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Epigenetic mechanisms in schizophrenia

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Summary

Epidemiological research suggests that both an individual's genes and the environment underlie the pathophysiology of schizophrenia. Molecular mechanisms mediating the interplay between genes and the environment are likely to have a significant role in the onset of the disorder. Recent work indicates that epigenetic mechanisms, or the chemical markings of the DNA and the surrounding histone proteins, remain labile through the lifespan and can be altered by environmental factors. Thus, epigenetic mechanisms are an attractive molecular hypothesis for environmental contributions to schizophrenia. In this review, we first present an overview of schizophrenia and discuss the role of nature versus nurture in its pathology, where 'nature' is considered to be inherited or genetic vulnerability to schizophrenia, and 'nurture' is proposed to exert its effects through epigenetic mechanisms. Second, we define DNA methylation and discuss the evidence for its role in schizophrenia. Third, we define posttranslational histone modifications and discuss their place in schizophrenia. This research is likely to lead to the development of epigenetic therapy, which holds the promise of alleviating cognitive deficits associated with schizophrenia.

Keywords

schizophrenia; environment; epigenetic; DNA methylation; histone; HDAC; DNMT; cognition

1. Introduction

Schizophrenia is a severely debilitating, stigmatized disorder occurring in up to 1% of the population worldwide. The pathology observed in patients includes three core disabilities: 1) positive symptoms, which include hallucinations, delusions, and thought disorder; 2) negative symptoms, which include apathy, inappropriate mood, and poverty of speech; and, 3) cognitive dysfunction, which includes impaired working memory and conceptual disorganization. Together these symptoms render patients difficult to employ and socially isolated. Schizophrenia patients are therefore some of the most disadvantaged members of society. Antipsychotic drug treatments only alleviate symptoms of schizophrenia so elucidating the

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pathophysiological mechanisms causing schizophrenia is likely to facilitate the development of more effective treatments.

2. Nature, nurture and their roles in the pathophysiology of schizophrenia

Schizophrenia is predicted via path analyses to have a multifactorial mode of inheritance. This model includes several genetic and non-genetic factors acting in combination to produce the disorder. Epidemiological studies have detected several non-genetic risk factors for schizophrenia, including marijuana use and obstetric complications, but these risk factors rarely have odds ratios exceeding 2 [1–5]. Attempts to consider gene x environment interactions have led to the finding that some candidate genes interact with severe obstetric complications to increase the risk of developing schizophrenia. A recent study by Nicodemus and colleagues detected association between four candidate genes for schizophrenia which are likely to play a role in hypoxic events, *AKT1*, brain derived neurotrophic factor (*BDNF*), metabotropic glutamate receptor 3 (*GRM3*) and dystrobrevin binding protein-1 (*DTNBP1*) [6]. These genes showed significant evidence for gene x environment interaction in a study of schizophrenia patients with or without obstetric complications [6].

The environmental risks and heritability of schizophrenia have also been studied separately. The latter has been established after genetic epidemiological investigations, based on family, twin, and adoption studies, which we discuss in the following sections.

2.1 Studies of the heritability of schizophrenia

Evidence for the aggregation of a trait in families suggests a hereditary component. Indeed, there is considerable evidence for a familial component in schizophrenia. The risk of schizophrenia and related disorders to first degree relatives is approximately 9 times greater than the risk of schizophrenia in the general population [7,8]. The degree of genetic identity to the proband is strongly associated with the degree of risk, such that first degree relatives have a greater risk of psychosis than more distant relatives of affected individuals [9].

Twin studies are typically used to estimate the heritability of a trait. Comprehensive studies of twins with schizophrenia have shown that the risk of schizophrenia in the co-twin of the proband is substantially higher for monozygotic (MZ, 53%) compared with dizygotic (DZ, 15%) twins, and that there is an overall heritability estimate of 68% for the underlying liability to schizophrenia [10,11]. MZ discordance for schizophrenia may be due to reduced penetrance of a schizophrenia genotype, which has been supported by the observation that there is an increased risk of schizophrenia among the offspring of the unaffected twin of discordant MZ pairs [10,11]. A possible explanation for these observations could lie in environmental factors altering the function of genes creating vulnerability to schizophrenia [12].

The cross-fostering adoption study design is a powerful approach to examine the joint contribution of genetic and environmental factors to psychiatric illness. This method has been employed to compare the rates of schizophrenia in two groups of adoptees: adoptees with schizophrenic biological parents but raised by normal adoptive parents, and adoptees with normal biological parents who have been raised by adoptive parents diagnosed with schizophrenia after the adoption process. In one report using this design, Wender and colleagues found that the rate of disorder in the adoptees in the former group was 18.8% compared with 10.7% in the latter group [13]. Therefore, this suggests that both genetic and environmental contacts with schizophrenia are risk factors, although environmental factors are likely to have a much lesser impact. In conclusion, family, twin, and adoption studies have confirmed the existence of a genetic component in the etiology of psychosis, but also highlight the importance of environmental factors.

2.2 Studies of environmental risks for schizophrenia

Genetic data demonstrate that even when identical twins are investigated, i.e. subjects with 100% genetic identity, there is not 100% concordance for schizophrenia. If one twin has schizophrenia, the other has approximately a 50% lifetime risk for developing the disease [10,11]. Similar data have arisen from studies of twins with bipolar disorder, major depression and anxiety disorders.

