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Epigenetic Perspective in Schizophrenia: DNA Methylation Patterns

Şizofrenide Epigenetik Bakış Açısı: DNA Metilasyon Modelleri

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ABSTRACT

Schizophrenia is a mental disorder characterized by delusions, hallucinations and various behavioral disorders. Affecting approximately 1% of the world's population, schizophrenia not only affects patients, but also other members of the society. Genetic and environmental factors play roles in the etiology of the disorder.

Genetics, neurodevelopmental disorder, drug use, urban life, alone or together can be counted as the factors that cause the disorder. Despite increasing studies in recent years, the factors causing the formation of schizophrenia have not been fully clarified and more research is needed. Although genetic factors are risk factors for schizophrenia, it is thought that some environmental factors affect the emergence of the disorder. Epigenetic mechanisms regulate gene functions without changing the nucleotide sequence of DNA. DNA methylation is associated with schizophrenia, and methylation status studies have been conducted in many schizophrenia candidate genes. Examination of DNA methylation states will contribute significantly to psychiatric research.

In this review, data published in global databases obtained from DNA methylation studies related with schizophrenia are summarized and their importance in schizophrenia is briefly discussed.

Keywords: schizophrenia - epigenetics – environmental factors–DNA methylation

ÖZET

Şizofreni, sanrılar, halüsinasyonlar ve çeşitli davranış bozuklukları ile karakterize bir zihinsel bozukluktur. Dünya nüfusunun yaklaşık %1'ini etkileyen şizofreni sadece hastaları değil toplumun diğer üyelerini de etkilemektedir. Hastalığın etiyolojisinde genetik ve çevresel faktörler rol oynamaktadır.

Genetik, nörogelişimsel bozukluklar, ilaç kullanımı, şehir hayatı, tek başına veya birlikte hastalığa neden olan faktörler sayılabilir. Son yıllarda artan araştırmalara rağmen şizofreni oluşumuna neden olan faktörler tam olarak aydınlatılamamıştır ve daha fazla araştırmaya ihtiyaç vardır. Genetik faktörlerin şizofreni için risk faktörleri olmasına rağmen, bazı çevresel faktörlerin hastalığın ortaya çıkışını etkilediği düşünülmektedir. Epigenetik mekanizmalar, DNA'nın nükleotid dizisini değiştirmeden gen fonksiyonlarını düzenler. DNA metilasyonu şizofreni ile ilişkilidir ve birçok şizofreni aday geninde metilasyon durumu çalışmaları yapılmıştır. DNA metilasyon durumlarının incelenmesi psikiyatrik araştırmalara önemli katkı sağlayacaktır.

Bu derlemede şizofreni ile ilgili DNA metilasyon çalışmalarından elde edilen global veri tabanlarında yayınlanan veriler özetlenmiş ve şizofrenideki önemi kısaca tartışılmıştır.

Anahtar kelimeler: şizofreni- epigenetik- çevresel faktörler- DNA metilasyonu

Introduction

Schizophrenia (SZ) is one of the most important public health problems faced by societies and is a serious mental illness with a lifelong prevalence. It can have devastating effects on patients and their relatives and impose enormous costs on healthcare systems. Since SZ is a brain disorder that manifests itself in the activities of the mind, it damages many mental functions. The disorder disrupts the normal physiology of the brain and causes symptoms. However, a single sign or symptom does not prove that the disorder is present[1,2].

The etiology of SZ is still not fully understood, and based on limited understanding of its biological origins, more research is needed to better identify the causes of various symptoms and to develop and confirm new

treatments[3,4]. The onset of SZ mostly occurs in late adolescence or early adulthood, on average, between the ages of 15-35[5]. Also, the gender difference plays an important role in SZ. Studies have concluded that the incidence of SZ is higher in men than in women. At the same time, women have a later age of onset, the disease is less severe, and they respond better to antipsychotics than men[6,7]. It affects approximately 1% of the world's population[3,8]. However, comparing to general population it is common to see individuals with SZ who have family history. While the risk of developing the disorder is 17% in children whose one parent has SZ, this rate is 35% if both parents have SZ[9].

