Perspective

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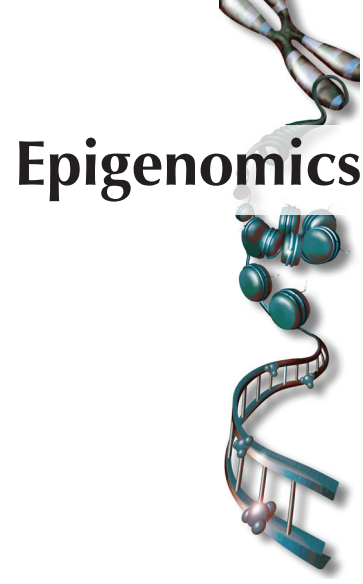

Understanding epigenetics of schizophrenia in the backdrop of its antipsychotic drug therapy

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The diatheses of gene and environment interaction in schizophrenia (SCZ) are becoming increasingly evident. Genetic and epigenetic backgrounds are being considered in stratifying and addressing phenotypic variation and drug response in SCZ. But how much of these epigenetic alterations are the primary contributing factor, toward disease pathogenesis and drug response, needs further clarity. Evidence indicates that antipsychotic drugs can also alter the epigenetic homeostasis thereby inducing pharmacoepigenomic effects. We re-examine the context of epigenetics in disease pathogenesis and antipsychotic drug therapy in SCZ to understand how much of these observations act as real indicators of the disease or therapeutic response. We propose that epigenetic viewpoint in SCZ needs to be critically examined under the genetic, epigenetic and pharmacoepigenetic background.

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Schizophrenia (SCZ) is a complex disorder that is influenced by both gene and environment. The interaction of risk gene and environment can result in presenting an aberrant epigenetic mechanism in SCZ. The hallmark of these epigenetic mechanisms is monitored through the altered state of methylation, histone modifications and miRNAs. The dynamic nature of epigenome and the reversibility of the epigenetic marks arouse the possibility that the epigenetic defects can be corrected by a wide range of interventions including therapeutics. Recent research in the area of epigenetic therapy has gathered hope for a better response. Accumulative evidence from human and animal studies suggests that antipsychotic drugs can also alter the epigenetic homeostasis. Therefore, there is a need to dissect the epigenetic crosstalk between disease and the drug in modulating a response. In this article, we first present the reported epigenetic landscape of SCZ and follow it up by emerging observations on the role of antipsychotic drug in modulating the epigenetic landscape. Finally, we present our perspective by evaluating the crosstalk between the two observations on epigenetic landscape. Our perspective might help in understanding the weak correlations and contradictions in epigenetic dysregulation in SCZ, and identify whether it is a feature of SCZ pathogenesis or a concomitant effect of antipsychotic drug-mediated manipulation of patient's epigenome.

Epigenetic mechanisms in schizophrenia

SCZ is a complex mental disorder that occurs with a global prevalence of 1% in general population. Family, twin and adoption studies provide strong evidence for the intersection of genetic and environmental factors in the etiology of SCZ [1]. Epigenetics provide

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a molecular link between genetic and environmental factors, thus contributing to the complex origin of the disease [2]. Several lines of research suggest that epigenetic mechanisms play a role in the pathophysiology of SCZ. A defective epigenome, characterized by altered miRNA expression or epimutations in the form of aberrant DNA methylation and histone modifications are reported to cause SCZ [3]. An overview of reported epigenetic landscape in SCZ is described below.

DNA methylation in schizophrenia

Global DNA methylation & maintenance in schizophrenia

DNA methylation is the best known epigenetic mechanism that regulates gene expression. DNA methylation is catalyzed by a family of related DNMTs that include *DNMT1*, *DNMT3A*, *DNMT3B* and *DNMT3L*. Methylation occurs when S-adenosyl-L-methionine donates a methyl group that is used by DNMTs to transfer the one carbon methyl group, producing 5-methylcytosine (5mC) and homocysteine. While 5mC is a marker for methylation index, 5-hydroxymethylcytosine (5hmC), 5-formylcytosine and 5-carboxylcytosine are the three additional DNA modifications that signify the active demethylation process. Methylation status of promoter proximal to CpG dinucleotides is in a dynamic balance between 5mC and 5hmC. 5hmC serves as an intermediate in the reaction of DNA demethylation or acts as a signal for chromatin factors. A family of ten-eleven translocase proteins hydroxylates 5mC to form 5hmC, 5-formylcytosine and 5-carboxylcytosine. Hydroxylation of 5mC is likely the first step in a mechanism by which cytosine methylation is reversed. Therefore, any interference in this system will influence the methylation process reflecting in altered methylation, demethylation and homocysteine levels. Here we present how alterations in global methylations and homocysteine are reported to impact the development of SCZ.

Global as well as gene-specific methylation changes have been reported in SCZ with inconsistent results across different populations (Table 1). Global DNA hypomethylation was reported in peripheral leukocyte of SCZ patients of European descent [4] while in Japanese population this hypomethylation was observed only in male SCZ patients [5]. The observation on global hypomethylation in SCZ and gender could not be replicated in Israel population [6]. In contrast to decreased global 5mC levels in SCZ brains, global 5hmC levels were observed to be increased in SCZ [7]. Global 5hmC levels are reported to have tissue-specific changes that may signify different tissue-specific routes to the pathogenesis of various psychotic disorders such as SCZ and bipolar disorder [8].

Homocysteine inversely correlates with the methylation levels. The accumulation of homocysteine can lead to neural damage and cognitive dysfunction [31]. Elevated plasma homocysteine has been reported to play a role in the pathogenesis of SCZ through alterations in DNA methylation [32]. High homocysteine level has also been reported in young male SCZ patients [9]. These global level methylation differences are known to be influenced by various environmental factors. At the same time, there are endogenous mechanisms that are responsible for maintaining these checks and balances in methylation patterns.

Several lines of research indicate region-specific alterations in the expression of various methyltransferases and demethylases in brains of SCZ patients. DNMT1 mRNA and protein levels are significantly increased in the telencephalic GABAergic interneurons of SCZ patients [28]. In SCZ patients, overexpression of *DNMT3a* is restricted to distinct GABAergic interneuron populations, while in peripheral blood lymphocytes both *DNMT1* and *DNMT3a* are overexpressed [29]. Recent studies have shown that there is increased ten-eleven translocase-1 mRNA and protein expression in the parietal cortices of psychotic patients [30]. This increase is associated with an increased genome-wide level of 5hmC and also an increase in 5hmC levels at *GAD67* and *BDNF* promoters [15,20]. Excerpts from various studies indicate that global methylation state does not reflect corresponding methyltransferases activity.