The general epidemiological statistics of schizophrenia are summarized in Table 1 [14–28]. Differences between genders in the age of onset of schizophrenia have been noted, with the onset in male patients 2–3 years earlier than in females. The protective effect of estrogen may explain this difference [29], although alternative variables such as marital status and premorbid personality traits are likely confounding factors between the sexes. For example, patients who have never married appear to have a 50-fold higher risk of developing schizophrenia if male, compared with a 15-fold higher risk if female [30].

Reports indicate that psychoses seem to aggregate in urban environments and in lower socioeconomic groups [14]. For example, Afro-Caribbean immigrants to the United Kingdom and especially their offspring have an approximate 10-fold increased risk of schizophrenia [23,24], and ethnic minorities in Britain have at least a 3-fold increase in the incidence of schizophrenia [31]. These observations have led some to propose that schizophrenia might be a disease of epidemiological transition, or in other words, a disease that rises in incidence during the development of a society [32]. Many hypotheses have been proposed to explain the increased rate of schizophrenia in second generation immigrants from third world countries born in industrialized countries. These hypotheses include the possibilities that industrialization may cause changes in maternal nutrition, obstetric complications and perinatal survival, maternal exposure to novel infectious agents, psychosocial stressors on both first- and second-generation immigrants, and gene-environment interactions that influence the development of schizophrenia [33].

The largest effect of environmental risk factors for schizophrenia has been detected for obstetric complications, such as preeclampsia and perinatal brain damage, while factors such as Rhesus incompatibility, unwanted pregnancy, malnutrition of the mother (during the first trimester), season of birth and maternal influenza (during the second trimester) could also be disruptive [34–37]. Furthermore, an excess of problems with speech and education, social anxiety, preference for solitary play and below average mothering skills have been reported to be predictive of schizophrenia later in life [38,39].

It is probable that examination of each environmental risk separately will not identify reliable predictors for developing schizophrenia. Instead, it may be more biologically relevant to classify each of these risks as an environmental stressor and consider these in combination with an individual's genotype. Indeed, an example of this strategy has been employed in a study of cannabis use in schizophrenia patients. Presence of the catecholamine-o-methyltransferase (*COMT*) valine158 allele was a predictor of psychotic symptoms and schizophreniform disorder only in subjects using cannabis, while subjects with the *COMT* 158 methionine homozygous genotype had no such adverse influence. Therefore the genetic vulnerability to psychosis produced by possession of the *COMT* valine158 allele was exacerbated by an environmental factor, cannabis use [40].

In summary, extensive epidemiological research does imply a supporting role of an individual's environment in schizophrenia. How then might the environment render an individual susceptible to schizophrenia? The answer may lie in the influence of environmental factors on epigenetic mechanisms, such as DNA methylation and histone modifications, which contribute to the regulation of gene activity in the CNS.

3. The role of epigenetic mechanisms in schizophrenia

Epigenetics refers to the covalent modifications of chromatin, which is the DNA-histone protein complex present in the cell nucleus. Epigenetic mechanisms not only perpetuate lasting variation in gene activity states in the CNS but also influence transient modulation in gene transcription that support activity-dependent changes in gene expression necessary for cognition [41–44]. Thus, the possibility of an epigenetic contribution to schizophrenia is an attractive molecular hypothesis, and indeed has been the focus of many studies which we will discuss in the following sections.

4. DNA Methylation

DNA methylation is a direct covalent modification of DNA, where at least three encoded enzymes known as DNA methyltransferases (DNMTs) catalyze the addition of a -CH₃ group to cytosine residues at the 5-position of the pyrimidine ring [45,46]. DNMT1 is responsible for perpetuating methylation marks after cell division, regenerating the methyl-cytosine marks on the newly synthesized complementary DNA strand that arises from DNA replication [45, 46]. Thus, DNMT1 is typically viewed as a “maintenance” DNMT. By contrast, DNMT3a and 3b place new methylation marks on DNA, for example, when genes are turned off as part of cell fate determination [45,46]. Consequently, DNMT3a and 3b are considered “de novo” DNMTs.

Cytosines that are followed by a guanine can be methylated, and these CpG dinucleotide sequences are typically found in and around gene regulatory regions in clusters known as CpG islands [45,46]. DNA methylation within regulatory regions is usually associated with the suppression of gene transcription [45,46]. In brief, methylated cytosines recruit methyl-DNA binding proteins, for example MeCP2, which help recruit histone deacetylases (HDACs). HDACs are enzymes that will remove acetyl groups from histone proteins. Together, these events compact chromatin structure, limit accessibility of transcriptional machinery, and in turn suppress gene transcription (Figure 1). However, it is important to note that while DNA methylation around gene promoters is typically associated with transcriptional suppression, recent work has indicated that DNA methylation can also be associated with transcriptional activation [47,48].

4.1 The stable vs. dynamic nature of DNA methylation

DNA methylation has been implicated in a number of developmental processes which are associated with long-lasting phenotypic changes, including genomic imprinting, cell differentiation, and X-chromosome inactivation [49]. Moreover, dysregulation of DNA methylation contributes to a number of neurodevelopmental disorders, including Rett syndrome and Fragile X mental retardation [50,51]. In these cases, the phenotypic outcomes are due to the programming of DNA methylation very early in development, and these patterns of DNA methylation remain very stable through the lifespan. Such cases fit the historic view of epigenetic regulation of gene expression, in that it is a static process following neural development and cell differentiation.