Gene polymorphisms and loci that are thought to play a role in the disorder have been identified by genetic studies[10]. Genetic findings have failed to understand the etiology of schizophrenia and have shown that risk factors are not caused by DNA sequence alone[11]. As a result of the researches until recent, we know that both genetic and environmental factors affect development of SZ. However, the nature, interactions, and pathogenic mechanisms of these two main etiological factors are not fully understood despite neurobiological, clinical and genetic research, and further research is needed[12].

In this review, we aimed to summarize the scientific studies on DNA methylation to understand their role in the molecular etiology of schizophrenia.

Genetic Factors Affecting Schizophrenia

Factors causing SZ are an important concern from past to present. SZ is a multifaceted disorder that does not occur for a single reason. However, we know that the most important risk factor is positive family history. They inherit several risk genes that interact with each other and with the environment to cause SZ when the critical threshold is exceeded[13].

The genetic basis of SZ has been partially clarified as a result of increasing research. We know that identical twins have a 40-50% concordance rate for the disorder, and that first-degree relatives of SZ patients have an approximately 10-fold increased risk of the disorder[14]. Researches have estimated that the heritability of SZ is 79-81%. We can say that not only genetic factors affect the development of SZ, but limited to a certain extent, and that the disorder develops as a result of the interaction of many environmental factors with genes.

Many studies have been conducted to identify susceptibility genes in schizophrenia. Strongly supported regions are 6p24–22, 1q21–22 and 13q32–34, while other promising regions are 8p21–22, 6q16–25, 22q11 12, 5q21–q33, 10p15 p11 and 1q42. There are several candidate genes known to be associated with schizophrenia. This suggests that dopamine receptors DRD3, DRD2 and serotonergic receptor HTR2A17 may pose a risk, albeit with extremely small effect sizes[15]. Some of the genes for which the evidence is strongest are NRG1, DTNBP1, DAOA, and DISC1[16]. The SZGene database (www.szgene.org) consists of all studies published in a peer-reviewed English-language journal on schizophrenia from 1965 to 2012. This platform consists of findings from genetic association studies for schizophrenia and lists more than 1,500 published studies for schizophrenia, the majority of which are candidate gene studies[17].

Polymorphisms and copy number variants (CNV) in SZ risk genes contribute to the high heritability of the disorder. By testing for differences in SNP frequency between cases and controls to identify risk alleles in schizophrenia, there are significant advances in understanding common genetic influences that contribute to many traits[18]. The Psychiatric Genomics Consortium published the most comprehensive GWAS report on schizophrenia in 2014, which included 36,989 patients and 113,075 controls. 108 independent genomic risk loci were identified, including genes that are promising targets for therapy (DRD2, GRM3), genes more commonly involved in glutamatergic neurotransmission (GRIN2A, SRR, CLCN3, and GRIA1), unexpected candidate mechanisms involving neuronal calcium signaling (CACNA1C, CACNA1l, CACNB2, RIMS1) and genes with broader synaptic function (KCTD13, CNTN4, PAK6)[19]. It is not yet known whether the factors influencing SZ are environmental or reflect genetic variation in the CNV region or risk variants elsewhere in the genome.

Molecular genetic studies in the field of psychiatry provide new ways to explore the mechanical aspects of disorders and new areas for treatments. Identification of SZ risk genes can shed light on the diagnostic uncertainties arising from the clinical heterogeneity of the disorder, and may be the basis for examining

altered brain functions as a result of the disorder, even if it does not directly affect the understanding of the disorder[2,20]. SZ risk genes are important for finding sensitive biomarkers of disorder and treatment effects and providing new targets and animal models for drug discovery[20]. Increasing evidence clearly indicates that risk genes are associated with certain features of SZ pathology.

Environmental Factors Affecting Schizophrenia

Epidemiological studies are generally concerned with the impact of environmental factors on the development of SZ[21]. Factors such as the season of birth, birth complications, advanced paternal age, infections and immune system, autoimmune diseases, ethnicity, cannabis use and urban life are among the environmental factors that alone or together affect the pathology of the disorder[22]. These factors may have a direct or indirect effect on the development of SZ. The combination of risk factors show us that high risk is not only the result of family history, but also high-risk individuals and environmental factors contribute significantly to the pathology of the disorder.