Gene-specific DNA methylation in schizophrenia

Impairment of neurotransmitter system due to differential methylation of genes of dopaminergic, serotoninergic and glutamatergic systems can result in SCZ condition. Increased promoter methylation of dopamine receptor genes including *DRD4*, *DRD5* and *DRD2* were reported in SCZ patients [10]. The two isoforms of a dopamine-metabolizing system, namely *MB-COMT* and *S-COMT*, are differentially methylated in SCZ. *S-COMT* has been reported to be hypermethylated [4], whereas *MB-COMT* is hypomethylated in SCZ [22]. Genes involved in serotoninergic pathways including serotonin receptors like *HTR1A* and *HTR2A* and serotonin transporter *5-HTT* are reported to be hypermethylated in SCZ [11–13]. *BDNF* promotes the development and function of serotonergic neurons. Decreased level of neural *BDNF* in SCZ is correlated with increased DNA methylation at the specific *BDNF* promoters. Also, the methylation status of *BDNF* promoters in peripheral blood was affected by gender, with methylation difference being prominent in

Table 1. Global and gene-specific DNA methylation reported in schizophrenia.

DNA methylation status	Major findings	Ref.
Global DNA methylation	Hypomethylation in peripheral leukocyte DNA	[4]
	Hypomethylation only in males with SCZ	[5]
	Correlation between plasma total homocysteine and genome-wide DNA methylation	[9]
	No correlation between global DNA methylation and SCZ or gender or plasma homocysteine level	[6]
Hypermethylation of SCZ-related gene promoters	*DRD2*, *DRD4*, *DRD5*	[10]
	S-COMT	[4]
	HTR1A, *HTR2A*	[11,12]
	5-HTT	[13]
	GRM2, *GRM5*, *GRIA3*	[14]
	GAD67, *RELN*	[15–19]
	BDNF	[20]
	SOX10	[21]
Hypomethylation of SCZ-related gene promoters	*MB-COMT*	[22]
Genome-wide methylation studies	Identified various differentially methylated loci in SCZ patients	[23–26]
Methylome-wide association study	Identified blood-based biomarkers in SCZ	[27]
Expression status of epigenetic genes	Overexpression of *DNMT1* and *DNMT3a* in GABAergic interneuron and peripheral blood lymphocytes of SCZ patients	[28,29]
	Overexpression of *TET1* mRNA and protein in the parietal cortices of psychotic patients	[30]

SCZ: Schizophrenia.

male SCZ patients [20]. Glutamatergic genes including *GRM2*, *GRM5*, *GRM8* and *GRIA3* are differentially methylated in SCZ. A study carried out in Iranian population showed that promoter methylation of the *GMR2* and *GMR5* genes greatly decreased the risk of SCZ whereas the promoter methylation of *GRIA3* gene highly increased the risk of SCZ [14]. The prefrontal cortical dysfunction in the pathophysiology of SCZ has been linked to alterations in GABAergic neurotransmission which are regulated by genes in the GABAergic pathway [33]. Downregulation of various GABAergic genes including *GAD67* and *RELN* in the prefrontal cortex and other brain regions are mediated by a hypermethylation of their gene promoters [15–19]. However, studies in a Japanese SCZ cohort *RELN* hypermethylation was not observed [34]. *SOX10*, a prime candidate for oligodendrocyte dysfunction in SCZ is highly methylated in brains of SCZ patients and is correlated with reduced expression of *SOX10* [21]. Recent technological developments using genome-wide methylation also revealed epigenetic changes in loci associated with GABAergic and glutaminergic neurotransmission, brain development and other processes that are functionally linked to etiology of the disease [23–25]. A recent innovative study suggests that DNA methylations associated with SCZ are more likely to be enriched near the genetic risk variants for SCZ and these can be traced as early as in fetal stage [24]. Another study has identified *FAM63B* as a potential marker for environmental insults in the blood of SCZ patients, which plays a role in neuronal differentiation and dopaminergic gene expression mediated by miRNA network [27]. Blood methylome of SCZ patients also indicated differentially methylated CpGs in inflammatory response genes such as *CD224*, *LAX1*, *TXK*, *PRF1*, *CD7*, *MPG* and *MPO* that are directly involved in activations of T cells, B cells and natural killer cells or, in cytotoxic reaction [26]. Thus differential gene-specific methylation seems to be a hallmark of SCZ but reported to vary among ethnicity, gender, tissue and brain regions. An appropriate study by considering factors that can influence methylations might stabilize the variability on observations.

Histone modifications in schizophrenia

Epigenetic signaling includes a host of opposing histone modifications occurring largely at histones 3(H3) and 4(H4) that includes phosphorylation, ubiquitination, acetylation and deacetylation, as well as methylation and demethylation. The amino acids along the histone tail, lysines (K) and arginines (R) are subject to methylation (me), while K is also a site for acetylation (ac). Specific modifications on histones can predict gene expression that is dependent on the local GC content of the gene promoters. This is reflected by the fact that histone modifications at H3K27ac and H4K20me1 are better predictors of gene expression that are driven from high GC content gene promoters while H3K4me3 and H3K79me1 are from low GC content promoters [35].

To date, very little is known about the histone modifications in the context of SCZ. Several lines of evidence suggest that histone modifications in the candidate genes of SCZ specific loci may contribute to the pathogenesis of prefrontal dysfunction in SCZ (Table 2). Increased expression of histone methyltransferases, *EHMT2* in lymphocytes and the *EHMT1* in both lymphocytes and postmortem parietal cortex are reported to be a significant predictor of SCZ [36]. A shift in histone methylation patterns from transcriptionally active H3K4me3 (trimethylation) to repressed H3K27me3 can play a role in regulating the expression of *GAD67*. In SCZ patients, sex specific deficits in H3K4me3 targeting the *GAD67* promoter resulted in decreased *GAD67* expression [37]. Histone H3K9K14 levels are hypoacetylated at the promoter regions of *GAD67*, *HTR2C*, *TOMM70A* and *PPM1E* genes in young subjects with SCZ [38]. High levels of H3-(methyl)arginine17 is associated with reduced expression of four metabolic genes (*CRYM*, *CYTOC/CYC1*, *MDH* and *OAT*) in the prefrontal cortex in a subset of subjects with SCZ [39]. Elevated levels of *HDAC1* are reported in the prefrontal cortex of SCZ patients [40]. Also, the mRNA expression level of GAD67 was negatively correlated with the mRNA expression levels of HDAC1, HDAC3 and HDAC4 levels. *HDAC9* is known to be implicated in SCZ with *HDAC* gene deletion being associated with a small subset of schizophrenic patients [41]. These studies do demonstrate that histone modifications are a feature of SCZ which seems to be age, gender and brain region specific. Consensus on these observations needs to be derived by considering an appropriate study design, which considers factors that can influence these changes.

miRNA in schizophrenia

The dysregulation of miRNAs contributes to the pathogenesis of SCZ. Multiple studies have identified numerous SCZ-associated miRNA (Table 3) [42–56]. Some of these miRNAs have been validated for their functional implication. Deletion of 22q11.2 results in alterations in brain miRNA biogenesis and downregulation of miR-185 in SCZ-associated brain regions [53]. This downregulation is reported to contribute toward deficits in dendritic spine developments in hippocampal neurons. miR-185 is known to interact with members of the RhoGTPase family (Cdc42, Rac1, RhoA) and spine-specific protein (Duo) that are critical for spine maintenance and spine formation which show decreased expression levels in the cortical gray matter of SCZ subjects. The expression of miR-195 is inversely correlated to BDNF protein levels, which in turn is positively correlated with neuropeptide Y (NPY), somatostatin and parvalbumin (PV) in the SCZ patients [54]. Computationally predicted targets of miR-195 are *GABRA1*, *GRIN1*, *HTR2C*, *HTR4*,

Table 2. Histone modifications reported in schizophrenia.