Unlike these disorders, however, the onset of schizophrenia occurs later in life, usually during or after adolescence, and the environment might have some role in this onset. Then, if DNA methylation is a mechanism contributing to the onset of the disorder, DNA methylation would also have to be a dynamic process that can be influenced by environmental factors. Data continue to highlight that DNA methylation is indeed dynamic and susceptible to environmental influences. For example, while DNMT activity is generally restricted to dividing cells during mitosis with the highest level of expression occurring early during development [52–56], there are robust levels of DNMT enzymatic activity observable in adult CNS neurons

[52,57,58]. Furthermore, methylation of DNA has been shown to be plastic in the adult CNS, and also appears to be important for the induction of synaptic plasticity and associative memory formation [59–62]. Finally, studies continue to highlight that the postnatal caregiving environment has a direct influence on DNA methylation patterns of several genes [63–65].

As a final note, since there is a high level of heritability of schizophrenia, one might ask whether DNA methylation patterns can also be transmitted from one generation to the next. This also appears to be the case, because reports continue to indicate that the epigenetic status of particular genes in the previous generation influences the next generation [64,66–71]. For example, the transmission of positive aspects of maternal behavior in rats, as well as adult stress responses, appears to be attributable to the methylation status of the promoter of the glucocorticoid receptor gene in the hippocampus of the mother, as well as the methylation status of the promoter of the estrogen receptor alpha gene in the medial preoptic area [42,66, 70]. Furthermore, changes in the methylation status of the *BDNF* gene due to early stress (abuse and neglect) can be transmitted to the next generation [64].

Then, it is reasonable to hypothesize that DNA methylation plays a role in schizophrenia. Indeed it has been previously postulated that the discordance observed in twin studies could be explained by epigenetic DNA modifications [72]. In the following sections, we discuss evidence for the role of DNA methylation in schizophrenia. To date, DNA methylation has been examined for only a handful of candidate genes, which are highlighted in Table 2 [73–85]. In the following sections we limit our discussion to the reelin gene (*RELN*), the glutamic acid decarboxylase 67 gene (*GADI*), the serotonin-2A receptor gene (*HTR2A*), the catechol-O-methyltransferase gene (*COMT*), and *BDNF*.

4.2 Genes influencing the GABAergic system

There is growing evidence that DNA methylation has a role in the dysfunction of GABAergic neurons in schizophrenia. In 2000, seminal observations were made that both *RELN* and the *GADI* genes were down-regulated in cortical [86] and hippocampal [87] GABAergic neurons in post-mortem samples from individuals with schizophrenia. Since then, reductions of *RELN* and *GADI* expression (both the mRNA and protein) have been one of the most consistent findings in the postmortem studies [88–94].

RELN encodes an extracellular matrix protein that is not only important for neural development and synapse integrity, but plays a necessary role in the long-term potentiation that supports synaptic and behavioral plasticity in the adult [95]. *RELN* is expressed predominately by GABAergic interneurons which regulate neighboring glutamatergic neurons [96]. The *GADI* gene encodes one of the enzymes, *GAD*₆₇, which synthesizes GABA from glutamate [97]. Down-regulation of either of these genes can certainly disrupt GABAergic neurotransmission, and altered GABA activity appears responsible for at least some of the clinical features of schizophrenia [98,99]. Moreover there are risk associated SNPs in *GADI* which are also associated with decreased expression in the hippocampus and dorsal prefrontal cortex of patients with schizophrenia [94]. However, this does not exclude the possibility that methylation of DNA may also contribute to the down-regulation of *RELN* and *GAD*₆₇ activity observed in schizophrenia.

Indeed there is mounting evidence to suggest that this may be the case. In adult cortical GABAergic interneurons, *RELN*, *GADI*, and *DNMT1* mRNAs co-localize [100,101]. Reports have shown that both *DNMT1* mRNA and protein levels are significantly increased in the cortex of individuals with schizophrenia [100–102], and that these increases parallel deficits in both *RELN* and *GADI* [101,102]. Similar results have been documented in GABAergic neurons located in the basal ganglia [103].

To examine whether changes in gene expression could explain altered methylation status of CpG sites within the regulatory regions of *RELN* and *GADI* in cortical structures, investigators have used a variety of approaches. Data suggest that the down-regulation of these transcripts is most likely due to hypermethylation of their gene promoters [104–106]. However it is important to note that one study has documented decreased methylation of the *GADI* promoter in schizophrenia patients with both repressive chromatin and lower levels of *GADI* mRNA [107]. A possible explanation for this apparent paradox is that down-regulation of *GADI* in the hippocampus also appears attributable to the up-regulation of an HDAC enzyme [108].

Using a genome-wide epigenetic approach, investigators have recently found there to be as many as 100 loci with altered CpG methylation in schizophrenia, including several other gene families related to the GABAergic system: glutamate receptor genes (*NR3B* and *GRIA2*), glutamate transporters (*VGLUT1* and 2), and a protein that regulates production of GABA receptors (*MARLIN-1*) [109,110]. Together, these studies and those reviewed above indicate that there are epigenetic changes associated with schizophrenia. But do these epigenetic changes contribute to the genetic dysfunction observed in the disorder?