Exposure to these environmental factors affects certain critical developmental periods of the brain. Exposures during fetal development affect overall neuronal organization, while exposures during early life and adolescence affect axonal growth, synaptogenesis, and synaptic pruning. Because environmental factors can cause permanent changes in gene expression through epigenetic mechanisms, it is thought to alter gene expression networks involved in neuronal development and synaptic regulation[23]. In line with these findings, we can say that epigenetics plays an important role in the investigation of the biological structure of SZ.

There is substantial evidence that SZ diagnoses are associated with environmental, relational, and social influences such as nutrition, pre- and postnatal complications, abuse, low socioeconomic status, and ethnicity. This emphasizes the importance of environmental factors in disorder pathology[24].

Epigenetics Overview

Epigenetics term was first used by Conrad Waddington in 1942. It was originally defined as the study of random interactions between genes and their products that control phenotypic changes that occur during development. However, later on, epigenetics was defined as the study of changes inherited during mitosis/meiosis without changing DNA sequences[25].

Epigenetic mechanisms include DNA methylation, histone modifications, and processes mediated by non-coding RNAs[26]. The most common epigenetic modification is DNA methylation, which is characterized by the covalent attachment of a methyl (CH3) group to a cytosine[27].

Genes are activated or silenced epigenetically in response to environmental factors, during or after gametogenesis and embryogenesis. Gene expression is regulated at the level of transcription by covalent modifications of gene promoters or after translation, covalent modifications of histone tails. Generally, methylation of the 5'cytosine in the DNA sequence located on gene promoters prevents binding of transcription factors and decreases gene expression. However, there is evidence that some methylation of gene promoters can also activate genes[12]. 5-methylcytosine is not found randomly in the genome. With some exceptions, cytosines preceding guanines can be methylated, and these regions are called CpG islands[28].

Compared to histone modifications, DNA methylation is a more stable epigenetic mechanism. DNA methylation is catalyzed by DNA methyltransferases (DNMTs)[29]. In mammals, three major DNA methyltransferases have been identified, these are DNMT1, DNMT3A and DNMT3B[30]. Besides these three main enzymes, DNMT3L is present and is not catalytically active, but is required for de novo methylation in germlines. DNMT3A and DNMT3B provide DNA methylation in embryonic development. In mammals, DNA methylation occurs mostly at CpG dinucleotides, and nonCpG methylation (eg, CpA and CpT) is rarely observed. Of the approximately 28 million CpGs in a human somatic cell, 60-80% are methylated[31].

Increasing evidence suggests that the addition of a methyl group to cytosine at CpG islands may play an important role in the pathogenesis of SZ. An increased mRNA expression of DNA methyltransferases (DNMT1 and DNMT3a) has also been observed in patients with SZ[32]. Epidemiological findings between

environmental risk factors and SZ can be an important guide to the potential molecular mechanisms of the disorder[33].

Techniques for Measuring DNA Methylation

Profiling DNA methylation across the genome or of disease -causing candidate genes is critical to understanding the impact of many disorder. It is important to choose the DNA methylation method in accordance with the purpose of the study. DNA methylation studies have evolved in recent decades, largely with the acquisition of new and dynamic technologies[34].

It is not easy to obtain reliable data with DNA methylation. Deciding which method will provide the best useful dataset and defining the advantages and disadvantages is an important point before starting work. Not all methods are equally easy and available equipment will often be considered when choosing a method. DNA quality and quantity are the leading limiting factors in DNA methylation analysis[28].

Many techniques are used for DNA methylation analysis. While the techniques are different, all methods are based on three different principles: digestion of DNA with methylation-sensitive restriction enzymes, enrichment of methylated genomic DNA fragments using anti-methylcytosine antibody or methyl-binding domain (MBD) proteins, and bisulfite-converted DNA sequencing[35].

Some of the methods used are: methylation sensitive PCR (MSP)[36], quantitative methylation-sensitive PCR (Q-MSP)[37], combined bisulfite restriction analysis (COBRA)[38], multiplex ligation-dependent probe amplification (MLPA)[39], methyLight assay[40], methylation-sensitive restriction endonucleas (MSRE) southern analysis and MSRE-PCR, methylation-specific single-strand conformation analysis (MS-SSCA)[28] and pyrosequencing[34].