Major findings on histone modifications in schizophrenia	Ref.
Disease and age-related changes in histone acetylation at gene promoters of several SCZ-related genes including *GAD67*, *HTR2C*, *TOMM70A* and *PPM1E*	[38]
A shift in histone methylation patterns from transcriptionally active (H3K4Me3) to repressed (H3K27Me3) at the *GAD67* gene in the SCZ cases	[37]
Association between high levels of H3-(methyl)arginine 17 and reduced expression of metabolic genes (*CRYM*, *CYTOC/CYC1*, *MDH* and *OAT*) in prefrontal cortex of a subset of subjects with SCZ	[39]
Elevated levels of HDAC1 in the prefrontal cortex of SCZ patients	[40]
Negative correlation between GAD67 mRNA expression level and mRNA levels of HDAC1, HDAC3 and HDAC4	[40]
HDAC9 gene deletion in SCZ	[41]

SCZ: Schizophrenia.

Table 3. miRNAs reported to be associated with schizophrenia.

miRNAs associated with schizophrenia	Expression	Ref.
miR-26b, miR-30b, miR-29b, miR-195, miR-92, miR-30a-5p, miR30d, miR-20b, miR-29c, miR-29a, miR-212, miR-24, miR-30e, miR-9-3p	Downregulated	[42]
miR-106b, miR-7	Upregulated	[42]
miR-181b, miR-26b, miR-107, miR-15a, miR-15b, miR-16, miR-195, miR-19a, miR-20a, miR-26b, let-7e, let-7d, miR-128a, miR-181a, miR-219, miR-27a, miR-29c, miR-7	Upregulated	[45]
miR-195	Downregulated	[54]
miR-346	Downregulated	[44]
miR-329, miR-31, miR-409-3p, miR-224, miR-432, miR-487b, miR134, miR-431, miR-150*, miR-99b, miR-1275, miR-335*, miR-200c, miR-486-3p, miR-29b-1*, miR-16-2*, miR-877, miR-107, miR-130b*, miR-544, miR-342-5p, miR-148b, miR-625*, miR-28-3p, miR-576-5p, miR-151-3p, miR-28-5p, miR-664, miR-128, miR-584, miR-574-3p, miR-181a, miR-30e*, miR-433, miR-654-5p, miR-193b, miR-485-3p, miR-370, miR-340*, miR-1271, miR-151, miR-15b*, miR-502-3p, miR-500*, miR-27b, miR-199a-3p, miR-199b-3p, miR-151-5p, miR-146a, miR-21, miR-30d, miR-127-3p, miR-98, miR-328, miR-181b, miR-378, miR-150, miR-323-3p, miR-874, miR-330-3p, miR-500, miR-181a-2*, miR-146b-5p, let-7b, miR-25, miR-92a, miR-410, miR-221*, miR-942, miR-664*, miR-20b, miR-628-3p, miR-152, let-7d, miR-154, miR-337-3p, miR-505, miR-625, miR-22*, let-7g, miR-1301, let-7d*, miR-766, let-7a	Downregulated	[48]
miR-34a, miR-449a, miR-564, miR-548d, miR-572, miR-652	Upregulated	[49]
miR-432	Downregulated	[49]
miR-148b, miR-151 miR-27b, miR-301, miR-545, miR-639	Upregulated	[50]
miR-106b, miR-138, miR-193b, miR-210, miR-22, miR-324-3p, miR-338, miR-339, miR-425	Downregulated	[50]
miR-519c, miR-409-3p, miR-652, miR-382, miR-532, miR-199a*, miR-17-5p, miR-542-3p, miR-199b, miR-592, miR-495, miR-487a, miR-425-5p, miR-152, miR-148b, miR-134, miR-150, miR-105, miR-187, miR-154, miR-767-5p, miR-548b, miR-590, miR-502, miR-452*, miR-25, miR-328, miR-92b, miR-433, miR-222, miR-512-3p, miR-423, miR-193a	Upregulated	[51]
miR-132, miR-212	Downregulated	[55]
miR-30b	Downregulated	[47]
miR-181b, miR-219-2-3p, miR-1308, let-7g, miR-346, miR-92a	Upregulated	[52]
miR-195, miR-17	Downregulated	[52]
miR-185	Downregulated	[53]

DRD1, *GRM7* and *FGF2*. These genes are known to be dysregulated in SCZ. Dysregulation of miR-132 and its mRNA targets are implicated in the etiology and pathology of SCZ. Downregulation of miR-132 in SCZ is linked to the altered regulation of SCZ- and neurodevelopment-associated genes that include *DNMT3A*, *GATA2* and *DPYSL3*. miR-212 which is cotranscribed with miR-132 is also downregulated in SCZ [55]. In SCZ, an upregulation of miR-181b in the temporal cortex was shown to be coupled with the downregulation of various SCZ-associated genes including the calcium sensor gene, *VSNL1* and the ionotropic AMPA glutamate receptor subunit (*GRIA2*) [43]. Recent studies have revealed that aberrant expression of serum miRNAs in SCZ indicates the relationship between circulating miRNAs and disease status [56]. Serum miR-181b, miR-195, miR-219-2-3p, miR-1308, miR-365, miR-520c-3p

and let-7g are identified as candidate biomarkers for diagnosis of SCZ [52]. Another study has identified a set of 7 miRNAs, hsa-miR-34a, miR-449a, miR-564, miR-432, miR-548d, miR-572 and miR-652 as potential blood biomarkers for SCZ [49]. miR-346, located in an intron of *GRID1* a SCZ-susceptibility gene, is upregulated in the serum and downregulated in dorsolateral prefrontal cortex of SCZ patients [52]. These altered miRNAs can influence the expression of genes associated with SCZ. Therefore, it is important to study these miRNAs in perspective, as miRNAs are known to be influenced by environmental factors.

Genetics of epigenetics

Gene–environment interaction manifests into a disease by synergistic coparticipation of the risk genotype and risk environment where the effect of one is conditional on the other. Most often the epigenetics has been discussed in isolation, without any reference to the genetic background of the epigenetic processes, which might determine the threshold of environmental insults. Various environmental factors have been reported to alter the epigenetic gene expression profile of DNMTs or chromatin modulators in SCZ which may be indicative of altered methylation events, chromatin modulation or miRNA expression. But these expression profile differences could also be mediated by the nature of polymorphism in these epigenetic genes or target regions of miRNA-binding sites and thereby modulate the epigenomic response. This compels us to investigate the role of genetic polymorphisms in epigenetic dysregulation in SCZ.

