition, maternal stress, child abuse, abnormal life events, immigration, head injury, drug abuse, adverse childhood experiences and extreme living conditions.

1.1.5 Diagnosis and treatment of schizophrenia

Diagnosing the disorders is the first step toward efficient treatment. Most of the disorders are diagnosed by some biochemical indicators in blood or other body fluids. In the case of psychotic disorders, there aren't any biochemical indicators from blood. Diagnosis criteria of schizophrenia were determined by The Diagnostic and Statistical Manual of Mental Disorders 5th Edition (DSM-5) published by the American Psychiatric Association in 2013 [32]. According to DSM-5 criteria, diagnosis requirements are given in the next title.

The treatment of schizophrenia is generally long-term. Neuroleptics are most common way of the treatment of schizophrenia. The neuroleptics are drug group that mainly effect the neurochemical balance of the brain and they stabilize the mood of individuals. Main effect mechanisms of the neuroleptics are back to neurochemical hypotheses of schizophrenia, especially dopamine hypothesis, and they block the receptors that found in the post-synaptic region. Antipsychotic drugs mainly can be divided into two groups, first is typical neuroleptics and the second is atypical neuroleptics. The main difference between typical and atypical neuroleptics is their receptors. While typical neuroleptics block the dopamine receptor D2, atypical ones effect the other receptors, primary other dopamine

receptors and 5HT2 receptors. There are also some advantages and disadvantages of these drugs. Dopamine receptor D2 blockers are just effective on positive symptoms of schizophrenia while atypical drugs have effect both on positive and negative symptoms. On the other hand, typical neuroleptics have serious side effects but atypical drugs have fewer side effects compared to typical ones [33]. In some cases, patients can show a resistance to treatment with antipsychotics. This type of schizophrenia is called treatment-resistant schizophrenia (TRS). Psychiatrists can prefer some alternative treatment methods like electroconvulsive therapy (ECT) against TRS. ECT is a treatment method used to treat certain psychiatric disorders. It includes the passing of a carefully controlled electric current to the brain and affects the brain's activity with the aim of relieving severe psychotic symptoms [34].

1.1.5.1 DSM-5 diagnostic criteria for schizophrenia

A. Two (or more) of the following, each present for a significant portion of time during a 1-month period (or less if successfully treated). At least one of these must be (1), (2), or (3):
    1. Delusions.
    2. Hallucinations.
    3. Disorganized speech (e.g., frequent derailment or incoherence).
    4. Grossly disorganized or catatonic behavior.
    5. Negative symptoms (i.e., diminished emotional expression or avolition).

B. For a significant portion of the time since the onset of the disturbance, level of functioning in one or more major areas, such as work, interpersonal relations, or self-care, is markedly below the level achieved prior to the onset (or when the onset is in childhood or adolescence, there is failure to achieve expected level of interpersonal, academic, or occupational functioning).

C. Continuous signs of the disturbance persist for at least 6 months. This 6-month period must include at least 1 month of symptoms (or less if successfully treated) that meet Criterion A (i.e., active-phase symptoms) and may include periods of prodromal or residual symptoms. During these prodromal or residual periods, the signs of the disturbance may be manifested by only negative symptoms or by two or more symptoms listed in Criterion A present in an attenuated form (e.g., odd beliefs, unusual perceptual experiences).

D. Schizoaffective disorder and depressive or bipolar disorder with psychotic features have been ruled out because either 1) no major depressive or manic episodes have occurred concurrently with the active-phase symptoms, or 2) if mood episodes have occurred during active-phase symptoms, they have been present for a minority of the total duration of the active and residual periods of the illness.
E. The disturbance is not attributable to the physiological effects of a substance (e.g., a drug of abuse, a medication) or another medical condition.
F. If there is a history of autism spectrum disorder or a communication disorder of childhood onset, the additional diagnosis of schizophrenia is made only if prominent delusions or hallucinations, in addition to the other required symptoms of schizophrenia, are also present for at least 1 month (or less if successfully treated).

1.2 Epigenetic Modifications

Epigenetics term was firstly used by Conrad Hal Waddington who is British developmental biologist to describe whole complex of developmental processes between genotype and phenotype in 1942. Research labelled "epigenetics" has still remained marginal until the 1990s. In modern biology, epigenetics is a field that is rapidly developed specially after end of the millennium. The modern term of epigenetics refers to the study of changes in gene expression, it is not caused by changes in the primary DNA sequence of organisms. The classical understanding of genetic disorders is mutations that cause the genetic disorder. Mutations simply can define as any changes in the sequence of the DNA. According to the central dogma of genetics, mutated DNA causes an abnormal phenotype. The scientists perceived an important fact in 20^{th} century. In some cases, abnormal phenotype can arise without mutated DNA. This phenomenon shows some other factors have a huge effect on gene expression except DNA sequence. Today, we know these factors as epigenetic factors [35].

Epigenetic modifications are basic mechanisms that are involved in the control of gene expression without any changes in DNA sequence. These mechanisms can be categorized in three main groups as DNA methylation, histone modifications and ncRNA mechanisms. DNA methylation is the most studied group of the epigenetic modifications and it refers adding a methyl group (CH_3) to DNA bases. Histone modifications are adding or removing some chemical groups like methyl, acetyl, phosphate or ubiquitin to histone proteins in the

structure of chromatins. Non-coding RNA mechanisms are also important part of the epigenetic mechanisms they take a crucial role in the control gene expression [36].

1.2.1 Histone modifications

Eukaryotic genomes are not immobile structures, instead they are highly active and they can change their structure with some modifications especially on their histone proteins. Histone proteins are special group of proteins they take crucial role in the eukaryotic genome organisation. There are four main types of the histone proteins, H2A, H2B, H3 and H4. These histones are coming together to form histone octamers and they take an important role in the core structure of nucleosomes [37]. Eukaryotic chromatin can be found on two main forms of it and they are euchromatin and heterochromatin. The main difference between these two forms is the different accessibility of DNA for transcription factors and polymerases. The transition between euchromatin and heterochromatin structures is mainly completed with histone modifications. Histone modifications are adding or removing some chemical groups to the tail part of the histones. These chemical groups are primary methyl, acetyl and phosphate groups. Except from those groups, there are some minor modifications also occurring in the histones.

1.2.1.1 Histone acetylation

Acetylation of histones was firstly reported by Vincent Allfrey and colleagues in 1964. Acetylation and deacetylation of lysine residues in histone tails are controlled by two different enzyme groups they show opposite action in fact. Histone acetyltransferases (HATs) are responsible enzyme groups for histone acetylation and they use acetyl CoA to catalyse the transfer of an acetyl group to the lysine side chain in histones [38]. The transfer of acetyl group to lysine side chains is neutralize the positive charge of lysine and provide a weak interaction between histone and DNA. There are two major groups of HATs as type-A and type-B. Although HATs are major enzymes that they responsible for histone acetylation, they often have to work together with large other multiprotein complex to complete their job. Histone deacetylases (HDACs) are enzyme group they have main role in histone deacetylation. Actually, they are working as a reverse HATs and they catalyse removing of acetyl group from the histon tails. The removing of acetyl helps the restoration of positive charge of lysine and support the gravitation between histones and DNA [39]. The key point about histone acetylation and deacetylation is changing the positive charge of lysine in histone tails. These changes provide more or less interaction between histone and DNA. Histone acetylation provides less interaction and it helps the transition of

heterochromatin to euchromatin. Because of this transition, DNA become more available to transcribe. In the opposite case, when HDACs remove the acetyl from histone tails, histones return their initial strong positive charge and this positive charge provides a strong gravity between histone and DNA. This gravity help stabilize the chromatin architecture in the region and DNA becomes less available to transcribe.

1.2.1.2 Histone phosphorylation

Phosphorylation of histones is another important group of histone modifications. Like other modifications, histone phosphorylation is highly dynamic and controlled. Histone phosphorylation is generally occurring in serine, threonine and tyrosine residues of the histone tails. Kinases and phosphatases control the level of histone phosphorylation and they are responsible for adding and removing, respectively. Histone kinases catalyse the transferring of a phosphate group from ATP to target amino acids in histone tails. Like histone acetylation, transferring the phosphate group to the target amino acid provides a change in the charge of amino acids. They add a highly negative charge to the target [40].