Choosing an appropriate method requires a balance between costs, advantages and disadvantages. The type of methylation (non-CpG, 5mhC or 5mC) and the extent of the methylomic region (candidate region vs. genome width) are important criteria in choosing the method[41].

Epigenetics in Schizophrenia: Focus on DNA Methylation

Epigenetic mechanisms are involved in regulating neurogenesis, neurodegeneration, neuronal activity, and important brain-related functions[42]. DNA methylation is a dynamic process that varies from one genomic region to another, cell to cell, tissue to tissue, and organism to organism, and DNA methylation changes during development and in response to environmental stimuli.

One of the greatest complexities of SZ is the undefined pathophysiology mechanism, diagnostic neuropathology, and biomarkers[43]. In recent years, it has been known that many disorder occur as a result of complex interactions between genetic and environmental insults. Epigenetic mechanisms are an inherited and dynamic way of regulating genomic functions[44]. Epigenetic factors provide an explanation for the pathogenesis of SZ from a different perspective[45]. Because genetic and environmental risk factors affect the normal brain development and maturation process[46].

Epigenetic researches has increased the understanding of gene-environment interactions by identifying molecular mechanisms that mediate environmental effects on gene expression and activity. These epigenetic findings are important for understanding not only SZ but also many psychiatric disorders[12].

Epigenetic mechanisms are critically important in neurodevelopment. It plays an important role in brain development, synaptic plasticity, learning and memory. Therefore, epigenetic research in the field of psychiatry has increased recently. There is substantial evidence that epigenetic mechanisms are a key determinant in the development of SZ[47].

Unlike the genome, the epigenome differs within the tissues of an organism, even between brain regions. Epigenetic regulation underlies normal cognition. In this case, disruption of epigenetic regulation reveals cognitive dysfunction. Environmental factors can influence genomic activities in the brain in synaptic signaling and organization, which underpins cognitive function[44].

Epigenetic modifications can reduce or exacerbate the expression of cell molecular functions, eventually resulting in SZ-associated behavioral changes[48]. Pathogenic changes in SZ mostly occur in the brain, but many studies are done with blood samples because access to brain tissue is not easy. Methylation markers in the blood may affect SZ epigenetically through processes not directly related to methylation in the brain[49].

In addition to clinical symptoms, SZ is characterized by changes in gene expression in the cerebral cortex and different brain regions. There are many genes with small to medium effect size that play an important role in SZ. Therefore, patients with SZ are expected to show epigenetic changes in the regulatory regions of genes with irregular expression in the brain. In studies conducted in this direction, the results of both postmortem human brain samples and peripheral blood samples examining changes in DNA methylation in candidate gene promoters are supported[29]. Methylation studies in SZ have focused on investigating the effect of DNA methylation as a global change in the methylome in the promoter regions of candidate genes or in the aggregate[47].

The first studies investigating the relationship between DNA methylation changes and SZ started with the examination of COMT, RELN, GAD1 and SOX10 genes in the early 2000s[50]. The RELN gene, which regulates microtubule function and neuronal migration in neurons, has been associated with abnormal methylation in schizophrenia. For the first time, in 2004, Veldic et al. showed hypermethylation in gene promoters based on data obtained from the brains of postmortem SZ patients[51].

SOX10, an oligodendrocyte-specific transcription factor, has been found to be highly methylated in the brains of schizophrenic patients. It was also found that DNA methylation status of SOX10 is associated with other oligodendrocyte gene expressions in schizophrenia[52].

COMT, one of the genes that affect schizophrenia, catalyzes the O-methylation of catecholamine neurotransmitters and catechol hormones. Abdolmaleky et al. is the first study to demonstrate that postmortem brain samples and MB-COMTpromoter DNA are frequently hypomethylated in SZ patients[53]. There have been many studies highlighting methylation changes in the COMT gene. Nohesara et al found that DNA from saliva samples of SZ patients was hypomethylated[54]. In addition, in a study including postmortem brain samples by Dempster et al. no difference was found in COMT methylation in SZ patients[55].