Extensive studies have been carried out on global and gene-specific methylations, but none of these studies have investigated whether the genetic polymorphisms in the genes that are responsible for endogenously maintaining the methylation have any role in modulating these events or could be a causative factor for the disease itself. A recent genome-wide study has suggested that DNA methylation quantitative trait loci (meQTL) can be used to refine genome-wide association study (GWAS) loci through the identification of discrete sites of variable fetal brain methylation associated with SCZ risk variants [24]. In our earlier observation, we demonstrated that the genetic events such as gene polymorphisms in *DNMT1* are associated with SCZ and *DNMT3b* and *DNMT3L* were found to be associated with early onset and positive family history of SCZ in patients from South India [57]. These DNMTs are responsible for endogenously maintaining methylation events. Similarly, epigenetic alterations in chromatin remodeling enzymes have also been reported for SCZ pathogenesis [36]. But here too, these alterations have not been investigated in the background of their genetic milieu. In a recent study, polymorphisms in the chromatin remodeling enzymes have been reported to disrupt the chromatin regulation leading to altered neuronal function and behavioral abnormalities [58]. However, till date no study has implied the genetic role of these chromatin modulators in SCZ. The polymorphisms in chromatin-modulating genes might also indicate tissue-specific threshold for triggering an epigenetic response.

miRNA expressions can also be modulated by the polymorphisms in the miRNA-binding site in the 3′-UTR of the target gene namely miRSNPs. Two SNPs rs17578796 and rs1700 in mir-206 and mir-198 showed nominal significant allelic association to SCZ in the Danish and Norwegian samples, respectively [59]. A recent study has identified a functional variant ss178077483 located in the pre-mir-30e, which is strongly associated with SCZ [60]. A recent GWAS reported that rs1625579, located in the primary transcript of a miRNA gene, hsa-miR-137, as the strongest new association for SCZ [61]. Interestingly, four SCZ loci-*CACNA1C*, *TCF4*, *CSMD1*, *C10orf26* were later experimentally validated as hsa-miR-137 targets. While screening for SNPs in miRNA genes on the X chromosomes of male schizophrenia patients, eight ultra-rare variants in eight distinct miRNA genes (three precursor and five mature miRNA sequences) were identified that were present in only 4% of the male SCZ patients [62].

These observations suggest that epigenetic events need to be understood in the backdrop of an individual's genetic architecture. While some of these events are being explored and investigated, we further wanted to understand whether the epigenetic alterations can also be mediated by antipsychotic drugs, which are conventionally used for the therapeutic response.

Epigenetic effect of antipsychotic drugs

The epigenome is highly dynamic in nature, and therefore reversibility of these epigenetic marks can raise the possibility of erasing the epigenetic defects by therapeutic intervention. The emerging field of pharmacoepigenomics is providing promising insights into the role of drugs in modulating host epigenome, as well as in addressing interindividual variability in drug response and drug-related adverse effects. Increasing lines of evidence indicate that antipsychotic drugs have the potential to target various epigenetic processes, thereby modulating the epigenome (Figure 1). Isolated studies on typical antipsychotic drugs like chlorpromazine, haloperidol and atypical drugs like amisulpride, clozapine, risperidone, olanzapine and quetiapine suggest that anti-

psychotics can modulate epigenome (Table 4). Clinical studies have shown that HDAC inhibitors are efficacious when given in combination with atypical antipsychotics. The clinical studies might be mired by multiple medications while *in vitro* or *in vivo* animal studies might indicate drug-specific effects.

Among typical antipsychotics chlorpromazine on prolonged treatment is reported to induce antinuclear antibodies that can react with histones, indicating the probability of histone modification [72]. Chlorpromazine is also reported to increase DNAseI susceptibility and enhance chromatin degradation indicating increased chromatin accessibility of the drug [73]. No specific study has been carried out to pinpoint the exact nature of epigenetic changes that are induced by chlorpromazine. Another typical antipsychotic, haloperidol is reported to induce global DNA hypermethylation in leukocytes of SCZ patients [4]. In rat models, it is reported to induce tissue- and sex-specific changes in DNA methylation [63]. It also induces DNA demethylation of the *DUSP6* gene in proliferative cancer cells [64]. In rat brain striatal extracts, haloperidol can induce phosphorylation of histone H3 at serine 10 and the acetylation of H3-lysine 14 (H3pS10-acK14) that is enriched at genomic sites with active transcription [65]. Haloperidol exposure can also mediate overexpression of three miRNAs: miR-199a, miR-128a and miR-128b in rats [42]. These studies clearly indicate that typical antipsychotics can induce epigenetic alterations.

Studies on atypical antipsychotics are just emerging. Clozapine is reported to reverse the methionine induced hypermethylation state of *RELN* and *GAD67* promoter by activating DNA demethylation process together with an increase in promoter-associated H3K9 and H3K14 acetylation [66]. Clozapine is shown to increase the GAD67-associated trimethylation of H3K4 in mouse cerebral cortex and human prefrontal cortex [39]. It can also increase the expression of the H3K4-specific histone methyltransferase gene Mll1, thereby increasing its occupancy in the promoter of *GAD67,* leading to its transcriptional activation. Clozapine can also reduce methylation at the Gadd45-β locus similar to the effect of brain-permeant mGlu2/3 receptor agonist LY379268 [74]. Clozapine can reverse the deficits in pre-pulse inhibitory action of dizocilpine by modulating the expression of miR-219 in the prefrontal cortex of mice brain [67]. Similar to haloperidol, ris-