1.2.1.3 Histone methylation

Adding the methyl group to histone tails is the most studied and maybe interesting type of the histone modifications. Histone methylation can occur in lysine and arginine side chains. Unlike the acetylation and phosphorylation, methyl groups cannot change the charge balance of the histones instead, they prevent the accessibility of DNA transcription factors and they block the gene expression. Methylation and demethylation of histones are catalyzed by methylases and demethylases, respectively. Another important exception during histone methylation is the different number of attached methyl groups in single lysine and arginine positions. Lysine residues can be methylated three times while arginine residues can be methylated two times in the same position [41].

1.2.2 Non-coding RNAs

Non-coding RNAs (ncRNAs) are a subgroup of RNA molecules that do not encode a protein and have some regulatory roles in the cells. Their roles in the control of gene expression at the level of epigenetics (there is no change in the original DNA sequence but they can affect the gene expression) are relatively newly figured out compared with histone modifications and DNA methylation. ncRNAs mainly can be divided onto two categories as small ncRNAs and long ncRNAs (lncRNAs). The difference between these two groups of ncRNAs is their size. The ncRNAs that are larger than 200 nts are classified as lncRNAs and

shorter than 200 nts ones are called small ncRNAs. The epigenetic activities of ncRNAs can affect the gene expression at different levels like transcriptional, translational or post-translational levels. For instance, piwi-interacting RNA (piRNA) that a type of ncRNAs, binds to piwi protein and this protein attaches to the genomic DNA to silence it. This example shows how piRNA molecules complete their role based on epigenetic regulation [42].

Another example to epigenetic activities of ncRNAs is miRNAs. miRNAs are typical type of small ncRNAs they generally about 22 nt length and they control the gene expression in the post-transcriptional level. miRNAs are generally bind to 3' untranslated region (3' UTR) of the mRNAs and they repress the translation. The Figure 1.2 show the how miRNAs block translation machinery and epigenetic activity of the miRNAs.

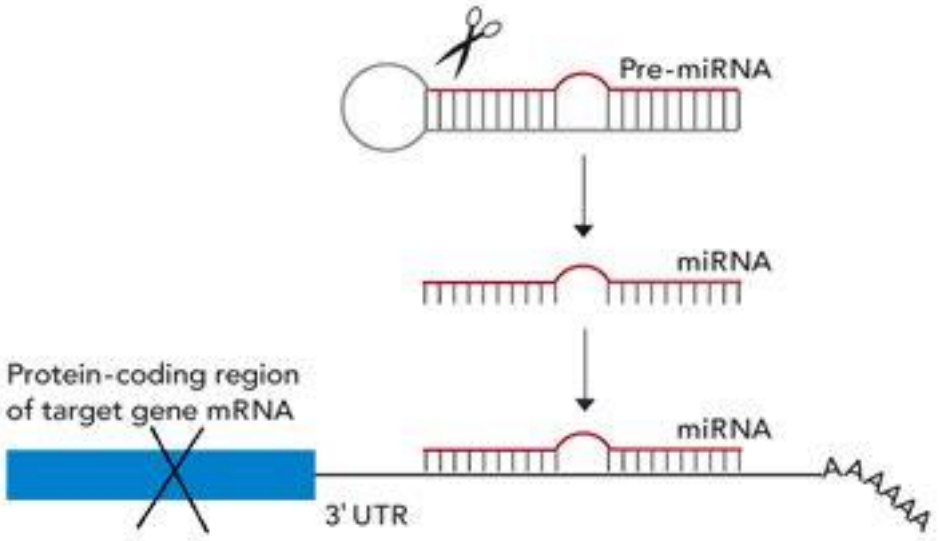

Figure 1.2 Epigenetic activity of miRNAs [43].

1.2.3 DNA methylation

DNA methylation is the most studied type of the epigenetic modifications. In the eukaryotic genome, most seen type of the methylation is cytosine methylation and it formed with the attachment of a methyl group to 5' carbon position of the cytosine. Methylated cytosine (5mC) was firstly reported by Rollin Hotchkiss in 1948 [44]. Although methylated DNA have some different roles, these are not clear until 1980s.

An important enzyme group called DNA methyltransferases (DNMTs) have active role in the catalysing of DNA methylation. DNMTs catalyse the transfer of a methyl group from S-Adenosyl methionine (SAM) to 5' carbon of the cytosine to form 5mC. There are five different DNMTs that are encoded by human genome and these are DNMT1, DNMT2 DNMT3A, DNMT3B and DNMT3L. DNMT1, DNMT3A and DNMT3B grouped as canonical DNMTs and show catalytic activity but DNMT2 and DNMT3L do not [45].

DNA methylation is a typical reversible modification and reversing of the DNA methylation called DNA demethylation. Two main mechanisms have an active role in DNA demethylation. The first mechanism is Ten-eleven translocators-mediated oxidation of 5mC and the other is thymine DNA glycosylase-mediated base excision repair[46].

The roles of DNA methylation are highly dependent on its location in the genome. CG dinucleotides can be seen frequently in some regions of the DNA. These regions are called CpG islands and they are highly related with the promotor regions of the genes. Most of the genes' promotors are found at CpG islands. These regions are generally weak based on the DNA methylation when transcription of the gene is active. DNA methylation in the promotor regions is generally repressing the gene expression with two main ways. The first is inhibiting the recruitment of transcription factors and the second is with recruitment of some special protein called methyl binding proteins (MBP) to block transcription machinery. Interestingly, DNA methylation is highly observed in the bodies of actively transcribed genes. As previously stated, the location of DNA methylation has a huge importance in role of methylation in gene expression. Except those, DNA methylation also takes crucial roles in the repressing of the germ-line specific genes, embryonic development and transposons. DNA methylation also have critical place in the protection of integrity of the eukaryotic genome. [47].

1.3 Synapsins and SYN2

Synapse is a special type of interaction between neurons or between neuron and other target cells. The main physiological parts of synapse can classify under 3 main parts. Presynaptic end, synaptic gap (a.k.a synaptic cleft) and postsynaptic end. Summarily, a group of signal molecules (called neurotransmitters) coming from the presynaptic region, are released the synaptic gap and they interact with their receptors that located in the postsynaptic region. In the presynaptic neurons, neurotransmitters are carried in a special type of vesicle that are called synaptic vesicle, before they are released to the synaptic gap.

An illustrated figure is given in Figure 1.2 that show main parts of the synapse. The control of the synaptic vesicle releasing is highly important for the health of humans. A large group of proteins they called synaptic proteins help to control synaptic vesicle release.

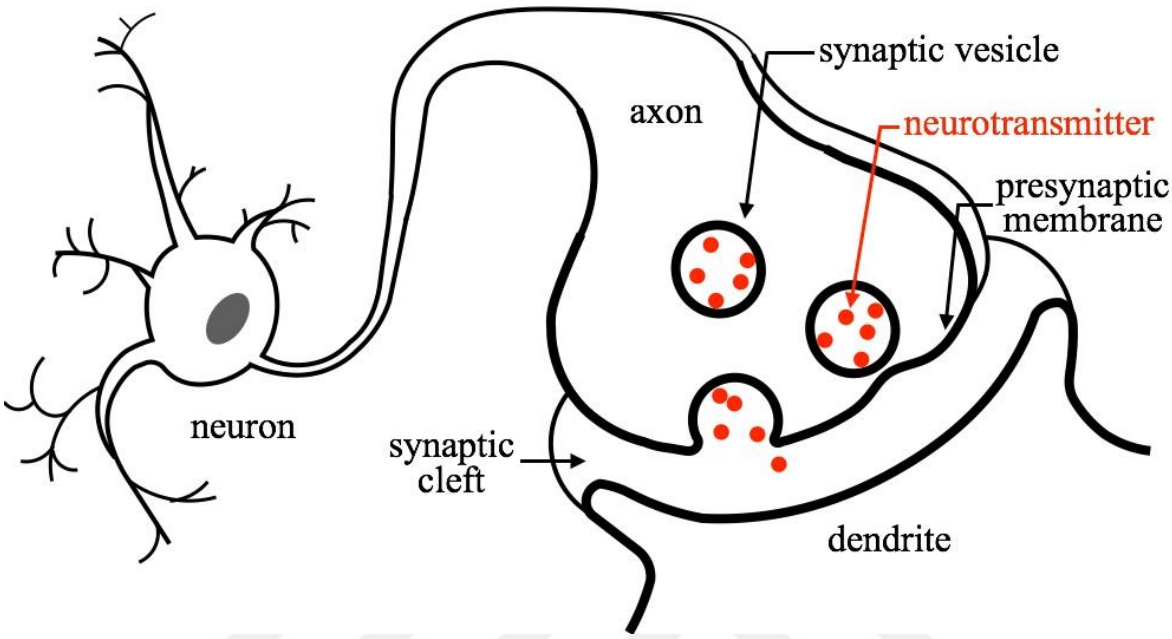

Figure 1.3 The main parts of synapse.