The brief answer is we do not know. However, basic research results help address this question, by demonstrating that DNA methylation and the associated chromatin remodeling could indeed play a pivotal role in the down-regulation of *RELN* and *GADI* documented in schizophrenia patients. Chronic treatment of mice with L-methionine (MET), a precursor necessary for DNMT catalytic activity, produces a schizophrenic-like phenotype [111,112]. This treatment also replicates some of the molecular aspects of schizophrenia, including an increase in methylation of the *RELN* promoter and the constituent down-regulation of *RELN* and *GADI* in GABAergic neurons [111,112]. Using these mice, investigators have also been able to show that both *RELN* and *GADI* promoters show increased recruitment of methyl-CpG binding proteins, such as MeCP2 [112]. Finally, using neuronal progenitor cells to further understand the epigenetic regulation of these genes, investigator have also shown that both DNMT and HDAC inhibitors activate *RELN* and *GADI* [113].

Though there is strong evidence for epigenetic changes in *RELN* and *GADI* in schizophrenia, it is not likely the case that their epigenetic dysfunction alone confers susceptibility to schizophrenia. Rather, it is likely that many genes are affected, as indicated by a study utilizing genome-wide epigenetic approaches [109,110].

4.3 Genes influencing the serotonergic system

Altered serotonergic function is hypothesized to increase vulnerability to psychiatric diseases, including schizophrenia, anxiety disorders and affective disorders. To date, fourteen serotonin receptors have been identified within the serotonin system, of which the serotonin-2A (5-HT_{2A}) receptor is one of three subtypes within the 5-HT₂ receptor group [114]. The involvement of serotonin in schizophrenia first emerged from the observation that lysergic acid diethylamide (LSD), a potent 5-HT₂ receptor agonist, had hallucinogenic properties [115, 116]. The LSD-induced psychosis includes both the hallucinations and delusions observed in schizophrenia patients, but not the negative symptoms (such as withdrawal, blunted affect and apathy). The affinity of many hallucinogenic drugs for 5-HT₂ receptor sites has been found to be closely related to their potency as hallucinogens in humans [117,118]. The atypical antipsychotic drugs and many antidepressants have high affinities for 5-HT₂ receptors [119].

Variation of the gene encoding the 5-HT_{2A} receptor (*HTR2A*) has been associated with schizophrenia in case control studies. The most extensively studied *HTR2A* polymorphism occurs in exon 1 and is a synonymous T/C change at position 102 (102 T/C). There are several findings of association between the *HTR2A* C102 allele and schizophrenia in Caucasian and Japanese studies [120–122]. The genetic association of this SNP with schizophrenia appears

to vary with ethnicity because studies of Chinese postmortem samples show increased frequencies of the *HTR2A* T102 allele in schizophrenia, which may indicate that a different SNP in linkage disequilibrium with 102 T/C is likely to be causative, or that epigenetic modification at this site could be confounding these analyses.

Initial evidence from analyses of postmortem brain indicated that there may be polymorphic differential expression of *HTR2A* alleles because 4 out of 18 heterozygous (T/C) subjects tested expressed only one allele at the 102 T/C site [123]. Another study has shown that the *HTR2A* is paternally imprinted in human fibroblasts and transcribed from the maternal allele only [124]. However, Polesskaya and Sokoloff [125] were not able to replicate this ‘on or off’ polymorphic imprinting, and a more recent study also found no evidence of imprinting of *HTR2A* [126]. Regardless, expression levels of *HTR2A* C102 have been shown to be reduced in the temporal cortex [125], and ligand binding studies have shown that subjects with the *HTR2A* C102 allele have reduced receptor binding in postmortem brain [127] and in platelets [128]. But it is important to note that there have been conflicting data [129–131], suggesting that these effects are not universal and could vary with brain region, ethnicity, and/or environmental influences.

Two polymorphic sites in *HTR2A* have been detected to have methylated CpG sites in recent studies by Polesskaya et al [132]. The first site is at the 102 T/C SNP and the second is at the -1438 A/G SNP in the promoter of the gene. The methylation of the *HTR2A* C102 allele was found to correlate with DNMT1 expression levels. Furthermore, methylation of the promoter correlated with *HTR2A* expression levels. However, De Luca et al. reported no differences in *HTR2A* methylation between controls and schizophrenia cases in a postmortem study [133]. These methylation events could provide explanations for the conflicting data generated by different research groups investigating *HTR2A* expression and genetic association with schizophrenia.

Epigenetic variation and imprinting of *HTR2A* in schizophrenia have yet to be extensively tested. The finding of differential DNA methylation within *HTR2A* could indicate variation in the activity of alleles 102 C and 102 T and thus confound genetic association studies of *HTR2A*, especially because the gene has not been intensively screened for methylated sites.

4.4 Genes influencing the dopaminergic system

Dysfunction of the limbic circuitry in schizophrenia and its influence on dopamine release is considered to be the primary pathophysiological mechanism of schizophrenia. Dopamine is involved in several brain functions, including attention, executive function (including working memory) and reward mechanisms. Therefore understanding the mechanisms of regulation of dopamine levels, particularly by catecholamine-*o*-methyltransferase enzyme or COMT, and the signal transduction of dopamine receptors within the limbic system is fundamental to the research of schizophrenia, addiction and related disorders [134].

The gene encoding the COMT enzyme (*COMT*) has been intensively investigated in genetic studies of schizophrenia. *COMT* is of particular interest because it is located in the schizophrenia ‘linkage hotspot’, 22q11, which is deleted in velocardio facial syndrome or VCFS (also called Di George syndrome or 22q11 deletion syndrome). VCFS patients have a range of symptoms, including psychosis, and together with replicated findings of linkage with schizophrenia in the 22q11 region, genes within this locus are strong candidates for investigation in schizophrenia research. Not only is *COMT* a strong positional candidate, it also has an important role in the regulation of monoamine metabolism and therefore is a strong physiological candidate gene for schizophrenia research [135].