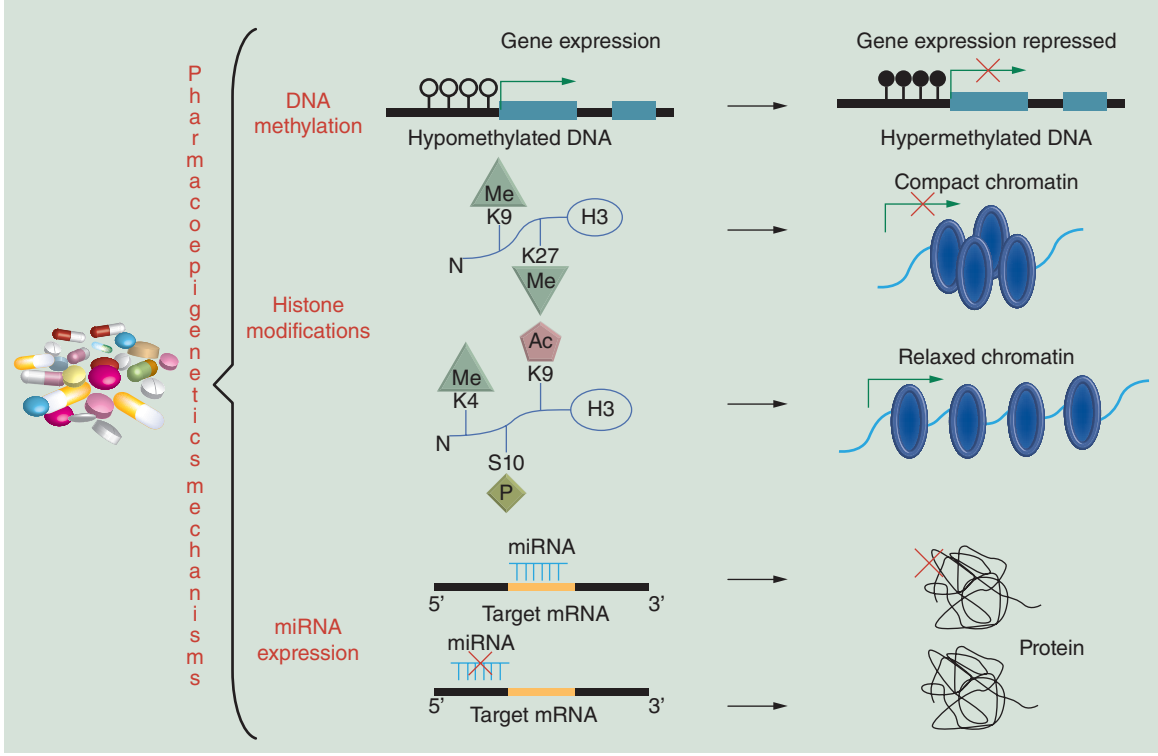

Figure 1. Modes of epigenetic modulation by antipsychotic drugs. Antipsychotic drugs can induce methylation (hypo/hyper) changes resulting in altered (increased/decreased) gene expression, differential histone modifications resulting in condensed or relaxed chromatin or miRNA expression (over/repressed) resulting in altered gene expression.

Table 4. Antipsychotic drugs and its reported role in epigenetic modifications.

Drug	Epigenetic modifications induced by antipsychotic drugs	Ref.
Haloperidol	Higher global DNA methylation in leukocytes of schizophrenia patients	[4]
	Induce tissue- and sex-specific changes in DNA methylation in rat models	[63]
	DNA demethylation of the *DUSP6* gene in proliferative cancer cells	[64]
	Phosphorylation of histone H3 at Ser 10 and acetylation of H3K14 in striatal neurons	[65]
	Overexpression of miR-199a, miR-128a and miR-128b in the haloperidol-treated rats	[42]
Clozapine	Increases GAD67-associated trimethylation of H3K4 by increasing expression of Mll1	[39]
	Increases promoter-associated H3K9 and H3K14 acetylation and cause demethylation of hypermethylated GABAergic gene promoters (i.e., *RELN* and *GAD67*)	[66]
	Modulate dizocilpine-induced effects on miR-219	[67]
Risperidone	Induces global phosphoacetylation of H3 in the striatum	[65]
	Modulate the expression levels of miR-346, miR-365 and miR-520c-3p in serum	[52]
Olanzapine	Facilitate chromatin remodeling at doses higher than used clinically	[68]
	Widespread and tissue-specific changes in genome-wide methylation in rats *in vivo*	[69]
	Increase the methylation of miRNA gene *mir125b-1* in cerebellum	[69]
Quetiapine	Facilitate chromatin remodeling at doses higher than used clinically	[68]
Sulpiride and amisulpride	Increases acetylation of H3 associated with *RELN* and *GAD67* gene promoters in the frontal cortices of mice	[70]
Valproic acid	Coadministration with clozapine and sulpiride accelerates the demethylation of *RELN* and *GAD67* genes	[66]
	Downregulation of DNMT1, 3A and 3B protein levels and DNMT enzyme activity	[70]
	Increase expression of HDAC 1, 2 and 3 and of MeCP2 in C6 glioma cell lines	[71]

peridone is also reported to induce both phosphorylation and acetylation of H3pS10-acK14 in nuclei of striatal neurons [65]. Risperidone can also modulate the expression levels of *miR-346*, miR-365 and miR-520c-3p in serum [52]. Among the new generation antipsychotics, olanzapine is reported to induce methylation changes in dopamine pathway genes that include *DRD1*, *DRD2*, *DRD5*, *COMT*, *SLC18A2* and *DDC8* in the rat model [75]. In a recent genome-wide methyation study, olanzapine is reported to induce widespread tissue-specific methylation changes in rats [69]. Olanzapine and quetiapine can facilitate chromatin remodeling at higher doses than used clinically [68]. Increase in methylation of miRNA gene Mir125b-1 in the rat cerebellum, post olanzapine treatment, is reported to alter the expression of the corresponding miRNA [69]. Sulpiride and amisulpride, two benzamide derivatives with antipsychotic properties are shown to increase acetylation of H3 associated with *RELN* and *GAD67* gene promoters in the frontal cortices of mice [70]. These studies clearly indicate that atypical antipsychotics can also induce epigenetic alterations.

In a conventional treatment protocol, antipsychotics are coadministered with other drugs and administering valproic acid is one of the most common practice. Coadministration of antipsychotic drugs (clozapine, sulpiride and amisulpiride) with valproic acid is reported to increase expression of the *GAD67* and *RELN* genes by altering histone methylation, increasing histone acetylation and decreasing DNA methylation associated with these genes [66]. Several lines of evidence suggest a possibility that various antipsychotics directly affect the expression and activity of epigenetic modifiers. Role of coadministered drug or drug for other co-morbid factors become critical in evaluating the epigenetic response as these drugs can also influence the host epigenome.

In silico curation of miRNAs linked to all major antipsychotic drugs was carried out using PharmacomiR database [76]. Pharmaco-miR is a web server which links miRNA expression and drug function by combining data on miRNA targeting and protein–drug interactions. A large number of miRNAs were observed to be targeted by antipsychotic drugs (Supplementary Table 1), thus establishing that these drugs can affect miRNA expression. Studies clearly demonstrate that both typical and atypical antipsychotic drugs and the commonly used coadministered drugs can modulate the host epigenome. The observations from animal or *in vitro* studies also clearly demonstrate the role of antipsychotic drugs in modulating host epigenome. However, validating these observations in human subjects need appropriate study design to avoid the crosstalk of other drugs. Considering these facts, we were now curious to understand how much of these epigenetic alterations reported in SCZ could be of real significance.