Synapsins are special type of synaptic phosphoproteins they help to control neurotransmitter release. Edward Johnson and colleagues firstly report them in rats in 1972. They were firstly reported in humans by Paul Greengard and colleagues as neuronal specific proteins in the end of 1970s [48]. Their most known function is the control of the synaptic vesicle exocytosis by attaching and releasing them. Synapsins have a critical relationship with kinases. In the unphosphorylated state of synapsins, they attach to the synaptic vesicles and prevent their exocytosis. When a kinase phosphorylates the synapsins, they stop the blocking of synaptic vesicles and the synaptic vesicle can do exocytosis to the synaptic gap [49]. They are the most abundant protein group in synaptic vesicles and they consist of 1% of total brain proteins. Many researchers have doubt about their relationship with neurological and psychological disorders like Alzheimer disease, autism, epilepsy and schizophrenia.

There are three types of synapsins encoded by human genome as Synapsin 1, Synapsin 2 and Synapsin 3 and they are encoded by genes SYN1, SYN2 and SYN3, respectively. All

types of synapsins have two isoforms as a and b. The genomic locations of these three genes are given in the Figures 1.3, 1.4 and 1.5 in below. Synapsin 1 is the main synapsin involved in axon elongation and regulation of the kinetics of synaptic vesicle fusion. It is the main type of synapsin that regulates synaptogenesis and neurotransmitter release.

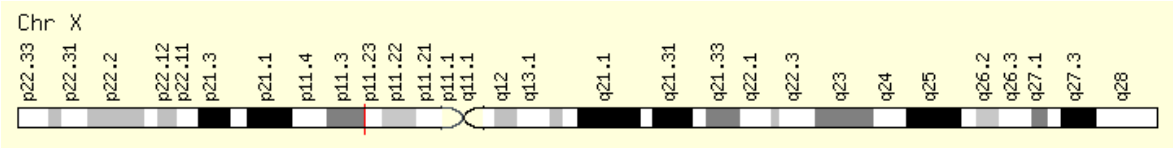

Figure 1.4 Genomic location of SYN1 gene.

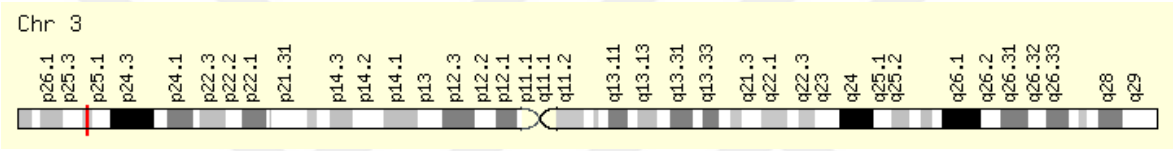

Figure 1.5 Genomic location of SYN2 gene.

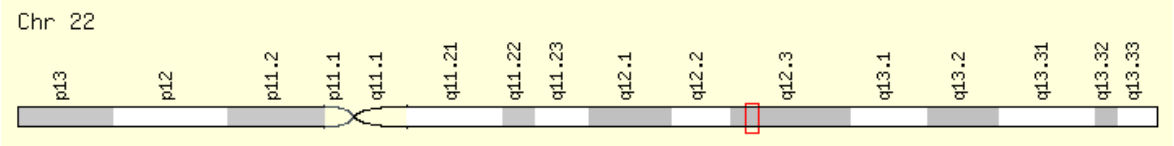

Figure 1.6 Genomic location of SYN3 gene.

In the light of all this theoretical knowledge, we decide to investigate the relationship between schizophrenia and the methylation status of the SYN2 gene with a strong doubt about a significant relationship. Both roles of Synapsin 2 protein and the importance of synapse for psychiatric disorders support our doubt. Bisulfite conversion and pyrosequencing technologies were used to investigate our hypothesis. Our aim is to prove this relationship and fill the gap in scientific literature. Methodological details of the study were given in the material and methods part of the thesis.

2. MATERIALS AND METHODS

In this study, we tried to understand the DNA methylation difference in 3 specific CpG positions in the SYN2 gene between healthy and schizophrenic subjects using pyrosequencing. DNA methylation analysis by pyrosequencing is a modern method to analysis of specific or global DNA methylation in human genome. Analysed sequence and CpG positions were 5'-CGCCCTCCCCGCATAGTCACGT-3' (CpG positions are shown in blue background). In accordance with this purpose, 39 volunteer schizophrenia patients who applied to the Department of Psychiatry in the Faculty of Medicine at Inonu University and 33 volunteer healthy controls were involved in this study. All of the subjects got information about the study and consent forms were collected. Consent forms for schizophrenia patients were collected from their *curator bonis*.

The first step of the research was determining the subjects. The blood of subjects collected by professional medical staff and bloods were used in isolation of genomic DNA. DNAs were isolated using a commercial isolation kit (PureLink™ Genomic DNA Mini Kit) according to the supplier's protocol. Obtained DNAs were stored at 4 °C until quantification. Quantification of the DNAs were complete with Denovix ds-11 fx+ fluorometer and they standardised to next step, bisulfite conversion. Bisulfite conversion is an important step in most of the DNA methylation analysis, also for pyrosequencing. As will be mentioned in detail later, after bisulfite conversion, cytosine nucleotides in the DNA that is not methylated will change to uracil while methylated cytosines stay as cytosine. After a few cycles of polymerase chain reaction (PCR), there is a noticeable difference between converted and not converted DNA by sequencing. Bisulfite conversion of DNAs was performed with another commercial kit (EpiTect Bisulfite Kit) according to the supplier's protocol. Before pyrosequencing procedure, our target region of DNA (3 specific CpG positions in SYN2 gene) was amplified by PCR. Amplified SYN2 genes were sequenced by pyrosequencing. After bisulfite conversion a special type of commercial kit was used in the rest of the study

(PyroMark CpG Assay). PCR and pyrosequencing steps were performed according to kit supplier's procedures. General experimental outline of the study is given in Figure 2.1.

Figure 2. 1 General experimental steps of study.

2.1 DNA Extraction

DNA isolation from the blood samples of subject was performed by PureLink™ Genomic DNA Mini Kit and suppliers' protocol was used during experiment. There are 4 main steps of DNA extraction in this protocol, lysis step, binding DNA, washing DNA and eluting DNA respectively.

Procedure was followed to isolate DNA given in below:

Lysis step:

1. Heat block was set at 55°C.
2. 200 µL blood sample was added to a sterile microcentrifuge tube.
3. 20 µL Proteinase K and 20 µL RNase A (both supplied with the kit) were added to the blood sample respectively and incubated for 2 minutes at room temperature after being mixed briefly.
4. 200 µL of PureLink® Genomic Lysis/Binding Buffer was added to the tube and mixed well by vortexing until obtaining a homogenous solution.
5. The mixture was incubated at 55°C for 10 minutes to promote protein digestion.
6. 200 µL of 96–100% ethanol was added to the lysate and mixed again to obtain a homogenous solution.
7. Immediately proceed to the next step, binding of the DNA.