COMT is an S-adenosylmethionine dependent methyltransferase enzyme which methylates catecholamines (including dopamine and norepinephrine) and catechol estrogens. Two isoforms of COMT enzyme have been reported; a membrane-bound COMT (MB-COMT) and soluble COMT (S-COMT), each with its own promoter. COMT is solely responsible for the metabolism of dopamine in the dorsolateral prefrontal cortex, a region important for working memory performance, which is dysfunctional in schizophrenia. The focus of genetic studies of COMT has been the valine to methionine substitution, which occurs at codon 158 in the MB-COMT isoform and at codon 108 in S-COMT. This polymorphism has been shown to alter the activity and thermal stability of the enzyme, so that subjects with the methionine homozygous genotype are estimated to have up to 50% reduction of COMT activity [136]. Dopamine signaling is therefore likely to be enhanced in subjects with the met 158 allele in comparison with subjects homozygous for the val 158 allele. A groundbreaking study by Egan et al. demonstrated that *COMT* val 158 homozygous subjects exhibited reduced prefrontal cognitive performance and efficiency, in comparison with met 158 homozygous individuals [137]. *COMT* SNPs in untranslated, promoter and intronic regions are thought to impact COMT function through altered gene expression [138]. While there have been many reports of association between the *COMT* val allele and schizophrenia, the data have not always been consistent. Combined genetic and epigenetic data are likely to produce more accurate predictors of psychiatric phenotypes than genetic variation alone and could be more correlated with gene x environment interactions in an integrated model.

DNA methylation analyses of *COMT* have been conducted in postmortem brain series. The promoter of MB-COMT has been found to be methylated and this isoform of COMT is predominantly responsible for the metabolism of dopamine in the brain. One study indicated that methylation of the MB-COMT gene promoter was reduced by approximately 50% in schizophrenia and bipolar disorder subjects compared with controls, especially in the left frontal lobe [139]. Furthermore, MB-COMT gene expression was raised in schizophrenia and bipolar disorder compared with the controls, and subjects with the 158 val allele had lower levels of MB-COMT promoter methylation. MB-COMT hypomethylation was also correlated with dopamine D2 receptor gene (*DRD2*) promoter hypomethylation in schizophrenia and bipolar disorder compared with controls [139]. Another study has indicated that the 158 val/val homozygote subjects had a greater degree of exonic DNA methylation in the frontal cortex, but there was no association between the level of methylation and psychosis in this region [110]. However, these studies did not specifically measure methylation within the dorsolateral prefrontal cortex, so whether methylation of *COMT* could be related to working memory in schizophrenia has not been established.

4.5 *BDNF*

BDNF is another gene known to play an important role in cognition, and aberrant regulation of this gene has been implicated in the etiology and pathogenesis of several cognitive and mental disorders, including schizophrenia [140–144]. The *BDNF* protein is synthesized from a gene that has a rather complex structure. The *BDNF* gene contains nine 5' non-coding exons (I-IX) linked to the common 3' coding exon (IX), which encodes for *BDNF* pre-protein [145, 146]. Despite the known importance of *BDNF* function and gene expression in normal neural processes and CNS disorders, there has been little investigation into the molecular mechanisms responsible for complex *BDNF* transcriptional readout in the brain.

Recently, several studies have begun to implicate DNA methylation as a provocative molecular mechanism contributing to ongoing regulation of *BDNF* transcription in the CNS to mediate synaptic plasticity and memory formation [59,60,62,146]. For example, Sun's group has shown that DNA methylation plays a role in activity-dependent *BDNF* gene regulation [147]. In addition, it has been shown that alterations in DNA methylation levels at the *BDNF* promoter

or intragenic regions occurs in response to fear learning [60]. Specifically, a Pavlovian learning paradigm (contextual fear conditioning) elicits changes in hippocampal DNA methylation across the *BDNF* gene, and that this mechanism is involved in differential *BDNF* transcript read-out necessary for long-term memory formation. Furthermore, pharmacologically inhibiting DNMT activity sufficiently alters basal *BDNF* transcript levels in hippocampus [60]. Together, these studies shed light on the potential role of epigenetic mechanisms, namely DNA methylation, in dynamic regulation of the *BDNF* gene in the adult CNS, and highlight the fact that altered *BDNF* regulation could contribute to schizophrenia [141].

In addition to regulation of gene expression changes supporting synaptic plasticity and memory formation, *BDNF* DNA methylation has also been shown to play a role in altered gene expression in response to environmental influences, such as social experiences [64]. In fact, stressful social experiences early in life have long-lasting effects on behavior, including increased anxiety, increased drug-seeking behavior, cognitive deficits, and altered affiliative behaviors. In relation to *BDNF*, depriving an infant of social interaction for example yields a reduction in hippocampal and cortical *BDNF* mRNA and protein levels that persists well into adulthood [148–150]. Finally, it was recently shown that social experiences early during the first postnatal week trigger lasting changes in DNA methylation across the *BDNF* gene that is associated with decreases in *BDNF* gene expression in the adult prefrontal cortex [64].

