Epigenetic Profiling in Schizophrenia and Major Mental Disorders

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Key Words

Epigenetic profiling · Major mental disorders · Schizophrenia

Abstract

Epigenetic alterations such as DNA methylation and histone modifications are important in the etiology or pathophysiology of mental disorders. Here, we review recent studies on the relationship between DNA methylation and major mental disorders. We will focus on the role of DNA methylation in postmitotic neurons, intra- and interindividual variations in DNA methylation, the possible involvement of methionine metabolism pathways, and candidate and genome-wide DNA methylation as they relate to mental disorders.

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Genetics and Epigenetics of Mental Disorders: Current Status

Despite decades of extensive research efforts, the etiology or pathophysiology of major mental disorders such as schizophrenia, bipolar disorder and major depression remains largely unclear. The development of high-throughput genetic technologies, together with collaborative multicenter efforts, has revealed a number of exciting findings focusing on the human genome and mental disorders. Several large-scale, genome-wide association studies have identified new genetic components that are

related to bipolar disorder [1–4] and schizophrenia [5, 6]. Surprisingly, most of the genes or loci identified had not been reported previously. After the discovery of copy number variation (CNV) throughout the normal human genome [7], certain genomic structural alterations such as microdeletions and rare CNVs were found to be involved in schizophrenia [8–11]. Nevertheless, it is becoming clear that genetic factors are not likely to fully explain the etiology of mental disorders, either because risk single nucleotide polymorphisms have a low or modest odds ratio [12], or risk CNVs are identified as a rare event [8–10]. Epigenetics includes DNA methylation and histone modifications, which are linked to long-lasting gene expression changes [13]. Epigenetic alterations can be caused by genetic or environmental factors and are important factors in the etiology or pathophysiology of mental disorders [14–19]. In this review, we focused on recent progress in understanding DNA methylation and its role in major mental disorders.

DNA Methylation

DNA methylation is an epigenetic event, which primarily occurs at the fifth position of the cytosine residue in CpG dinucleotides in mammals. Cytosine residues in the dinucleotides are generally methylated, while most CpG-rich regions in the genome, called ‘CpG islands’, remain unmethylated. This covalent modification is linked to the silencing of gene expression and is involved in X

inactivation and genomic imprinting [13]. CpG islands are frequently located in the promoter or first exon of a gene, and their methylation is often involved in tissue-specific gene expression. The mammalian genome undergoes drastic DNA methylation changes during development, including global DNA demethylation in early embryogenesis followed by de novo DNA methylation [20]. Aberrant DNA methylation causes disruption of normal development [21] and can cause diseases such as cancer [13, 22]. Recent studies suggest that DNA methylation and demethylation are actively controlled to regulate gene expressions from certain promoters [23, 24].

DNA Methylation and Neuroscience

DNA methylation plays an important role in brain development. In early brain development, DNA methylation changes are associated with neuronal and glial differentiation from neural stem cells [25]. The neural stem cells of mice lacking methyl-CpG-binding domain protein 1 show reduced neuronal differentiation [26]. The coordinated gene expression changes, and a stable expression of cell type-specific genes, are required for later stages of brain development. Aberrations of DNA methylation may also be involved in human neurological and psychiatric diseases [18]. Mutations in methyl-CpG-binding protein 2 and DNA methyltransferase 3B lead to Rett syndrome [27] and immunodeficiency-centromeric instability-facial anomalies syndrome [28], respectively, and those in the imprinted genes are involved in the spectrum of mental disorders and autism [29].

In addition, accumulating evidence suggests that DNA methylation also plays an important role in adult post-mitotic neurons. The DNA methylation status differs globally or locally depending on the brain region or subregions and it influences various neuronal activities and behaviors. Ladd-Acosta and colleagues [30] examined the DNA methylation level of about 1,500 CpG sites and found that the human cerebral cortex showed a clear epigenetic signature distinct from the cerebellum. The global DNA methylation level also differed across subregions of the rat hippocampus; CA1 and dentate gyrus showed hypermethylation compared with CA2 and CA3 [31]. Blocking the DNA methyltransferase activity resulted in the loss of long-term potentiation [32] and a decrease in the frequency of miniature excitatory synaptic currents [33] with demethylation of the neuronal genes in the mouse hippocampus. In addition, membrane depolarization induced DNA methylation changes in the promoter

region of the brain-derived neurotrophic factor gene [34, 35]. At the behavioral level, fear conditioning induced DNA methylation changes in the protein phosphatase 1 and reelin genes in the rat hippocampus [36]. In addition, increased nurturing behavior of rat mothers altered DNA methylation in the promoter of a glucocorticoid receptor gene in the hippocampus of offspring [37].