Binding DNA step:

1. PureLink® Spin Column in a Collection Tubes was removed from the package with enough count.

2. All of the lysate (~640 µL) was added to the PureLink® Spin Column.
3. The column was centrifuged at 10,000 × g for 1 minute at room temperature.
4. Collection tube was discarded and spin column was placed into a clean PureLink® Collection Tube supplied with the kit.
5. Proceeded to the next step, washing DNA.

Washing DNA step:

1. 500 µL of Wash Buffer 1 prepared with ethanol was added to the column.
2. The column was centrifuged at room temperature at 10,000 × g for 1 minute.
3. Collection tube was discarded and spin column was placed into a clean PureLink® Collection Tube supplied with the kit.
4. 500 µL of Wash Buffer 2 prepared with ethanol was added to the column.
5. The column was centrifuged at 13000 rpm for 3 minutes at room temperature and the collection tube was discarded.
6. Proceeded to Eluting DNA step.

Eluting DNA step:

1. The spin column placed in a sterile 1.5 mL microcentrifuge tube.
2. 50 µL of PureLink® Genomic Elution Buffer was added to the middle of column.
3. Incubated at room temperature for 1 minute.
4. Column was centrifuged at 13000 rpm for 1 minute at room temperature. The tube contains purified genomic DNA.

Obtained DNA was stored at 4 °C and quantification step was performed with Denovix ds-11 fx+ fluorometer. The DNA samples that have available purity and concentration were prepared for next step of the study, bisulfite conversion.

2.2 Bisulfite Conversion

With the epigenetics studies become interesting more and more, DNA methylation analysis studies was accelerated. There are a lot of different methods used for analysis of global or specific DNA methylation in human genome. Important group of these methods are classified as bisulfite conversion based methods and bisulfite conversion is still “gold standard” for DNA methylation analysis studies [50].

Bisulfite conversion method is firstly reported by Marianne Frommer and colleagues in 1992 [51]. The key point of the method is back up to converting the unmethylated cytosine

to uracil by treatment of sodium bisulfite in low pH. The converted fragment of DNA can be analysed after the PCR cycle by sequencing technologies. The difference between methylated and unmethylated cytosine and reactions belonging to the conversion are given in Figure 2.2.

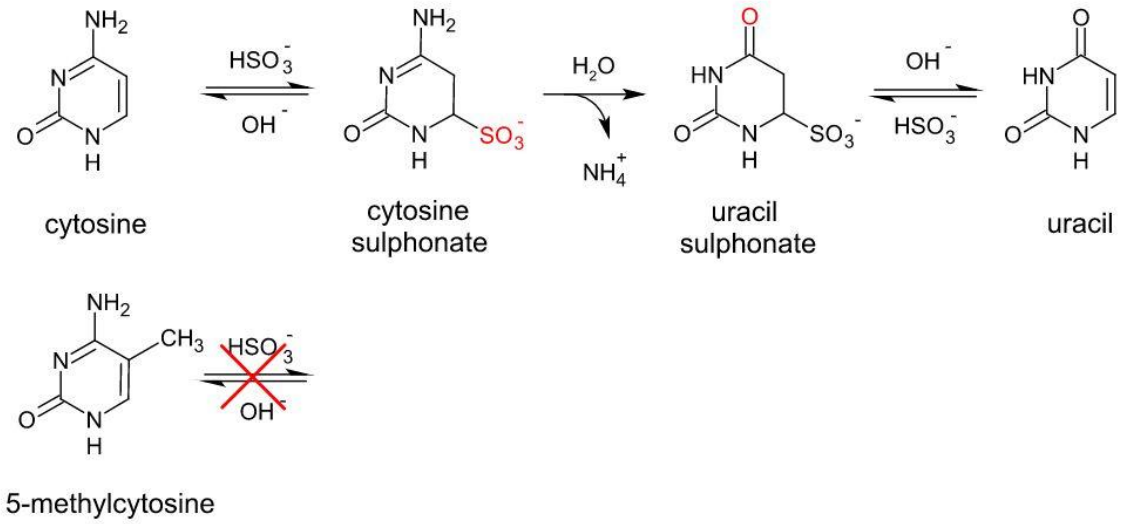

Figure 2. 2 Effects of bisulfite conversion in methylated and unmethylated cytosine

Bisulfite conversion of DNAs were performed with the help of another commercial kit, EpiTect Bisulfite Kit from Qiagen. Manufacturer's protocol was used during all conversion experiment. There are two main steps of conversion. The first part of the experiment is involved in bisulfite conversion of the DNAs and the second part of it is involved in clean-up of bisulfite converted DNAs for next steps.

Procedure that used to bisulfite conversion of DNAs and clean-up DNAs was given below:

Bisulfite DNA conversion:

1. DNA to be used in the bisulfite reactions was thawed. Required number of aliquots of Bisulfite Mix (supplied with the kit) resolved by adding 800 µl RNase-free water (supplied with the kit) to each aliquot. Vortexed until the Bisulfite Mix is completely dissolved.
2. In 200 µl PCR tubes, bisulfite reactions were prepared by adding 10 µl of the DNA solution, 10 µl of RNase-free water, 85 µl of Bisulfite Mix (from the previous step) and 35 µl of DNA Protect Buffer, respectively.

3. PCR tubes were closed and mixed the bisulfite reactions well at room temperature.
4. Bisulfite DNA conversion was performed using a thermal cycler. The thermal cycler was programmed according to the Table 2.1 given below and bisulfite conversion was started.

Table 2. 1 Conditions of thermal cycler for bisulfite conversion

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

Bisulfite converted DNAs was obtained in the end of thermal cycle and these DNA samples immediately cleaned by following cleaning procedure.

Clean-up of bisulfite converted DNA:

1. After the bisulfite conversion is done, PCR tubes containing the bisulfite reactions were centrifuged shortly, and then all of the bisulfite reactions were transferred to clean 1.5 ml microcentrifuge tubes.
2. 560 µl freshly prepared Buffer BL (supplied with the kit) containing 10 µg/ml carrier RNA was added to each sample. The solutions were mixed well and spun briefly by centrifugation.
3. EpiTect spin columns (supplied with the kit) and collection tubes were prepared in a suitable rack. All of the mixtures were transferred from the tube in the previous step into the middle of the EpiTect spin columns.

4. Spin columns were centrifuged at 13000 rpm for 1 minute. The flow-through was discarded and spin columns were placed back into the collection tubes.
5. 500 µl of Buffer BW (supplied with the kit) was added to each spin column and centrifuged at 13000 rpm for 1 minute. The flow-through was discarded and spin columns were placed back into the collection tubes.
6. 500 µl of Buffer BD (supplied with the kit) was added to each spin column and incubated for 15 minutes at room temperature.
7. The spin columns were centrifuged at 13000 rpm for 1 minute. The flow-through was discarded and spin columns were placed back into the collection tubes.
8. 500 µl Buffer BW (supplied with the kit) was added to each spin column and centrifuged at 13000 for 1 minute. The flow-through was discarded and spin columns were placed back into the collection tubes. (This step was repeated one more time. Two times in total).
9. The spin columns were placed into new 2 ml collection tubes and centrifuged at 13000 rpm for 1 minute to remove any residual liquid.
10. The spin columns were placed into clean 1.5 ml microcentrifuge tubes. 20 µl of Buffer EB (supplied with the kit) was dispensed onto the centre of each membrane. Purified and converted DNAs were eluted by centrifugation for 1 minute at 12000 rpm.

Obtained bisulfite conversed and cleaned DNAs can be stored at 4 °C up to 24 hours. Manufacturer's protocol recommends the DNAs store at – 20 °C if it is longer than 24 hours. Obtained DNAs in our study stored at 4 °C until the next step.

2.3 PCR Amplification

PCR amplification was important step to amplify our target region, gene SYN2. The primers that used in PCR amplification was supplied with PyroMark CpG Assay kit. Bisulfite converted DNA was used as template and SYN2 region was amplified by PCR. Manufacturer's protocol was followed to perform PCR step of the study. When the PCR is complete, amplified DNAs was stored at -20 ºC until perform the next step. Protocol is given in below.

