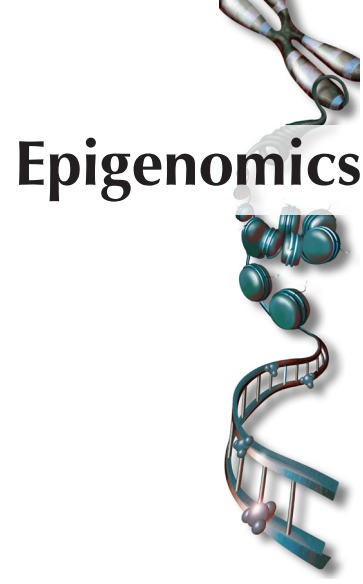
Perspective



DNA methylation in psychosis: insights into etiology and treatment

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Evidence for involvement of DNA methylation in psychosis forms the focus of this perspective. Of interest are results from two independent sets of experiments including rats treated with antipsychotic drugs and monozygotic twins discordant for schizophrenia. The results show that DNA methylation is increased in rats treated with antipsychotic drugs, reflecting the global effect of the drugs. Some of these changes are also seen in affected schizophrenic twins that were treated with antipsychotics. The genes and pathways identified in the unrelated experiments are relevant to neurodevelopment and psychiatric disorders. The common cause is hypothesized to be aberrations resulting from medication use. However, this needs to be established by future studies that address the origin of methylation changes in psychosis.

Keywords: antipsychotics • DNA methylation • environment • epigenomics • exposure • mental disorders • monozygotic twins • olanzapine • psychosis

It is now apparent that the manifestation of the genetic code into psychiatric phenotypes including mental disorders is not determined solely by DNA sequence [1,2]. The causation of psychiatric disorders involves complex interactions involving chromatin, where epigenetic signals superimpose a regulatory role. In fact, it has been suggested that the missing heritability seen in neuropsychiatric disorders could be due in part to the effect of epigenetic patterning [1,2]. This perspective suggests that the epigenome is in a dynamic state influenced by both deterministic as well as stochastic processes. This complexity also makes it difficult to tease apart the underlying factors that contribute to its state at any given time [3]. It represents a major challenge for future studies. For now, and for a variety of reasons, this research is accentuated by studies on epigenetic processes involving DNA methylation. DNA methylation in mammals involves the modification of cytosine to methylated cytosine (or its equivalent) in the genome. The phenomenon is sequence specific and needed for the proper functioning of the genome. DNA methylation provides regulatory roles in cellular functioning via regulation of gene transcription [4], genomic imprinting [5], gene splicing [6] and chromatin structure and stability [7]. Indeed, any aberration from normal patterns of methylation may cause abnormal cellular functioning including disease phenotypes [8]. Potentially, DNA methylation profiles can be altered by various factors including seasonal, social and environmental factors as well as chemicals and drugs [9,10]. This dynamic property may help to further the understanding of disease processes including mechanisms of actions of drugs that are used to treat disease. For example, it remains unknown how antipsychotic drugs control emotional and behavioral symptoms. The most accepted explanation is that antipsychotics have their own receptor-binding profiles, pharmacologic profiles and mechanisms of action [11–14]. Often, the treatment protocol involves 'testing and trying' toward finding a suitable drug and its appropriate dose for each patient. Some patients fail to respond to one antipsychotic but subsequently show a robust response to a different drug despite the fact that both

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block the D2 receptor, which is believed to be their mechanism of action [15]. Also, adverse effects of antipsychotic drugs vary greatly across patients [16]. Further, the delayed response of antipsychotics [17] and the associated variable metabolic side effects remain poorly understood [18–20]. It is however understood that drugs used to treat psychiatric disorders may cause epigenetic changes in the genome [21,22]. These changes have been identified in patients with psychosis that were on antipsychotic medication as compared with controls who were not on any medication. In fact, there have been a number of reports on epigenetic profile differences in psychosis patients, but the role of epigenetic changes in the causation of psychosis remains poorly understood [23]. The questions remain, whether these changes are the causes or the effects of the disease process and what is the role, if any, of drugs used by patients? In this perspective, we will discuss the role of DNA methylation in the etiology, pathophysiology and treatment of psychosis.

The etiology of psychiatric disorders involves DNA methylation

Psychiatric disorders, such as schizophrenia, may run in families as well as result from the response to a variety of drugs and chemicals. Schizophrenia has a high heritability (~80%) but monozygotic twins show a high discordance rate (~50%) [24]. The most extensive study performed on schizophrenia to date, has identified 108 loci, most of them with additive effects, that may contribute to this disease [25]. Also, some of the associated markers may contribute to the disease by affecting gene expression. These results argue for the potential involvement of regulatory mechanisms, particularly DNA methylation, in the development of the disease [26]. These mechanisms may underlie aberrations in neurodevelopment known to exist in a number of mental disorders [27–29]. The direct involvement of DNA methylation in schizophrenia has been assessed using a variety of inventive approaches, such as post-mortem human brains, familial relatedness including monozygotic twins discordant for the disease and animal models. In this perspective, we will use selected results to make the point that the epigenome is involved in the development of psychiatric disorders in general and in particular in schizophrenia, as summarized below.