There has been little investigation into whether *BDNF* DNA methylation is altered in schizophrenia. Postmortem reports have indicated that in the prefrontal cortex and hippocampus of schizophrenic patients there is both decreased *BDNF* protein [143,151,152] and *BDNF* mRNA levels [140,143,144]. Whether DNA methylation is a mechanism responsible for the abnormal regulation of the *BDNF* gene is certainly not clear. However, one study to date by Mill and colleagues suggests this may indeed be the case [110]. They found modest evidence for an association between DNA methylation and the *BDNF* genotype at a nonsynonymous SNP (rs6265 or val66met) which affects exonic CpG sites.

Overall, data suggest that DNA methylation may indeed be an epigenetic mechanism that contributes to the aberrant regulation of genes associated with schizophrenia. Another epigenetic mechanism we will now consider is the posttranslational modification of histone tails, as the functional significance of histone modifications in the regulation of genes involved in schizophrenia is increasingly becoming the focus of vigorous research.

5. Histone Modifications

In the nucleus, DNA is wrapped around an octamer of 8 different histone proteins (H2A, H2B, H3, and H4), with H1 serving as a linker protein. Histone tail amino acid residues are subject to covalent modifications, including lysine acetylation, methylation, SUMOylation, and ubiquitinylation; arginine methylation; serine phosphorylation; and proline isomerization [153]. Depending on the amino acid residue modified there can be different effects on gene transcription. For example, acetylation is linked with gene activation, while SUMOylation is associated with gene repression. However, some histone modifications are more complex, such as methylation, and can be associated with either gene activation or repression dependent upon the nature of the modifications.

Histone methylation at lysine residues can be up to three forms: mono-, di-, and tri-methylated. For example, tri-methylation of lysine 4 of histone H3 is localized at gene promoter regions, and is linked with active gene transcription. Additional examples of the complexity of lysine methylation include methylation of lysine 9 and 27 of histone H3 and methylation of lysine 20 of histone H4. Mono-methylation is associated with active transcription while di- and tri-methylation at these lysine residues are associated with transcriptional repression [154]. Figure

2 illustrates some specific histone modifications that are associated with gene activation or gene suppression.

5.1 Histone Modifications in schizophrenia

To date, far less is known regarding histone modifications in comparison to DNA methylation in schizophrenia. However, histone modifications are beginning to receive attention for their likely contribution [155,156]. Importantly, histone marks are relatively preserved in human postmortem tissue [157].

In 2005, Akbarian's group provided the first demonstration that there are histone modifications associated with schizophrenia [158]. Specifically, they found in a small subset of prefrontal cortex samples that there was significantly higher open chromatin-associated H3-methylation (at arginine 17) in schizophrenic patients than controls [158]. Since their seminal study, they and others have provided further evidence for histone modifications in schizophrenia. For example, there is altered H3-lysine 4 and 27 trimethylation [107,159] and increased HDAC1 expression in the prefrontal cortex of schizophrenic patients [160].

Most of what we know regarding histone regulation of gene activity comes by way of studies in the learning and memory field indicating that specific histone modifications at gene promoters are an important control mechanism for transcriptional regulation in the adult CNS [60,161–166]. To date, this has been studied for the *BDNF* gene. Specifically, histone H3 acetylation and phosphorylation is increased at *BDNF* gene promoters in association with exon-specific *BDNF* gene expression in an electroconvulsive seizure model [166]. Other forms of histone modifications at *BDNF* promoters, such as histone H3 dimethylation, have also been described in transcriptionally repressed *BDNF* transcripts in a mouse model of depression [165]. Thus, an overall emerging hypothesis is that post-translational modification of histones can alter chromatin structure at gene promoter regions and subsequently control transcription of the genes in response to environmental cues. These data along with postmortem analyses suggest that histone modifications might also contribute to the aberrant regulation of genes associated with schizophrenia.

6. Conclusions and future directions

It is becoming increasingly clear that epigenetic mechanisms not only provide a molecular mechanism responsible for stable changes in brain function and behavior, but also have some necessary role in the dynamic nature of the adult CNS in response to the environment. Thus, an epigenetic contribution to schizophrenia has become an attractive molecular hypothesis, and evidence gathered to date appears to support this hypothesis. Unfortunately, postmortem studies make it difficult to address whether epigenetic mechanisms are causally related to the pathogenesis of schizophrenia. Thus, rodent models of neuropsychiatric disorders will likely have to fill this void in future studies.

One of the most important questions that future studies will likely address is whether epigenetic drugs can alleviate the cognitive deficits associated with schizophrenia. Encouragingly, studies on abnormal gene expression in postmortem brain have revealed that histone methylation may be a viable avenue for early detection for some cases of schizophrenia [155]. To date there is limited clinical and basic research data examining the role of DNMT and HDAC inhibitors in alleviating cognitive deficits [167–170]. However, beneficial effects of DNMT or HDAC inhibition have been observed in both alleviating some of the molecular and behavioral manifestations of cognitive dysfunction in rodents [156].

Such observations hold promise that drugs that can modify chromatin will be a viable therapy for treating schizophrenia. The continued study of epigenetic marks in normal brain

development, in the regulation of normal cognition, and in postmortem tissue promises a future where we will be able to unravel the molecular mysteries underlying schizophrenia. Elucidating these pathophysiological mechanisms will certainly facilitate the development of more effective therapeutic strategies.