PCR protocol to perform bisulfite converted SYN2 amplification:

1. The master mix was prepared by adding 12.5 µl of PyroMark PCR Master Mix, 2.5 µl of CoralLoad Concentrate (which includes Taq DNA Polymerase), 2.5 µl of PyroMark assay primers A and B and 5.5 µl of Molecular Biology Grade Water, per reaction.
2. 23 µl of the mix prepared was aliquoted in 3 to the appropriate number of 0.2 ml (PCR) tubes.
3. 2 µl of Molecular Biology Grade Water was added to the NTC tubes and 2 µl of bisulfite converted DNA was added to the remaining tubes.
4. Tubes were centrifuged briefly and loaded into a thermal cycler. The thermal cycler was programmed with the following conditions given in Table 2.2.

Table 2. 2 PCR conditions to amplify SYN2.

Step	Time		Temperature
Initial	15 min		95 ºC
Denaturation	45 cycles	30 s	94 ºC
Annealing		30 s	55 ºC
Extension		30 s	72 ºC
Final Extension	10 min		72 ºC
Hold	∞		4 ºC

2.4 Pyrosequencing

Sequencing technologies have critical roles to improve our knowledge in the field of genetics. With the invention of Sanger Sequencing technology by Frederick Sanger in 1977, genetics enter its new era. Sanger Sequencing system was firstly commercialized by Applied Biosystems in 1986 by the combining system with capillary electrophoresis [52]. Until the Human Genome Project was completed, at the beginning of the 2000s, Sanger Sequencing was helping a lot of important scientific development but because of some disadvantages of the system, scientists tried to develop new sequencing systems called Next-generation Sequencing (NGS) systems. NGS systems was firstly development by Lynx Therapeutics in

2000 with the Massively Parallel Signature Sequencing (MPSS). After this step, there are a lot of difference NGS technologies improved in biotechnology industry.

Sequencing-by-synthesis (SBS) methods are reliable methods and their principle is the sequencing of the DNA strand during synthesis. Only if the added nucleotide is complementary to the template DNA, it can be incorporated by a DNA polymerase into the strand and this event is monitored in real-time with special types of cameras [53]. Pyrosequencing is a modern and efficient SBS technology to uses the sequencing of nucleic acids. Pyrosequencing is mainly using the releasing of PPi during addition of dNTPs by DNA Polymerase. Released PPi is converted to ATP by special type of enzyme, ATP sulfurylase. ATP and luciferin are used as substrate by Luciferase that is a special type of enzyme and convert the substrate to oxyluciferin and visible light. This visible light is recorded by charge-coupled device (CCD) camera and sequencing is complete for specific position. The third enzyme called apyrase helps to destroy leftovers from dNTPs. The results of sequencing are recorded as pyrograms and analysis can be completed by researchers. The enzyme cascade of the pyrosequencing system is given in the Figure 3.3.

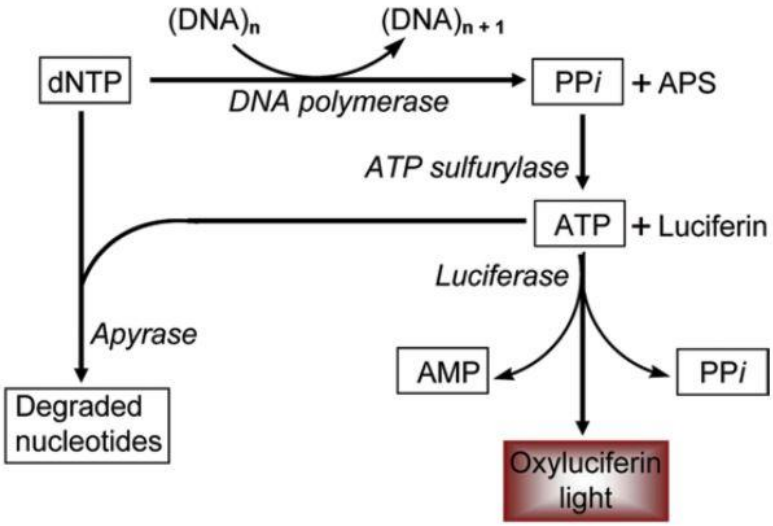

Figure 2. 3 The enzyme cascade of pyrosequencing [54].

Bisulfite converted and target specific amplified DNAs were used for sequencing by pyrosequencing in our study. All steps of the pyrosequencing were completed by the following of manufacturer's instructions. PyroMark Q24 system was used for sequencing. The procedures were given in follow that followed to perform sequencing.

Pyrosequencing protocol:

1. The bead mixture was prepared by adding 40 µl of binding buffer, 29 µl of Molecular Biology Grade Water and 1 µl of beads per reaction.
2. 70 µl of this mixture was added to 22 wells of a 24-well PCR plate. The last two wells were left empty for negative controls and did not put beads mixture into these wells.
3. 10 µl of PCR product was added into the corresponding well of the 24-well PCR plate.
4. The wells were closed with a cap and mixed by shaker at 1400 rpm for 20 minutes.
5. 20 µl of 1 x sequencing primer in annealing buffer was added to 22 corresponded wells on the PyroMark Q24 plate.
6. The last two wells were reserved for the primer controls. The sequencing primer was added to one of these wells. A mixture of biotinylated PCR primer (2 µl) and sequencing primer (20 µl) was added to the other well.
7. PyroMark Q24 plate was placed on the vacuum preparation workstation and all the trays of the station were filled with the corresponding solutions.
8. The template for the plate was prepared on the PyroMark Q24 Advanced software.
9. The volumes to add to the cartridge for each nucleotide, enzyme mix and substrate mix were checked and added then placed on the PyroMark Q24 Advanced instrument.
10. The vacuum switch was switched on in the suction probe, and placed on the prime tray with distilled water, aspirating approximately 70 ml.
11. The 24-well PCR plate was removed by avoiding shaking, the lids took off and placed in the vacuum preparation workstation.
12. The suction probe was placed into the PCR plate wells with the vacuum switched on and all the solution from the wells carefully captured for about 15 seconds.
13. The beads/samples were washed with 70% ethanol for 5 seconds.
14. The tray was washed with denaturing solution for 5 seconds.
15. The Washing Buffer tray was aspirated for 10 seconds and the suction probe tilted to beyond vertical 90º for a few seconds.
16. The vacuum suction probe was placed on top of the PyroMark Q24 plate without touching the liquid.
17. The vacuum switched off and the handle on the PyroMark Q24 plate was geared lower.

18. The suction probes were shaken gently from side to side for about 1 minute, to release the beads on the PyroMark Q24 plate containing sequencing primers.
19. The vacuum suction probe was rinsed on the rinse tray containing distilled water, by agitating for 10 seconds.
20. 70 ml of distilled water was aspirated on the prime tray.
21. The suction probe was stayed beyond 90º vertical for a few seconds and disconnected from vacuum.
22. The plate was placed on the metal tray and heated at 80 ºC for 5 minutes.
23. The sequencing plate was placed on the PyroMark Q24 Advanced instrument, the lid was closed and started the run.
24. When the run is complete, the PyroMark Q24 plate was discarded and the unused contents of the cartridge were removed by inverting it and cartridge was rinsed with deionized water.
25. Each well of the cartridge was filled up with water and applied pressured to the top with fingers to force the water to pass through the small needles at the bottom. This step was repeated 3 times for each well.
26. It dried at room temperature.
27. All trays were removed from the vacuum preparation workstation and rinsed with deionized water. Dried at room temperature.

The results of the sequencing were taken as pyrograms from PyroMark Q24 Advanced software.

2.5 Statistical Analysis of Data

Statistical analysis of data was performed to pyrogram results from 33 volunteer controls and 39 volunteer schizophrenia patients. Compliance of numerical data with normal distribution was examined with the Shapiro-Wilk test. Median, minimum and maximum values were used as descriptors. Comparisons of the groups were made with the Mann-Whitney U test. The two-way significance level was accepted as 0.05 in all tests.