Crossroads of epigenomics & pharmacoepigenomics in schizophrenia

In the earlier sections, we discussed the role of epigenetics in disease pathogenesis and how drugs modulate host epigenetics. To date, majority of the studies have handled these topics in isolation. We present our perspective by merging the two concepts and understand the influence of pharmacoepigenomics by interrogating the study design and subject selection strategy in the studies that reported epigenetic changes in SCZ. Majority of the investigations on epigenetic markers were performed in the peripheral blood and in some cases in postmortem brains. Most of these studies have not mentioned the medication history of the patients or refer to a conventional therapeutic background of the patient sample, indicating that the patients were on the conventional treatment protocol. SCZ subjects in a majority of the studies were under the antipsychotic drug, monotherapy or polytherapy unless mentioned as drug naive. Very few studies have been performed in drug naive SCZ patients. The statistical tools implemented in some of these studies were based on regression analysis to account for the possible confounding factors like age, gender, diet, drug, ethnicity, substance use, which can influence the epigenetic pattern while antipsychotic medication was rarely considered as a confounding variable in these studies. Coadministration of other drugs or comorbid factors of the disease was never discussed in any of the studies. Since antipsychotic drugs or coadministered drugs can exert a confounding effect on the host epigenome, it is advisable to investigate this aspect and its crosstalk with epigenetic patterns in SCZ patients. To understand this crosstalk we will be reviewing the articles that report global and gene-specific methylations, histone modifications and miRNA expressions and understand whether these articles do refer to antipsychotic drug effects in SCZ.

While considering studies on global methylations we observe that global DNA hypomethylation in peripheral leukocyte of SCZ patients was based on a patient population that was undergoing antipsychotic drug medication. One of such studies has also shown that patients treated with haloperidol had higher global DNA methylation compared with patients treated with other main antipsychotic drugs [4]. Another study which showed sex-dependent differences in the global methylation profile of peripheral leukocyte DNA in SCZ patients mentions that this observation might be a secondary effect of antipsychotic medication. They further indicated that the male patients might have received multiple drugs and higher doses than female patients, which might have reflected in hypomethylation [5]. On the contrary, a study which excluded patient subjects treated chronically with medications that may alter DNA methylation like valproate failed to demonstrate any difference in global DNA methylation between patients and control [6]. Also, this study suggests no association between global leukocyte DNA methylation and homocysteine levels in SCZ patients, whereas other studies which included subjects treated with antipsychotic drugs have shown a relationship between plasma total homocysteine and DNA methylation patterns in the peripheral leukocytes of patients with SCZ [32]. This indicates that the reported global DNA methylation profile in SCZ patients could be due to an impact of antipsychotic medication.

Not many studies have correlated the methylation changes with gene expression alterations in drug naive SCZ patients. A recent study had correlated the reduction in serotonin transporter *5-HTT* expression with DNA hypermethylation of its promoter in drug naive SCZ patients [13] and interestingly their follow-up study demonstrated that antipsychotic drug attenuates aberrant DNA methylation of *DTNBP1* promoter in saliva and postmortem brain of the same set of SCZ patients [70]. In another study, *MB-COMT* promoter hypomethylation was reported with no significant difference in methylation frequency in drug naive and antipsychotic drug treated patients [22]. Interestingly, the same authors have demonstrated genotype-specific differential methylation in *HTR2A* gene promoter in postmortem brain samples that reflect different psychiatric condition [12]. It is possible that observations in these postmortem brain samples might be mired by medi-

cation effects. In a study that reported altered methylation state of glutamatergic genes and dopaminergic genes in SCZ, it is not very clear whether these observations reflect drug-naive SCZ patients or patients from conventional treatment background [10,14]. Very few studies have clearly mentioned this conflict between antipsychotic usage in SCZ and drug-naive SCZ patients while addressing epigenetic aberrations in SCZ patients. Methylation in neuronal genes like *RELN* reported no correlation between methylation and drug equivalents for SCZ [15,34]. Another study reported downregulation and methylation of *SOX10* but expressed inability to completely rule out the effect of medication on *SOX10* oligodendrocyte gene expressions in the study. However, they also demonstrate that the gene expression and DNA methylation levels of *SOX10* were not significantly correlated with a lifetime antipsychotic dose in the SCZ group [21]. In the studies that reported increased expression of *DNMT1* and *DNMT3a* in SCZ, the patients were undergoing treatment with typical and atypical antipsychotics and valproic acid [29]. However, they suggest that these changes were unaffected by the dose, the duration or the type of antipsychotic treatment. Interestingly, in an earlier study, it has been demonstrated that the genotype patterns of *DNMT1* and *DNMT3a* are themselves implicated in SCZ which might reflect on differences in methylation patterns [57]. None of the studies on epigenetic modifications have correlated their observation with individual drugs or have been matched with their drug levels or average chlorpromazine equivalences. These studies indicate that methylation and gene expression differences in SCZ need to be established further with an appropriate design to rule out the influence of genotype effect and antipsychotic drugs.

The role of confounding factors in differential methylation of certain genes has been reported, although to a very limited extent. A study, showing increased methylation of *HTR1A* gene promoter, has not shown clinical data on antipsychotic drug usage [11]. Similarly, the effect of medication on DNA methylation changes in *BDNF* gene promoter could not be assessed as the medical history was unavailable for most of the patients [20]. Therefore, the association of gender with the methylation status of certain genes like *BDNF* might be because of sex-specific differences in drug reactions. Sex-specific differences have been reported in treatment response to antipsychotics. Stanley Foundation Neuropathology Consortium [77] maintains an exhaustive demographic data of SCZ samples including antipsychotic drug information. Very few studies have procured the brain samples from this consortium [12,18,19,21,23]. In SCZ, *GAD67* expression has been reported to be decreased [18]. Antipsychotic drugs are reported to increase expression of the *GAD67* by epigenetic modifications [37,66]. We performed an exploration of data from this consortium and tested for the role of antipsychotics as a confounding variable. We observed wide variability in *GAD67* gene expression by considering antipsychotics as a confounding variable, and this might have reflected on the lack of correlation with an increase in drug concentration. This variability in *GAD67* gene expression across samples could be mediated by differential epigenomic or pharmacoepigenomic influence. Therefore, it is possible that antipsychotic drugs can also act as a confounding factor for epigenomic modifications to modulate therapeutic response.

In many of the genome-wide methylation studies, the role of antipsychotic drug in altering the methylation events cannot be ruled out. One study did mention the chlorpromazine equivalent of antipsychotic drugs but did not consider it as confounding variable while evaluating DNA methylation signatures in peripheral leukocytes in SCZ [32]. Another study on genome-wide DNA methylation did report that the patients were on antipsychotic medication as a limitation of their study [25]. A study on epigenomic profiling revealed that DNA methylation changes associated with SCZ has no false discovery rate (FDR)-significant correlation between DNA methylation and lifetime illicit drug use [23]. Interestingly, in the same study, a strong correlation between DNA methylation in the *MEK1* gene promoter region and lifetime antipsychotic use in SCZ patients has also been reported [23]. However, it is also important to note that clozapine has been reported to increase MEK/ERK signal transduction pathway, thereby pointing to the fact that antipsychotic drug usage influences key genes in the signaling pathway by methylation events [71].