First, studies on brains from patients with schizophrenia and matched controls have identified differences in DNA methylation [30]. The results are comparable to similar studies on blood samples from schizophrenia patients [30]. The questions of both tissue specificity and the effect of drugs are critical and present concerns in studies on methylation involving human brain disorders. Indeed, a recent study on medication free patients [31] suggests that the methylation effect is indeed a part of the complexity of epigenetic studies on schizophrenia. Also, some nonbrain tissues may serve as markers for abnormalities in the brain [32]. Further, the genes affected in patients are related to a number of pathways particularly the glutamatergic and GABAergic neurotransmission pathways, which have been previously implicated in psychosis [33,34].

Second, results on DNA methylation analysis of blood DNA from monozygotic twins discordant for schizophrenia further support the involvement of DNA methylation in psychosis [35]. Methylation of genomic DNA and promoter methylation of specific genes in blood samples of twins discordant for schizophrenia showed hypermethylation and hypomethylation of several genes [34]. These findings are consistent with the global increase and decrease in methylation of promoter regions of several genes in brain tissues in a rat model [36]. These findings suggest that a common epigenetic regulation mechanism may be applicable both in the brain and in peripheral tissues of schizophrenia patients [37]. Also, observed changes in methylation in all these studies report epigenetic changes that may have resulted in the disorder and also changes induced by the drugs administered to treat the patients. In addition, studies involving medication-free schizophrenia patients suggests that altered DNA methylation could be involved in the pathophysiology of schizophrenia [31]. Differences in methylation between identical twins have been identified as early as in newborn twin pairs [38]. Also, these differences change over time, supporting the potential for neurodevelopmental programing and reprograming in the causation of this disease [35]. Additional contributions to the discordance of monozygotic twins may involve *de novo* mutations [39–41] and epimutations [42], strengthening the case for dynamic processes including DNA methylation in psychosis. These processes are likely directed by genetic as well as random and environmental contributors over the lifetime [43]. We assessed the blood DNA methylation in two pairs of unrelated monozygotic twins discordant for schizophrenia using Methylated DNA Immunoprecipitation (MeDIP) [Castellani *et al.*, Unpublished Data]. The genomic DNA was processed at ArrayStar Inc (MD, USA); this included the MeDIP, sample labeling and hybridization to the NimbleGen Human DNA Methylation Promoter Plus CpG Island 720k Array. DNA was extracted from whole blood samples and the arrays were analyzed using Partek Genomics Suite® version 6.6 (MO, USA), Partek Pathways (Fishers Exact Test) and Ingenuity Pathway Analysis (Ingenuity Systems Inc., CA, USA). The results show that the monozygotic twins differ in DNA methylation. Interestingly, differentially methylated genes affect common pathways in

the schizophrenic twins [Castellani CA *et al.*, Unpublished Data]. Specific pathways identified include cell death and survival, cellular movement and immune cell trafficking network. In addition, Ingenuity Pathway Analysis (IPA) has identified protein kinase A signaling (p = 3.09E-04), granzyme A signaling (p = 6.83E-03), G protein signaling (p = 1.24E-02), serotonin receptor signaling (p = 1.72E-02) and UVB-induced MAPK signaling (p = 2.12E-02) as canonical pathways affected in this disease. Additionally, the top physiological system functions identified were nervous system development and function, immune cell trafficking and behavior. These results on blood DNA argue that DNA methylation in schizophrenia is common. Its pattern predominates the pathways that are compatible with their manifestation [Castellani CA *et al.*, Unpublished Data].

Third, evidence is emerging from human as well as animal models that antipsychotic drugs may function via their effect on DNA methylation. For example, a significant increase in DNA methylation (60.5 vs 37.6%) has been observed in schizophrenic patients who were treated with antipsychotic medication versus those that were not [34]. Similar results have been found in twins with major depressive disorder and on medication as compared with their unaffected co-twin not exposed to medication [44]. The authors also suggest that this difference could be due to antidepressants rather than as a cause or result of the disease alone [44]. It argues that methylation changes are not only involved in the etiology of psychosis, but also play a role in the response to antipsychotics [45,46]. Interestingly, antipsychotics were reported to have improved the efficacy of histone deacetylase (HDAC) inhibitors when administered in combination, through downregulation of genes such as *mGlu2* [47]. Also, previous reports suggested that antipsychotic drugs impacted DNA methylating enzymes only when the HDAC inhibitor, valproate, was administered concurrently [48]. This suggests that the impact of antipsychotics on DNA methylation may involve indirect mechanisms [47,48]. However, the specific mechanisms of actions of antipsychotics are not yet fully understood. Further study is required to investigate the possible mechanisms by which DNA methylation functions in regulating chromatin structure, stability and thereby gene expression.