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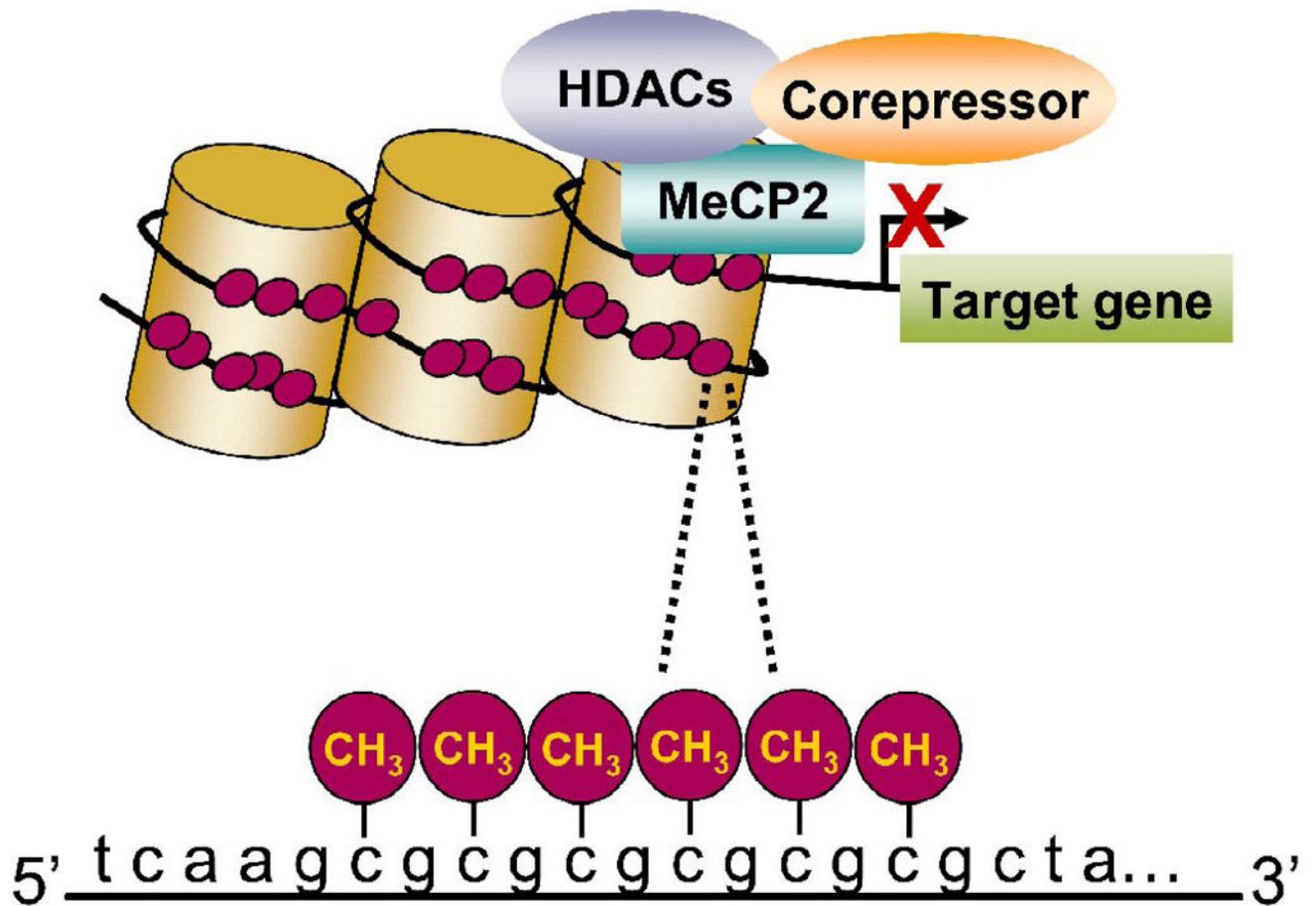


Figure 1. Schematic of transcriptional suppression by DNA methylation. Methylated cytosines bind, for example, methyl-CpG-binding protein (MeCP2), which recruits co-repressors and histone deacetylases (HDACs). Together, this leads to compaction of chromatin structure, which hinders gene transcription.