Taken together, the different cortical cells are likely to contain distinctive epigenome information, which reflects both their developmental history and environmental inputs such as chemical, nutritional and social factors [38, 39].

DNA Methylation: Intra- and Interindividual Variations

Estimating intra- and interindividual variations in DNA methylation levels in controls and patients is essential. At the sequence level, a bioinformatics approach revealed greater interindividual variations in DNA methylation of CpG-poor regions as compared with CpG-rich regions [40]. However, factors affecting variations in DNA methylation remain largely unclear.

Currently, aging is often reported to be a major factor in DNA methylation changes. Global DNA methylation levels are known to decrease with aging [41]. Fuke et al. [42] examined global DNA methylation levels of large groups of subjects whose ages ranged from 4 to 94 years and confirmed an age-dependent decrease in global DNA methylation levels in the peripheral leukocytes. They also found large interindividual variations in the methylation level. In contrast, Bjornsson et al. [43] examined longitudinal DNA methylation changes. They examined global methylation levels in 2 independent sample sets whose average sampling interval was more than 10 years. They found that global methylation levels changed over time, but the direction of change was not predictable: it both increased and decreased. Consistent with age-dependent DNA methylation changes, Fraga et al. [44] examined the epigenetic status of peripheral samples derived from 40 monozygotic twins and found that epigenetic differences became larger as twins aged. Flanagan et al. [45] examined the DNA methylation status of sperm cells from a total of 46 subjects. They found considerable intraindividual variation and age-dependent gene-specific DNA methylation changes. This included both hyper- and hypomethylations. Aging also affects the DNA methylation status in the brain. Siegmund et al. [46] found that DNA methylation status in the human cerebral cortex was con-

tinuously regulated during development. Through quantifying the DNA methylation status of 50 genes in 125 subjects, they identified 4 typical patterns of DNA methylation changes during development: age-dependent linear increase, biphasic distribution in which change occurred at some age point, stochastic accumulation, and decrease in DNA methylation in the first half of the life span [46]. In the patient samples, Shimabukuro et al. [47] examined global methylation levels of blood DNA in 210 patients with schizophrenia and 237 controls. They found global hypomethylation in male patients with schizophrenia.

It is not known whether global hypomethylation with aging involves DNA methylation changes at specific genomic sites, especially in patients. It will be very important to examine whether intra- and interindividual epigenetic variations in the sperm cells, which accumulated with aging, affect the methylation status of offspring, because advancing paternal age has been reported as a risk factor for bipolar disorder [48] and schizophrenia [49, 50].

DNA Methylation: Methionine Metabolism Pathway

L-Methionine is a precursor of S-adenosylmethionine, a methyl donor. After the transfer of the methyl group, S-adenosylmethionine is converted to S-adenosylhomocysteine. S-Adenosylhomocysteine, an inhibitor of DNA methyltransferase, is then hydrolyzed to homocysteine [51]. Therefore, factors affecting this pathway may be potentially important for the pathophysiology of mental disorders from an epigenetic viewpoint.

Meta-analysis revealed that L-methionine exacerbates psychotic symptoms of schizophrenia [52] and increased the level of homocysteine, which may be caused by a polymorphism of the gene for methylenetetrahydrofolate reductase, is linked to an increased risk of schizophrenia [53]. In animal models, methionine administration induces DNA methylation changes and behavioral alterations such as in prepulse inhibition and social interaction [54, 55].

An elevated level of plasma homocysteine in young males has been repeatedly found in schizophrenia and bipolar disorder [56–59], and elevated prenatal homocysteine levels are reported to be a risk factor for schizophrenia [60]. However, no association was found between plasma homocysteine levels and global DNA methylation levels [61]. In addition, an elevated homocysteine level was also reported to be involved in dementia and Alz-

heimer's disease [62], suggesting that this may not be a mental disorder-specific change. Consideration of the role of homocysteine is also important in that this may act as an agonist/partial antagonist of N-methyl-D-aspartate receptor [63] rather than as an epigenetic modulator.

Further studies that investigate global and site-specific DNA methylation changes will elucidate the relationships between the DNA methylation-related metabolism pathway and the pathophysiology of mental disorders.

DNA Methylation Analysis in Psychiatry: From Candidate Gene to Genome-Wide Analysis

Dysfunction of the cortical γ -aminobutyric acid (GABA)-ergic interneurons, as revealed by transcriptome and histochemical studies [64], is considered to be related to the pathophysiology of schizophrenia [65]. It has been reported that increased expression of DNA methyltransferase 1 is associated with a decreased expression of glutamate decarboxylase 1 and reelin genes in the GABAergic neurons [66]. In addition, hypermethylation of reelin was reported by the same [67] as well as different groups [68], although several recent studies failed to confirm it [69–71]. Altered histone modification of GABAergic gene promoters in brains of patients with schizophrenia has also been reported [72, 73].