The GAD1 gene, which plays a role in schizophrenia, catalyzes the production of GABA and expresses glutamic acid decarboxylase 67 (GAD67). Huang et al. study with postmortem brain samples showed hypermethylation in gene promoter regions of SZ patients[56].

A study on EGR1 and EGR3 genes, which play a role in synaptic plasticity, which is considered a potential candidate gene for SZ, showed that methylation patterns were not significantly different between SZ patients and control samples[57,58].

Another study examining DNA methylation in the human type A gamma-aminobutyric acid (GABA) receptor β2 subunit gene (GABRB2) used zebrafish, one of the model organisms, unlike blood, saliva and postmortem brain samples. It showed developmental increases in global DNA methylation and decreased GABRB2 promoter methylation in zebrafish[59].

Brain-derived neurotrophic factor (BDNF), which is the main regulator of the survival, development, function and plasticity of neurons, is one of the genes that play an important role in the pathology of schizophrenia[60]. Results from post-mortem brain samples from SZ patients who committed suicide showed greater methylation in the BDNF promoter IV (Keller et al. 2014). A 2016 study by Çöpoğlu et al from peripheral blood samples showed no difference between SZ patients and controls[61].

In another study with blood samples, it was observed that DNA methylation increased in the promoter region of the serotonin receptor 5HTR1A gene[62]. Another study found differentially methylated CpG regions in the HTR1E gene in SZ patients[63].

In a study using CpG island microarrays to identify DNA-methylation changes in the frontal cortex and germline associated with SZ, the SCG2 gene was seen as hypomethylated. In the VGLUT1 and VGLUT2 genes, different methylation patterns were observed in males and females[64].

Another recent study on the gene SLC6A4 encoding the serotonin transporter (5-HTT) found significant hypermethylation at one CpG site in male patients with SZ[65]. A recent study by Gao et al. showed that MMP9, which plays a role in the regulation of the extracellular matrix, is hypomethylated in SZ patients with deficiency compared to non-deficient SZ patients[66]. In a recent study conducted in 2020, CpG regions in FAM63B were found to be significantly hypomethylated in blood samples from SZ patients[67].

As a result of the study on BRD1, which plays a role in the development of the central nervous system and embryo, SZ patients were found to be hypermethylated[68].

In 2007, Zhang et al found no statistically significant difference in the frequency of site-specific cytosine methylation modification of DRD2 in SZ patients. No significant relationship was found between methylated cytosines of DRD2[69].

In a study conducted by Mill et al. in 2008 on frontal-cortex postmortem brain samples, the GRIA2 gene, WDR18 gene, MARLIN1 gene and KCNJ6 gene were hypomethylated, while the WNT1 gene and NR4A2 gene were differentially methylated[64].

It is known that dysfunction of glutamatergic neurotransmission and its receptors play a role in SZ. Kordi-Tamandani et al. In 2013, he showed that the GMR2, GMR5, GMR8 and GRIA3 genes were hypermethylated[70]. In a study conducted by the same study group in 2012, no significant difference was found between SZ patients and the control group, in which the methylation status of CpG islets located in the promoter of the dopamine transporter (DAT1) gene were examined[71].

DNA methylation studies in peripheral blood and postmortem brain tissues of SZ patients and animal experiments related with SZ are summarized in Table 1.

Table 1. Studies of DNA methylation patterns in SZ.