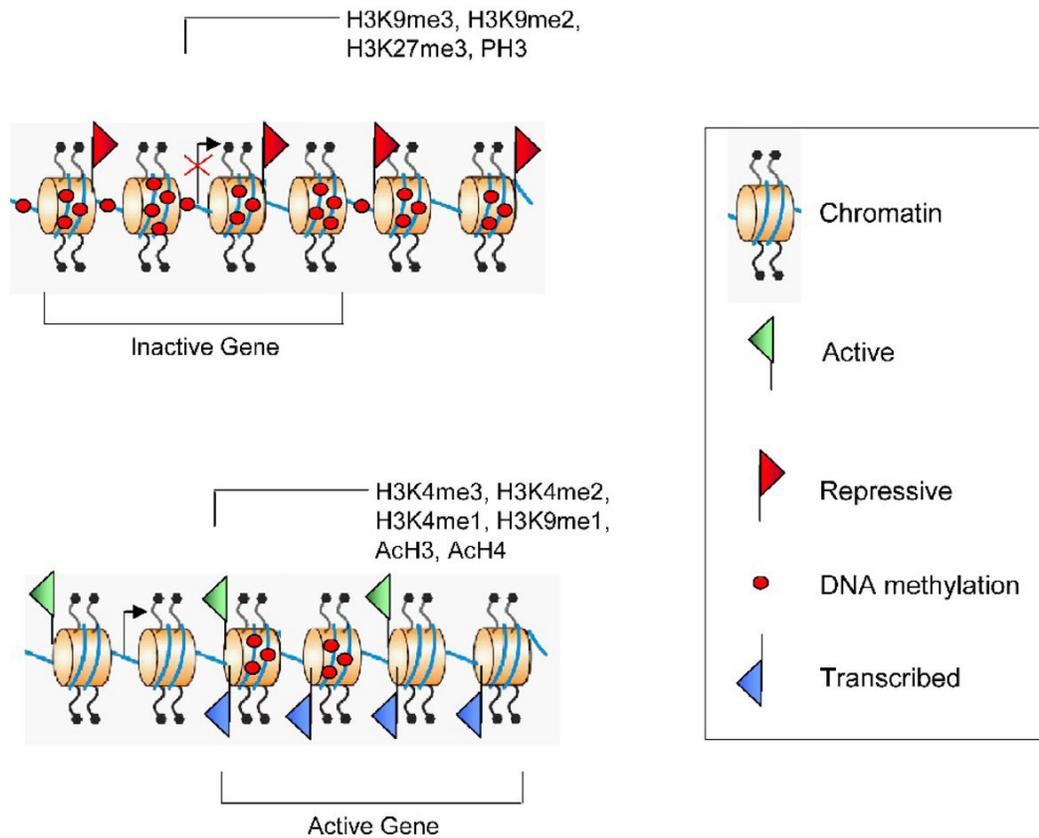


Figure 2.

The nucleosome core histones (H2A, H2B, H3, and H4) are subject to a variety of covalent modifications including: lysine acetylation, methylation, SUMOylation, and ubiquitylation; arginine methylation; and serine phosphorylation. Chromatin can be divided into accessible regions named euchromatin or inaccessible regions called heterochromatin. Some histone marks are associated with gene activation and others with gene repression. For example, histone H3, H4 acetylation (AcH3, AcH4), and phosphorylation (PH3) are associated with activation. However, other histone marks, such as histone methylation, have different effects depending on the lysine residue. The H3K4me3, H3K4me2, H3K4me1, and H3K9me1 mark the active transcription start site regions of a gene, while H3K27me3 is found in regions that encompass inactive genes. Histone lysine 9 mono- and dimethylated (H3K9me2 and H3K9me3) is also associated with the heterochromatic regions.

Table 1

Summary of epidemiological studies of Schizophrenia

Lifetime prevalence	0.5–1.72%, broad diagnostic category 0.26–0.54%, narrow diagnostic category
Onset of illness	Male mean age of onset at 26 yrs Female mean age of onset at 31 yrs
Socio-economic risk factors	Inverse relationship between socio-economic status and incidence of schizophrenia Higher prevalence in urban environments, especially males Downward drift seen in patients, especially in males
Seasonal differences in onset	Some evidence for seasonal patterns in incidence of the disease
Ethnic differences	Higher incidence of schizophrenia among migrant and ethnic minority groups living in large cities

Table 2

Candidate genes for schizophrenia which are epigenetically modified.

Gene	Locus	Linkage	Association	References
Glutamic acid decarboxylase (<i>GADI</i>)	2q31	2q37 [73] 2q33.3 [74]	Several SNPs and haplotypes associated with SCZ	<i>Discussed in section 4.2</i>
Reelin (<i>RELN</i>)	7q22	-	Association in Caucasians [75]	<i>Discussed in section 4.2</i>
Serotonin _{2A} receptor (<i>HTR2A</i>)	13q14-21	-	<i>HTR2A</i> -1438 G/A associated with SCZ	<i>Discussed in section 4.3</i>
Monoamine oxidase A (<i>MAOA</i>)	Xp11.3	-	No association *	[76]
Tyrosine hydroxylase (<i>TH</i>)	11p15.5	-	11p14.1 associated with SCZ in GWA [77]	[76]
Catechol-o-methyltransferase (<i>COMT</i>)	22q11	Strong linkage with SCZ	158 val/met associated with SCZ	<i>Discussed in section 4.4</i>
Dopamine D2 receptor (<i>DRD2</i>)	11q23	-	rs6277 associated with SCZ *	<i>Discussed in section 4.4</i>
Dopamine D3 receptor (<i>DRD3</i>)	3q13.3	-	No association *	[78]
Dopamine transporter (<i>SLC6A3</i>)	5p15.3	-	No association *	[76]
Brain derived neurotrophic factor (<i>BDNF</i>)	11p13	-	<i>BDNF</i> 66 val/met associated with SCZ	<i>Discussed in section 4.5</i>
Glucocorticoid receptor (<i>NR3C1</i>)	5q31.3	Within region of strong linkage [79]	No association *	[80,81]
Potassium-chloride Co-Transporter 3 (<i>SLC12A6</i>)	15q13-14	Linkage of 15q14- 15 with BPD and SCZ	Association with BPD and SCZ [82]	
Sex-determining region Y-box containing gene 10 (<i>SOX10</i>)	22q13.1	Linkage with SCZ	Association of rs139887 with SCZ in Japan [83]	[84]
Synapsin III (<i>SYN III</i>)	22q12.3	Linkage with SCZ	No association *	[85]

* SZGene at www.schizophreniaforum.org

Abbreviations: SCZ=schizophrenia, BPD=bipolar affective disorder, GWA=genome-wide association