3. RESULTS

Three specific CpG regions in the SYN2 gene were examined based on methylation status both in healthy controls and schizophrenia patients in this study. The CpG methylation rates both from 33 healthy control and 39 schizophrenia patients were statistically analysed to understand relationship between methylation status and schizophrenia. The statistical results showed significant relationship with schizophrenia and CpG methylation in average CpG methylation and CpG position 2 and 3. The Table 3.1 show the summary the results of analyses. The pyrograms of two subjects (one of them is control and the other is patient) are also given in Figure 3.1 and Figure 3.2. Methylation ratios of all control subjects and patients subjects are given as tables in Table 3.2 and Table 3.3, respectively.

Table 3. 1 General results of statistical analysis.

	Control (n=33)	Schizophrenia (n=39)	p
Position 1 Meth (%)	28 (9-97)	37 (5-69)	0,176
Position 2 Meth (%)	100 (54-100)	100 (76-100)	**0,006**
Position 3 Meth (%)	60 (18-97)	75 (30-85)	**0,003**
Average	62 (32,33-97,67)	70,33 (38-83,67)	**0,014**

* Data were shown with median (minimum-maximum) values.

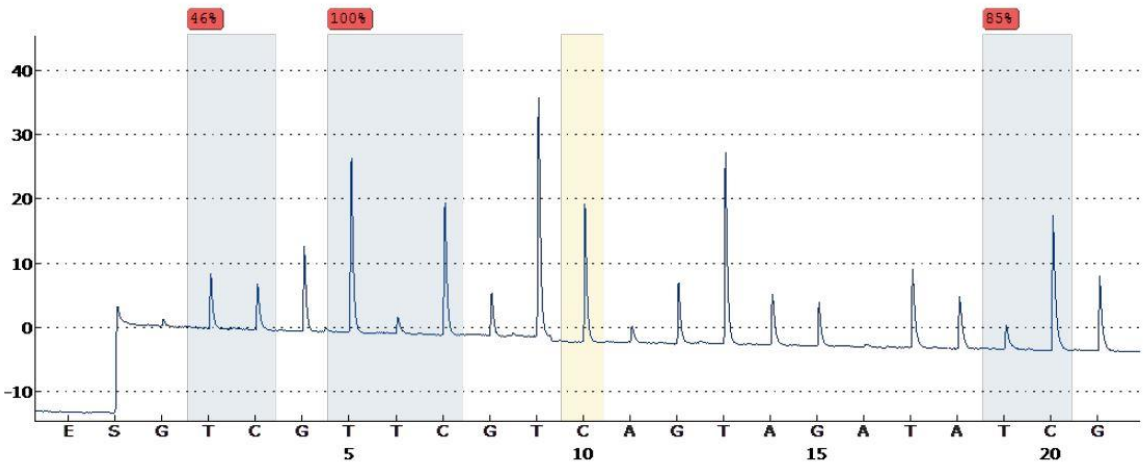

Figure 3. 1 Pyrogram result of a patient (Sample ID: E6).

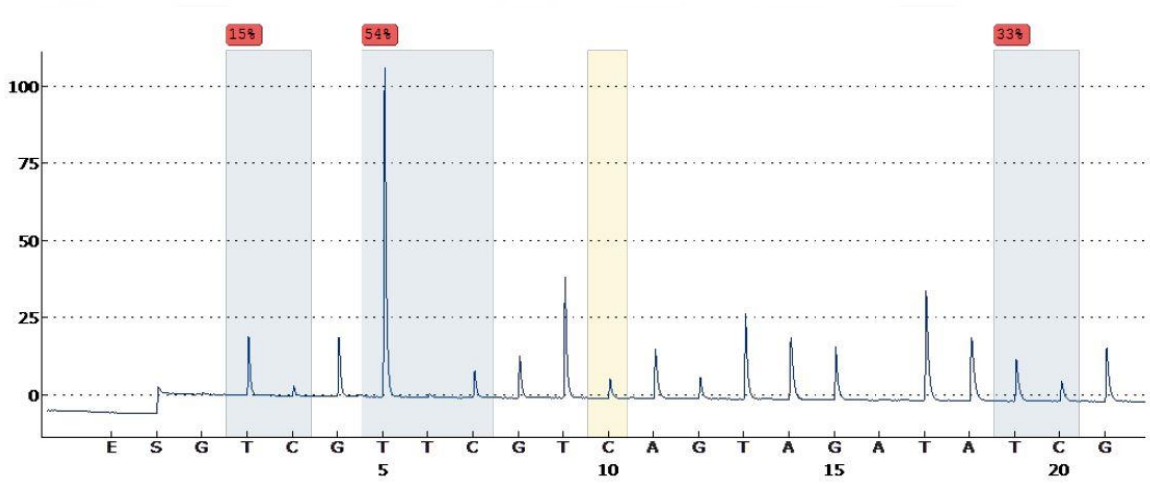

Figure 3. 2 Pyrogram result of a control (Sample ID: A22).

Table 3. 2 Methylation ratios of all control group.

Sample ID	Position 1 Meth (%)	Position 2 Meth (%)	Position 3 Meth (%)
A79	18	98	44
A96	40	100	46
A23	35	100	69
A98	9	100	79
A24	32	100	68
A29	20	85	33
A20	30	100	63
A40	15	67	28
A99	24	100	60
T3	21	100	45
T11	60	100	97
T12	69	100	77
A37	20	61	32
T9	97	100	96
A97	19	97	66
A81	21	88	43
A82	17	76	36
A95	28	100	54
A37	39	100	52
A83	14	75	45
A88	16	85	36
A22	15	54	33
A93	19	87	40
A80	18	94	43
EK1	38	100	73
EK2	42	100	78
EK3	41	100	74
EK4	21	58	18
EK5	44	100	78
EK6	36	100	74
EK7	41	100	72
EK8	39	100	72
EK9	32	100	67

Table 3. 3 Methylation ratios of all schizophrenia group.

Sample ID	Position 1 Meth (%)	Position 2 Meth (%)	Position 3 Meth (%)
3	27	100	65
21	13	100	76
15	20	100	59
48	35	100	72
5	26	89	37
24	7	78	41
57	27	100	57
28	14	99	59
34	5	100	59
58	43	100	85
56	15	100	53
22	22	100	51
74	8	76	30
27	19	99	45
E1	42	100	75
E2	55	100	83
E3	42	100	81
E4	48	100	76
E5	38	100	78
E6	46	100	85
E7	69	100	82
E8	42	100	80
E9	48	100	75
E10	37	100	74
E11	38	100	70
E12	42	100	78
E13	44	100	80
E14	44	100	80
E15	38	100	74
E16	42	100	74
E17	33	100	75
E18	39	100	80
E19	31	100	80
E20	33	100	82
E21	29	100	81
E22	39	100	71
E23	27	100	54
E24	23	100	53
E25	42	100	76

4. DISCUSSION AND CONCLUSION

Psychiatric disorders are an important threat to public health and there are a lot of scientific research performed in the scientific world to understand and get over them. The genetic basis of psychiatric disorders is one of the critical research focus of today's science. Psychogenetics is a field that rise from the studies about the understanding of genetic basis of psychiatric disorders. The genes that have potential roles in etiology of mood disorders are classified as candidate genes. The thousands of candidate genes are started to be identified for schizophrenia and other mood disorders with the understanding of genetic background of mood disorders. The genes that are expressed in nerve tissues are naturally number one candidate for schizophrenia.

The synapsins are an important group of phosphoproteins that are produced in the brain and they have a huge responsibility in releasing of neurotransmitters and plasticity of the nerve cells. SYN2 gene encode the protein Synapsin 2 in human genome and it was shown that SNPs in SYN2 gene are associated with schizophrenia by Hee Jae Lee and colleagues in Korean population [55]. Association studies was continued in different populations like North Europeans and Chinese. In November 2007, Viatcheslav Saviouk et. al., tried to understand role of SYN2 in schizophrenia in families from North Europe [56]. All these studies showed the association between schizophrenia and SYN2 gene.

With the development of epigenetics field as a scientific discipline, epigenetic basis of disorders become an attractive stuff to scientist from all around the world. CpG region analysis and association studies of these CpG methylations with disorders started a new era for research on the genetic basis of disorders. Hyper and hypomethylation in many CpG regions (especially in promotor regions) were associated with different disorders.