Catechol-O-methyltransferase (*COMT*) encodes an enzyme that metabolizes catecholamines including dopamine and is suggested to be involved in schizophrenia from its function and chromosomal location, 22q11, where several mental disorders have been reported to be associated with its deletion [74]. Abdolmaleky et al. [75] reported hypomethylation of the *COMT* gene in brains of patients with bipolar disorder and schizophrenia. In contrast, Murphy et al. [76] found hypermethylation of the specific CpG site in a blood sample of a schizophrenia patient with prominent negative symptoms, but they did not observe altered methylation in brain samples. Dempster et al. [77] also reported no evidence of altered *COMT* expression and methylation in cerebellum samples of patients with bipolar disorder, schizophrenia and major depression.

One approach for detecting epigenetic differences is to use samples from monozygotic twins discordant for schizophrenia or bipolar disorder [16]. This included differential methylation of retroviral elements [78] and the dopamine D₂ receptor [79] in schizophrenia, and differential methylation of spermine synthase and peptidylp-

rolyl isomerase E-like gene in bipolar disorder [80]. Zhang et al. [81] reported no significant difference in DNA methylation levels in the dopamine D₂ receptor promoter region in blood samples derived from patients with schizophrenia compared with normals. In addition, differential DNA methylation of the X chromosome was found in the female monozygotic twins of bipolar disorder [82]. Considering that both genetic [83] and epigenetic [44] differences are expected to exist in monozygotic twins, it will be important to clarify whether an identified difference has a pathophysiological role.

In the brains of suicide victims, overexpression of DNA methyltransferase genes as well as hypermethylation of the γ -aminobutyric acid receptor- α_1 and its decreased expression was reported [84]. Interestingly, hypermethylation of a ribosomal RNA promoter was found in the brains of suicide victims, suggesting that epigenetic alteration may be directly linked to the aberration of protein synthesis in the suicide brain [85].

DNA microarray techniques, by which expression thousands of genes can be monitored, has an advantage in identifying the genes or cascades involved in mental disorders [86]. A number of reports have already been published. One of the most consistent findings using postmortem brains is the decreased expression of oligodendrocyte-related genes in schizophrenia [87, 88]. This was an important line of evidence for the oligodendrocyte dysfunction hypothesis in schizophrenia [88–90]. We previously found that the DNA methylation status of the CpG island of the SRY-related HMG-box gene 10 (*SOX10*) gene negatively correlated with the expression of *SOX10* and other oligodendrocyte-related genes such as oligodendrocyte lineage transcription factor 2, myelin oligodendrocyte glycoprotein, myelin-associated glycoprotein, cyclic nucleotide phosphodiesterase and myelin-associated oligodendrocyte basic protein in the postmortem brain [91]. Therefore, the DNA methylation status of a single gene may account for systematic gene expression changes in patients with mental disorders. The cause of epigenetic change remains unclear and will need to be studied further from the viewpoint of genetics and cellular heterogeneity. At least, we confirmed that genetic variations within the *SOX10* gene were not associated with expression or methylation [91] and were not associated with schizophrenia in a Japanese population [92].

Siegmund et al. [46] performed a real-time PCR-based quantitative methylation assay using postmortem brains of patients with schizophrenia and Alzheimer's disease. They found higher DNA methylation in the paired box gene 8 gene in schizophrenia compared with controls, al-

though this was not considered significant after a multiple testing correction. Mill et al. [69] first performed genome-wide DNA methylation analysis of brains of patients with schizophrenia and bipolar disorder using the CpG island microarray, which contained 7,834 spots. They found that there are numerous epigenetic differences in the patients compared with controls. Although most of the differences are gender and disease dependent, ontology analysis showed that genes related to glutamate, GABA and brain development were affected.

Perspectives

Cancer cells exhibit both global hypomethylation and local or site-specific hypermethylation [93]. The former is thought to be related to genome instability, and the latter may contribute to the silencing of tumor suppressor genes. In contrast, reproducible global changes or on/off-like site-specific methylation changes have not been found in patients with mental disorders. It has been proposed that subtle and multiple changes are important for the mental disorders [69, 94]. With this in mind, it will be advantageous to employ high-throughput assays such as microarrays and next-generation sequencers. In addition, cellular heterogeneity in the brain will be a critical issue in interpreting the detected epigenetic changes in the brain. To deal with this, dissection of brain subregions [95] and/or sorting-based separation of neuronal nuclei would be helpful. The latter approach has proved to be useful for studying aneuploidy [96], assessment of the age of neurons [97] and DNA methylation [46] in the human brain. Application of high-throughput techniques to the dissected or separated brain samples will further reveal the role of epigenetics on the etiology or pathophysiology of major mental disorders.

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