The studies that performed histone profiling in SCZ were reviewed to understand whether the patients were on antipsychotic drug therapy. In a study that reported deficits in H3K4 trimethylation associated with GABAergic gene promoters, the study subjects selected were on atypical antipsychotic drug treatment [36]. Interestingly, the same study further demonstrated that clozapine treatment can increase the H3K4 (tri)methylation and Mll1 occupancy in mouse brain [37]. In a study that reported a correlation between high histone acetylation at lysine 14 and reduced metabolic gene expression in SCZ, the patient subjects were under typical and atypical drug treatment. This points to a possibility that it might be the effect of drug therapy that the metabolic gene expression is downregulated, thereby result-

ing in drug-related adverse effects [39]. In another study that reported disease and age-related changes in histone acetylation at gene promoters of *GAD67*, *HTR2C*, *TOMM70A* and *PPM1E* in SCZ, patients were treated with antipsychotic drugs [38]. However, in this study, they ruled out the possibility of valproic acid, a known HDAC inhibitor, by not considering patients on valproic acid. Medication effects are known to influence the HDAC mRNA expression in the prefrontal cortex of SCZ patients. Articles that report increased HDAC mRNA expression in SCZ patients suggest that confounding effects of psychotropic medication cannot be entirely resolved in clinical studies as they are on multiple medications and the duration of treatment is also a matter of concern [40]. Patients treated with valproic acid had significantly elevated levels of HDAC9 compared with other subjects (controls and patients taking other drugs) [41]. However, in this study too, the authors suggest that the confounding effects of antipsychotics and antidepressant medications could not be ruled out. Interestingly, chronic administration of atypical antipsychotic drugs has been shown to selectively upregulate the expression of HDAC2 in both mouse and human frontal cortex and transcriptional repression by HDAC inhibitors can improve atypical antipsychotic response [78]. HDAC2 is known to modulate metabotropic glutamate receptor. It has also been demonstrated that atypical antipsychotics downregulate the expression of mGlu2 receptor in the frontal cortex through decrease histone acetylation at the promoter regions of the receptors. This prohibits pomaglumetad, an agonist for mGlu2 receptors, to act efficiently in the presence of atypical antipsychotics while with the dopaminergic drug it produces a better response [79]. These studies clearly indicate the existence of overlapping patterns of histone modifications in SCZ and antipsychotic drugs, as demonstrated by the use of inhibitors, which can reverse the overexpression induced by antipsychotics.

In the studies reporting dysregulation of miRNA expression in SCZ, the patient population selected for the study were under antipsychotic or neuroleptic treatments [46,47]. Toxicology report presented in some articles clearly unravels the presence of the drug in patient samples [43,45,48,51]. It has been demonstrated that modulating the levels of circulating plasma miR-134 in bipolar disorder by antipsychotic medication can enhance therapeutic response [80]. Contradictions are evident while considering observations from individual miRNAs, such as miR-199a and miR-346, has been reported to be upregulated as well as downregulated in SCZ [44,48,51–52]. Interestingly, haloperidol and risperidone have also been reported to upregulate these miRNAs [42,52]. Similarly, miR-128a was found to be upregulated in SCZ and haloperidol treatment [42,45]. Bayesian model averaging method used in one of the studies revealed that demographic variables and psychiatric phenotype were unlikely to influence miRNA expression in postmortem brain samples [50]. Interestingly, in the same study, antipsychotic drug treatment was excluded from Bayesian model averaging, with a prejudice that inclusion of this variable could obscure the detection of disease-related alterations in miRNA expression. We performed a comparison of miRNAs that are dysregulated in SCZ and modulated by antipsychotic drugs. miRNAs that were dysregulated in SCZ were extracted from literature (Table 3) while miRNAs that were modulated by antipsychotic drugs were predicted using Pharmaco-miR (Supplementary Table 1). We identified a large number of overlapping miRNAs that were found to be altered in SCZ and also by antipsychotic drugs (Table 5). This clearly justifies that the reports on miRNA expression in SCZ might be heavily mired by the influence of ongoing treatment. To overcome this scenario, it would be ideal to have drug-naive SCZ patients, which in conventional clinical setup seems almost impossible. The emerging observations on pharmacoepigenomics might help in resolving the real epigenomic signature of pathogenesis from drug response.

Conclusion

The role of epigenetic dysregulation in SCZ has often been investigated in clinical subjects. The recent understandings in epigenetics and the technological developments in the field has provided deeper insights in identifying epigenetic patterns of histone modifications, methylations and miRNAs in the pathogenesis of SCZ. Accumulative evidence from human, animal, *in vitro* and *in silico* studies suggests that antipsychotic drugs can also alter the epigenetic homeostasis. Although the studies reporting epigenetic dysregulation in SCZ has taken into consideration various demographic variables like age, gender, post-mortem interval; the effects of medication is often neglected. Antipsychotic drug therapy related issues that include dosage regimen, combination therapy and duration of therapy can also influence the epigenetic machinery. In clinical subjects, it is difficult to resolve the potential confounding effect of antipsychotic medication because of the study design in existing studies and their ethical considerations. Therefore, considering the epigenetic effects of antipsychotic drugs and their overlapping patterns with SCZ pathogenesis, we propose the possibility of epigenetic dysregulation reported in SCZ could be mired by antipsychotic drugs. It is also possible that the pharmacoepigenomic

response can result in attenuation of the actual epigenetic alterations by antipsychotic drugs. Hence, to get a novel insight into the epigenetics of SCZ, the nonoverlapping epigenomic patterns between antipsychotic drugs and SCZ might be of interest.

Future perspective

In this perspective article, we intend to draw attention toward the pharmacoepigenomic influence of antipsychotic drugs, and distinguish its significance from therapeutic response and SCZ pathogenesis. We suggest that an appropriate longitudinal study design of SCZ patients starting from drug naive to treatment follow-up might help in dissecting the real significance of epigenomics and pharmacoepigenomics in pathogenesis and therapeutic response. Whereas for addressing confounding role of antipsychotics in retrospective studies, a careful evaluation of the crosstalk between

Table 5. Overlapping miRNA that are associated with schizophrenia and predicted to have alterations in response to antipsychotic drugs (pharmaco-miR database).