Methylation studies of SYN2 gene is one of the important gaps in scientific literature. Although SYN2 is candidate gene for different disorders like ASD (autism spectrum disorder), MDD (major depressive disorder), epilepsy and schizophrenia, there is no enough study about its methylation status. In 2006, Cristiana Cruceanu and colleagues tried to understand the roles of methylation of CpG regions in SYN2 in bipolar disorder and MDD and they find out interesting results [57]. They use post-mortem brain tissues and the specific CpG regions that found in the promoter of the SYN2 was hypomethylated and expression of SYN2 was increased in patients with bipolar disorder and MDD.

DNA methylation studies in schizophrenia is modern and hot topic field in psychogenetics. Although nerve and synapse related genes are potential candidate for schizophrenia, SYN2 was an ignored gene based on association and methylation studies in literature. Considering all of these reasons, we decide to understand the role of the methylation status of SYN2 in schizophrenia and add new data to the literature to fill the gap.

As mentioned before, the results of our studies show a relationship between methylation status of SYN2 and schizophrenia. The CpG regions in SYN2 are more methylated in schizophrenia patients compared with healthy controls. These results can be potential keys to understanding the molecular basis and etiology of schizophrenia. Moreover, methylation studies can be supported and combined with expression studies. The biggest problem in the combining of the methylation studies with expression studies for SYN2 gene is collecting of the subjects from population. SYN2 gene is not expressed in the blood instead it is highly expressed in tissues that are related to nerves. Post-mortem tissues are necessary for understanding of relationship between methylation status and gene expression of SYN2. Except those, studies of miRNAs that effect expression of SYN2 is also another potential research subject. Walter Lukiw and colleagues associate the miRNA-125b with regulation of SYN2 gene in human brain cells. miRNA studies related with SYN2 gene in schizophrenia can support to our understanding in molecular basis of schizophrenia.

REFERENCES

[1] R. S. Kahn *et al.*, "Schizophrenia," (in English), *Nature Reviews Disease Primers*, Article vol. 1, p. 23, Nov 2015, Art. no. 15067.

[2] S. R. Marder and T. D. Cannon, "Schizophrenia," (in English), *New England Journal of Medicine*, Review vol. 381, no. 18, pp. 1753-1761, Oct 2019.

[3] J. McGrath, S. Saha, D. Chant, and J. Welham, "Schizophrenia: A Concise Overview of Incidence, Prevalence, and Mortality," (in English), *Epidemiologic Reviews*, Article vol. 30, no. 1, pp. 67-76, Nov 2008.

[4] M. J. Owen, A. Sawa, and P. B. Mortensen, "Schizophrenia," (in English), *Lancet*, Review vol. 388, no. 10039, pp. 86-97, Jul 2016.

[5] R. Lardenoije *et al.*, "The epigenetics of aging and neurodegeneration," (in English), *Progress in Neurobiology*, Review vol. 131, pp. 21-64, Aug 2015.

[6] J. W. Wei, K. Huang, C. Yang, and C. S. Kang, "Non-coding RNAs as regulators in epigenetics," (in English), *Oncology Reports*, Review vol. 37, no. 1, pp. 3-9, Jan 2017.

[7] A. J. Pocklington, M. O'Donovan, and M. J. Owen, "The synapse in schizophrenia," (in English), *European Journal of Neuroscience*, Review vol. 39, no. 7, pp. 1059-1067, Apr 2014.

[8] Adityanjee, Y. A. Aderibigbe, D. Theodoridis, and W. V. R. Vieweg, "Dementia praecox to schizophrenia: The first 100 years," (in English), *Psychiatry and Clinical Neurosciences*, Review vol. 53, no. 4, pp. 437-448, Aug 1999.

[9] S. Heckers, "Bleuler and the Neurobiology of Schizophrenia," (in English), *Schizophrenia Bulletin*, Article vol. 37, no. 6, pp. 1131-1135, Nov 2011.

[10] K. S. Kendler, "Eugen Bleuler's Views on the Genetics of Schizophrenia in 1917," (in English), *Schizophrenia Bulletin*, Article vol. 46, no. 4, pp. 758-764, Jul 2020.

[11] M. J. Tueth, "Schizophrenia: Emil kraepelin, Adolph Meyer, and beyond," *The Journal of Emergency Medicine*, vol. 13, no. 6, pp. 805-809, 1995/11/01/ 1995.

[12] W. G. Rosen *et al.*, "Positive and negative symptoms in schizophrenia," *Psychiatry Research*, vol. 13, no. 4, pp. 277-284, 1984/12/01/ 1984.

[13] C. Acar, M. M. Sozen, H. Gozukara, K. Orman, and S. Kartalci, "Lack of association between catechol-O-methyltransferase and schizophrenia in a Turkish population," (in English), *Turkish Journal of Biochemistry-Turk Biyokimya Dergisi*, Article vol. 40, no. 3, pp. 205-209, 2015.

[14] E. J. Bromet and S. Fennig, "Epidemiology and natural history of schizophrenia," *Biological Psychiatry*, vol. 46, no. 7, pp. 871-881, 1999.

[15] R. C. Schwartz and D. M. Blankenship, "Racial disparities in psychotic disorder diagnosis: A review of empirical literature," (in English), *World Journal of Psychiatry*, Review vol. 4, no. 4, pp. 133-140, Dec 2014.

[16] G. Davies, J. Welham, D. Chant, E. F. Torrey, and J. McGrath, "A systematic review and meta-analysis of Northern Hemisphere season of birth studies in schizophrenia," (in English), *Schizophrenia Bulletin*, Review vol. 29, no. 3, pp. 587-593, 2003.

[17] E. Vassos, C. B. Pedersen, R. M. Murray, D. A. Collier, and C. M. Lewis, "Meta-Analysis of the Association of Urbanicity With Schizophrenia," (in English), *Schizophrenia Bulletin*, Article vol. 38, no. 6, pp. 1118-1123, Nov 2012.

[18] J. J. McGrath, L. Petersen, E. Agerbo, O. Mors, P. B. Mortensen, and C. B. Pedersen, "A Comprehensive Assessment of Parental Age and Psychiatric Disorders," *JAMA Psychiatry*, vol. 71, no. 3, pp. 301-309, 2014.

[19] A. Mendrek and A. Mancini-Marïe, "Sex/gender differences in the brain and cognition in schizophrenia," *Neuroscience & Biobehavioral Reviews*, vol. 67, pp. 57-78, 2016/08/01/ 2016.

[20] J. G. Barbee, P. D. Clark, M. S. Crapanzano, G. C. Heintz, and C. E. Kehoe, "ALCOHOL AND SUBSTANCE ABUSE AMONG SCHIZOPHRENIC-PATIENTS PRESENTING TO AN EMERGENCY PSYCHIATRIC-SERVICE," (in English), *Journal of Nervous and Mental Disease*, Article vol. 177, no. 7, pp. 400-407, Jul 1989.

[21] P. Batel, "Addiction and schizophrenia," (in English), *European Psychiatry*, Review vol. 15, no. 2, pp. 115-122, Mar 2000.

[22] P. F. Sullivan *et al.*, "Family History of Schizophrenia and Bipolar Disorder as Risk Factors for Autism," (in English), *Archives of General Psychiatry*, Article vol. 69, no. 11, pp. 1099-1103, Nov 2012.

[23] E. Walker, L. Kestler, A. Bollini, and K. M. Hochman, "Schizophrenia: Etiology and course," (in English), *Annual Review of Psychology*, Review vol. 55, pp. 401-430, 2004.

[24] R. A. McCutcheon, J. H. Krystal, and O. D. Howes, "Dopamine and glutamate in schizophrenia: biology, symptoms and treatment," (in English), *World Psychiatry*, Article vol. 19, no. 1, pp. 15-33, Feb 2020.

[25] A. Carlsson, L. O. Hansson, N. Waters, and M. L. Carlsson, "Neurotransmitter aberrations in schizophrenia: New perspectives and therapeutic implications," (in English), *Life Sciences*, Review vol. 61, no. 2, pp. 75-94, Jun 1997.