Gene Name	Function	Result	Reference
RELN	Brain development	Hypermethylation (brain)	Veldic et al (2004)[51]
SOX10	Transcription factor, nerve development	Hypermethylation (brain)	Iwamoto et al (2005)[52]
MB-COMT	Catalyzes the O-methylation	Hypomethylation in SZ (brain)	Abdolmaleky et al (2006)[53]
GAD1	Catalyzes the production of GABA	Gene promoter hypermethylation (brain)	Huang et al (2007)[56]
GRIA2	Glutamate receptor	Hypomethylation in SZ (brain)	Mill et al (2007)[64]
DRD2	Dopamine Receptor D2	No difference in SZ (blood)	Zhang et al (2007)[69]
VGLUT1, VGLUT2	Packs glutamate into synaptic vesicles	Differential methylation in SZ (brain)	Mill et al (2008)[64]
SCG2	Neuroendocrine protein of the granin family that regulates the biogenesis of secretory granules	Hypomethylation in SZ (brain)	Mill et al (2008)[64]
HTR1A	G-protein coupled receptor for 5-hydroxytryptamine	Hypermethylation in SZ (blood)	Carrard et al (2011)[62]
DAT1	Dopamine transporter	No difference in SZ (blood)	Kordi-Tamandani et al (2012)[71]
GMR2,GMR5,GMR8, GRIA3	Glutamate metabotropic receptor	Hypermethylation in SZ (blood)	Kordi-Tamandani et al (2013)[70]
HTR1E	G-protein coupled receptor for 5-hydroxytryptamine	Differential methylation in SZ (blood)	Nishioka et al (2013)[63]
GABRB2 promoter	Formation of functional inhibitory GABAergic synapses	Hypomethylation in SZ (model organism: zebrafish)	Wang et al (2016)[59]
BDNF	Brain Derived Neurotrophic Factor	No difference in SZ and control (blood)	Copoglu et al (2016)[61]
EGR3	Synaptic plasticity, learning, and memory	No difference in SZ and healthy control (blood)	Hu et al (2017)[58]
BRD1 promoter	Embryogenesis and CNS development	Hypermethylation in SZ (blood)	Dyrvig et al (2017)[68]
FAM63B	Ubiquitin carboxyl-terminal hydrolase	Hypomethylation in SZ (blood)	Sugawara et al (2017)[67]

MMP9	Matrix Metallopeptinase	Hypomethylation in deficit SZ compared to non-deficit SZ (blood)	Gao et al (2018)[66]
EGR1	Synaptic plasticity	No difference in SZ and healthy control (blood)	Hu et al (2019)[57]
SLC6A4	Serotonin transporter	Hypermethylation in SZ (blood)	Ikegame et al (2020)[65]

Differences in the expression of genes involved in the regulation of brain function, such as neurogenesis, synaptic plasticity, and neurotransmitter transmission, indicate that they play an important role in the onset of SZ.

Genes undergoing epigenetic modification in SZ correspond to those affected by antipsychotic treatment. Therefore, epigenetic studies in SZ are of great importance. It has been shown that antipsychotics affect epigenetic mechanisms, but studies on this subject are insufficient[47].DNA hypermethylation in promoter regions in SZ is associated with down-regulation of gene expression, thus DNA methylation is important among epigenetic modifications[72].

Conclusion

Genetic studies on SZ have confirmed the importance of genes in etiology, but studies so far have not identified the relationship between genetic risks and specific DNA variants, protein changes, or biological processes. Epigenetic mechanisms together with genetic studies are important molecular mechanisms to explain the neuropathology of SZ. All the results show that epigenetic mechanisms are a promising field in psychiatry, involving links between genetic architecture and environmental factors. However, all genetic studies in the field of schizophrenia only help us understand the etiology of the disorder. It is predicted that many genes and mechanisms associated with schizophrenia will be found with rapidly advancing molecular genetic studies. Since schizophrenia is a multifactorial and complex disorder in which genetic and environmental factors play a role, there is currently no gene or gene region in which we can diagnose or treat schizophrenia. However, with rapidly increasing developments in genetics, neuroscience and psychopathology, significant changes can be expected in both classification and treatment.

It is clear that the pathophysiology of SZ can be understood with a general approach that takes into account the interaction between genes and the environment[48]. It is extremely important that we look at the big picture so that we can understand the underlying causes of the disorder and the phenotype. With the advancement of the science of genetics and the rapid increase in research, a different perspective on psychiatric disorders have been developed in recent years.

Looking at SZ from an epigenetic point of view allows us to look at the big picture behind the etiology from a different perspective. While the number of contents investigating the connection of SZ with epigenetics in databases was negligible before the 2000s, the number of studies has increased considerably after the 2000s.

Studies to date have shown the importance of DNA methylation in the psychopathology of psychiatric disorders. It has also demonstrated the potential of methylation sites as targets for drug development [47]. SZ-related research is a new field of research examining the link between epigenetic changes and treatment outcomes. Therefore, it is promising to identify new diagnostic steps and treatments that may contribute to the molecular pathogenesis of SZ.

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