Drugs	miRNAs that are common in schizophrenia and pharmaco-miR database
Aripiprazole	miR-15a, miR-15b, miR-16, miR-195, miR-330-5p, miR-9
Chlorpromazine	let-7b, let-7b, let-7d, let-7e, let-7g, miR-101, miR-103, miR-107, miR-125b, miR-1271, miR-133b, miR-134, miR-148a, miR-153, miR-155, miR-15a, miR-15b, miR-16, miR-193a-3p, miR-193b, miR-195, miR-19a, miR-200c, miR-210, miR-219-5p, miR-22, miR-221, miR-222, miR-25, miR-26b, miR-27a, miR-30a, miR-30b, miR-30d, miR-30e, miR-330-5p, miR-342-3p, miR-370, miR-382, miR-410, miR-455-5p, miR-494, miR-495, miR-520c-3p, miR-590-3p, miR-874, miR-9, miR-92a, miR-92b, miR-98
Clozapine	let-7b, let-7d, let-7e, let-7g, miR-101, miR-103, miR-106b, miR-107, miR-125b, miR-1271, miR-130a, miR-130b, miR-133b, miR-138, miR-155, miR-15a, miR-15b, miR-16, miR-17, miR-181a, miR-181b, miR-186, miR-192, miR-195, miR-200c, miR-20a, miR-20b, miR-22, miR-221, miR-222, miR-23a, miR-23a, miR-24, miR-25, miR-26b, miR-27a, miR-27b, miR-29a, miR-29b, miR-29c, miR-301a, miR-301b, miR-30a, miR-30a, miR-30b, miR-30d, miR-30e, miR-330-5p, miR-339-5p, miR-33a, miR-340, miR-34a, miR-373, miR-382, miR-433, miR-449a, miR-494, miR-495, miR-519d, miR-590-3p, miR-874, miR-9, miR-92a, miR-92b, miR-96, miR-98
Haloperidol	let-7b, let-7d, let-7e, let-7g, miR-101, miR-103, miR-107, miR-125b, miR-1271, miR-133b, miR-148a, miR-148b, miR-150, miR-152, miR-153, miR-155, miR-15a, miR-15b, miR-16, miR-181a, miR-181b, miR-181d, miR-186, miR-192, miR-193a-3p, miR-193b, miR-195, miR-197, miR-19a, miR-200c, miR-219-5p, miR-219-5p, miR-22, miR-24, miR-25, miR-26b, miR-27a, miR-27b, miR-29a, miR-29b, miR-29c, miR-30a, miR-30b, miR-30d, miR-30e, miR-340, miR-342-3p, miR-370, miR-382, miR-433, miR-455-5p, miR-494, miR-495, miR-520c-3p, miR-520d-3p, miR-544, miR-590-3p, miR-7, miR-874, miR-9, miR-92a, miR-92b, miR-98
Olanzapine	let-7b, let-7d, let-7e, let-7g, miR-101, miR-106b, miR-125a-5p, miR-125b, miR-1271, miR-128, miR-132, miR-133b, miR-138, miR-155, miR-15a, miR-15b, miR-16, miR-17, miR-192, miR-195, miR-20a, miR-20b, miR-212, miR-22, miR-221, miR-222, miR-223, miR-23a, miR-24, miR-26b, miR-27a, miR-27b, miR-29a, miR-29b, miR-29c, miR-30a, miR-30b, miR-30d, miR-30e, miR-330-5p, miR-339-5p, miR-340, miR-34a, miR-373, miR-449a, miR-519d, miR-590-3p, miR-874, miR-9, miR-98
Quetiapine	miR-103, miR-107, miR-133b, miR-26b, miR-27b, miR-31, miR-340, miR-874
Risperidone	let-7b, let-7d, let-7e, let-7g, miR-101, miR-103, miR-106b, miR-125b, miR-1271, miR-128, miR-130a, miR-130b, miR-132, miR-133b, miR-134, miR-148a, miR-148b, miR-152, miR-155, miR-15a, miR-15b, miR-16, miR-17, miR-181a, miR-181b, miR-181d, miR-185, miR-186, miR-193a-3p, miR-193b, miR-195, miR-19a, miR-200c, miR-20a, miR-20b, miR-210, miR-22, miR-221, miR-222, miR-23a, miR-24, miR-25, miR-26b, miR-27a, miR-27b, miR-29a, miR-29b, miR-29c, miR-301a, miR-301b, miR-30a, miR-30b, miR-30d, miR-30e, miR-31, miR-329, miR-330-5p, miR-340, miR-34a, miR-370, miR-373, miR-382, miR-410, miR-433, miR-449a, miR-494, miR-495, miR-519d, miR-520c-3p, miR-590-3p, miR-7, miR-9, miR-92a, miR-92b, miR-96, miR-98
Ziprasidone	miR-103, miR-107, miR-133b, miR-192, miR-24, miR-26b, miR-27b, miR-31, miR-330-5p, miR-340, miR-874, miR-9

antipsychotic-induced pharmacoepigenetic events and epigenetic events including the meQTLs in SCZ is required. The present perspective article might provide a basis for background check on this crosstalk between epigenetic alterations by antipsychotic drug and SCZ pathogenesis. However, a more comprehensive understanding of each antipsychotic drug and its influence on the epigenome might help in resolving the issue of therapeutic response, side effects and pathogenesis of SCZ. This comprehensive observation can subsequently be used to resolve the role of antipsychotics as a confounding factor from retrospective studies.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/full/10.2217/epi-2016-0106

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Executive summary

- Schizophrenia (SCZ) is a complex disorder that is influenced by gene and environment.
- Family, twin and adoption studies provide strong evidence for the intersection of genetic and environmental factors in the etiology of SCZ.
- Epigenetics provides a molecular link between genetic and environmental factors thus contributing to the complex origin of the disease.

Epigenetics in schizophrenia

- Epimutations in the form of aberrant DNA methylation, histone modifications and miRNA expression are reported to be associated with SCZ.
- Differential global as well as gene-specific changes in DNA methylation have been reported in SCZ with contradictions.
- Histone modifications in few candidate genes may contribute to the pathogenesis of prefrontal dysfunction in SCZ.
- Increased expression of histone methyltransferases has reported to being a significant predictor for diagnosis of SCZ.
- Aberrant expression of serum miRNAs and postmortem brain in SCZ indicate the relationship between circulating miRNAs and disease status.
- Genetic polymorphisms in the methylation machinery genes and chromatin remodeling enzymes have been reported to be associated with behavioral abnormalities.

Pharmacoepigenetics of antipsychotic drugs

- Haloperidol has been shown to induce changes in DNA methylation, histone modifications and miRNA expressions.
- Clozapine has also been reported to alter expression of histone modifier genes, gene-specific methylation and miRNA expressions.
- Similar observations have been made with risperidone, olanzapine and quetiapine, indicating epigenetics effects of these antipsychotic drugs.

Future perspective

- We suggest that a careful evaluation of pharmacoepigenetic observations may provide better clarity on epigenetics of SCZ.
- A longitudinal study design might help in dissecting the real significance of epigenomics and pharmacoepigenomics in SCZ pathogenesis and therapeutic response.
- The emerging observations on pharmacoepigenomics might help in distinguishing the epigenomic signatures of pathogenesis and drug response, by using antipsychotic drug as a confounding factor.
- The present perspective article might provide a basis for background check on the crosstalk between epigenetic alterations by antipsychotic drug and SCZ pathogenesis.
- A comprehensive prospective study on the pharmacoepigenetic effects of antipsychotics can subsequently be used to resolve the role of antipsychotics as a confounding factor from retrospective studies.

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