[26] C. I. Lau, H. C. Wang, J. L. Hsu, and M. E. Liu, "Does the dopamine hypothesis explain schizophrenia?," (in English), *Reviews in the Neurosciences*, Article vol. 24, no. 4, pp. 389-400, Aug 2013.

[27] A. E. Eggers, "A serotonin hypothesis of schizophrenia," *Medical Hypotheses,* vol. 80, no. 6, pp. 791-794, 2013/06/01/ 2013.

[28] B. Moghaddam and D. Javitt, "From Revolution to Evolution: The Glutamate Hypothesis of Schizophrenia and its Implication for Treatment," (in English), *Neuropsychopharmacology,* Review vol. 37, no. 1, pp. 4-15, Jan 2012.

[29] P. V. Gejman, A. R. Sanders, and J. Duan, "The Role of Genetics in the Etiology of Schizophrenia," (in English), *Psychiatric Clinics of North America,* Article vol. 33, no. 1, pp. 35-+, Mar 2010.

[30] H. J. Williams, M. J. Owen, and M. C. O'Donovan, "New findings from genetic association studies of schizophrenia," *Journal of Human Genetics,* vol. 54, no. 1, pp. 9-14, 2009/01/01 2009.

[31] H. J. Kang *et al.,* "Decreased expression of synapse-related genes and loss of synapses in major depressive disorder," *Nature Medicine,* vol. 18, no. 9, pp. 1413-1417, 2012.

[32] R. Tandon *et al.,* "Definition and description of schizophrenia in the DSM-5," (in English), *Schizophrenia Research,* Review vol. 150, no. 1, pp. 3-10, Oct 2013.

[33] J. Buxeraud and S. Faure, "Antipsychotics," (in French), *Actualites Pharmaceutiques,* Review vol. 59, no. 594, pp. 25-29, Mar 2020.

[34] R. T. Espinoza and C. H. Kellner, "Electroconvulsive Therapy," (in English), *New England Journal of Medicine,* Review vol. 386, no. 7, pp. 667-672, Feb 2022.

[35] U. Deichmann, "Epigenetics: The origins and evolution of a fashionable topic," (in English), *Developmental Biology,* Article vol. 416, no. 1, pp. 249-254, Aug 2016.

[36] A. Portela and M. Esteller, "Epigenetic modifications and human disease," (in English), *Nature Biotechnology,* Review vol. 28, no. 10, pp. 1057-1068, Oct 2010.

[37] Z. W. Jiang, X. G. Liu, and Z. J. Zhou, "The Regulation of Histone Modifications," (in Chinese), *Progress in Biochemistry and Biophysics,* Review vol. 36, no. 10, pp. 1252-1259, Oct 2009.

[38] K. Ito and I. M. Adcock, "Histone acetylation and histone deacetylation," (in English), *Molecular Biotechnology,* Article vol. 20, no. 1, pp. 99-106, Jan 2002.

[39] Y. X. Li and E. Seto, "HDACs and HDAC Inhibitors in Cancer Development and Therapy," (in English), *Cold Spring Harbor Perspectives in Medicine,* Article vol. 6, no. 10, p. 34, Oct 2016, Art. no. a026831.

[40] S. L. Hammond *et al.,* "Mitotic phosphorylation of histone H3 threonine 80," (in English), *Cell Cycle,* Article vol. 13, no. 3, pp. 440-452, Feb 2014.

[41] K. Hyun, J. Jeon, K. Park, and J. Kim, "Writing, erasing and reading histone lysine methylations," (in English), *Experimental and Molecular Medicine,* Review vol. 49, p. 22, Apr 2017, Art. no. e324.

[42] M. U. Kaikkonen, M. T. Y. Lam, and C. K. Glass, "Non-coding RNAs as regulators of gene expression and epigenetics," (in English), *Cardiovascular Research,* Review vol. 90, no. 3, pp. 430-440, Jun 2011.

[43] V. Ts, *Statistical Analysis of micro RNA: classification, identification and conservation based on structure and function*. 2015.

[44] L. D. Moore, T. Le, and G. P. Fan, "DNA Methylation and Its Basic Function," (in English), *Neuropsychopharmacology,* Review vol. 38, no. 1, pp. 23-38, Jan 2013.

[45] F. Lyko, "The DNA methyltransferase family: a versatile toolkit for epigenetic regulation," (in English), *Nature Reviews Genetics,* Review vol. 19, no. 2, pp. 81-92, Feb 2018.

[46] X. Wu and Y. Zhang, "TET-mediated active DNA demethylation: mechanism, function and beyond," *Nature Reviews Genetics,* vol. 18, no. 9, pp. 517-534, 2017/09/01 2017.

[47] M. V. C. Greenberg and D. Bourc'his, "The diverse roles of DNA methylation in mammalian development and disease," (in English), *Nature Reviews Molecular Cell Biology,* Review vol. 20, no. 10, pp. 590-607, Oct 2019.

[48] F. Cesca, P. Baldelli, F. Valtorta, and F. Benfenati, "The synapsins: Key actors of synapse function and plasticity," (in English), *Progress in Neurobiology,* Review vol. 91, no. 4, pp. 313-348, Aug 2010.

[49] F. J. Mirza and S. Zahid, "The Role of Synapsins in Neurological Disorders," (in English), *Neuroscience Bulletin,* Review vol. 34, no. 2, pp. 349-358, Apr 2018.

[50] S. Kurdyukov and M. Bullock, "DNA Methylation Analysis: Choosing the Right Method," (in eng), *Biology (Basel),* vol. 5, no. 1, Jan 6 2016.

[51] M. Frommer *et al.*, "A GENOMIC SEQUENCING PROTOCOL THAT YIELDS A POSITIVE DISPLAY OF 5-METHYLCYTOSINE RESIDUES IN INDIVIDUAL DNA STRANDS," (in English), *Proceedings of the National Academy of Sciences of the United States of America,* Article vol. 89, no. 5, pp. 1827-1831, Mar 1992.

[52] J. M. Heather and B. Chain, "The sequence of sequencers: The history of sequencing DNA," (in English), *Genomics,* Review vol. 107, no. 1, pp. 1-8, Jan 2016.

[53] J. Tost and I. G. Gut, "DNA methylation analysis by pyrosequencing," (in English), *Nature Protocols,* Article vol. 2, no. 9, pp. 2265-2275, 2007.

[54] C. Delaney, S. K. Garg, and R. Yung, "Analysis of DNA Methylation by Pyrosequencing," in *Immunosenescence: Methods and Protocols*, vol. 1343, A. C.

Shaw, Ed. (Methods in Molecular Biology, Totowa: Humana Press Inc, 2015, pp. 249-264.

[55] H. J. Lee *et al.*, "Association study of polymorphisms in synaptic vesicle-associated genes, SYN2 and CPLX2, with schizophrenia," (in English), *Behavioral and Brain Functions,* Article vol. 1, p. 7, 2005, Art. no. 15.

[56] V. Saviouk, M. P. Moreau, I. V. Tereshchenko, and L. M. Brzustowicz, "Association of synapsin 2 with schizophrenia in families of Northern European ancestry," (in English), *Schizophrenia Research,* Article vol. 96, no. 1-3, pp. 100-111, Nov 2007.

[57] C. Cruceanu *et al.*, "DNA hypomethylation of Synapsin II CpG islands associates with increased gene expression in bipolar disorder and major depression," (in English), *Bmc Psychiatry,* Article vol. 16, p. 5, Aug 2016, Art. no. 286.

CURRICULUM VITAE

Name and Surname: İbrahim FETTAHOĞLU

Educational Status:

Primary School:

2004-2011, Atatürk Primary School, Osmaniye/TURKEY

High School:

2011-2015, Kozan Anadolu Öğretmen High School, Adana/TURKEY

Bachelor of Science:

2015-2020, Inonu University, Faculty of Arts and Science, Department of Molecular Biology and Genetics, Malatya/TURKEY

Master of Science:

2020-Ongoing, Inonu University, Graduate School of Natural and Applied Sciences, Department of Molecular Biology and Genetics, Malatya/TURKEY

Languages:

Turkish – Native

English – Intermediate

German – Elementary