We used a rat model to evaluate the effect of an antipsychotic drug (olanzapine) on genome-wide DNA methylation using methylation chips [36]. It offers for the first time, a published assessment of methylation differences between brain regions (hippocampus and cerebellum) and liver as a nonbrain reference in the same individual. The results show that olanzapine causes differential methylation that is tissue specific [36]. This response is similar but not identical between hippocampus and cerebellum and very different in the liver. Explicitly, olanzapine caused methylation changes in genes encoding for members of the dopamine pathways. They include *Drd1, Drd2, Drd5*, *Slc18a2*, *Ddc8* and *Comt*. Most of these genes (17/19) showed an increase in methylation in their promoter regions with *in silico* analysis [49]. These results strongly indicate the potential to suppress transcription, particularly in brain regions [49,50]. The findings support the dopamine hypothesis of psychosis and argue that antipsychotic drugs may mediate disease symptoms by their effect on the methylation of genes of critical pathways that include dopamine. Interestingly, the observed methylation alterations in the liver are compatible with the adverse effects of olanzapine that include metabolic syndrome and increased body weight. We conclude that DNA methylation may play an important role in the efficacy, as well as side effects, of the drugs used to treat psychosis.

Methylation changes in psychiatric disorders affect multiple pathways

Psychiatric disorders are highly heterogeneous in manifestation and causation. They involve multiple mechanisms including a large number of genes affecting relevant pathways. In fact, schizophrenia is associated with > 108 genes that participate in a number of pathways [25]. Most of these pathways are consistent with leading pathophysiological hypotheses. Some of these pathways are rare while others may be relatively common, such as the dopamine pathway. Such conclusions drawn from genome-wide associations are also compatible with methylation studies that have reported hypermethylation of the serotonin transporter gene promoter particularly in schizophrenia patients [50]. Some pathways are shared by a number of manifestations. As an example, *CDK5* and *CREB* signaling is often reported in schizophrenia [51], in excessive anxiety induced by stress [52], as well as in depressive-like behavior [53]. Interestingly, altered DNA methylation of genes involved in the *CREB* pathway has been reported in a recent study [54]. We feel that this complexity is complemented by differences in methylation across relevant tissues as well as by differential responses to different drugs. For example, the effect of a second-generation antipsychotic, blonanserin, causes hypermethylation in *DRD2* and *HTR2A* in human neuroblastoma cells [55]. Also, methylation at a transcription factor-binding site of the *5-HT1A* gene was reported to be associated with the treatment response to negative symptoms in schizophrenia patients [56]. In addition, a number of genes including *Drd2* and *Drd3* were downregulated in the nucleus

accumbens and prefrontal cortex of rat brains due to antipsychotic treatment, while *GLRA1* was found to have a hypermethylated promoter region [57]. Further, *DRD2, DRD4* and *DRD5* promoters were significantly methylated in schizophrenia patients as compared with healthy controls [58], suggesting that the dopamine network is actively involved in an increased risk for psychosis. We conclude that alterations in DNA methylation are in fact important to the etiology of psychosis. Also, alterations in DNA methylation may mediate the efficacy and side effects of antipsychotic drugs. The results also argue that not all pathways involved may have an equal contribution to psychosis. We will address this issue under two separate headings using results generated from our rat model [36,49].

DNA methylation & dopaminergic pathways in psychosis

Olanzapine-induced DNA methylation changes in rats included hypermethylation of genes of dopamine synthesis, receptors, transporters and metabolism [49]. It follows a report on olanzapine-induced methylation changes in the promoter regions of genes involved in the dopaminergic pathways in humans [59] arguing that transcriptional repression of those genes may be critical [60]. A similar conclusion might be applicable for other genes of this pathway including *DRD5* [61-64] and *COMT* [65]. Interestingly, the observed methylation peaks often overlapped with genomic regions containing CTCF binding sites, which are frequently associated with gene promoters and involved in genome organization [66]. The efficacy of antipsychotic drugs may represent an indirect effect via alterations in DNA methylation, which may take time to act. These results from different experimental setups suggest that the dopaminergic pathway is likely to serve as an essential framework in the etiology as well as treatment of psychosis.

DNA methylation & nondopaminergic pathways in psychosis

Results on olanzapine-induced methylation changes in our rat model identified six of the 18 genes to be involved in the GABAergic, glutamatergic and cholinergic pathways [36]. Among them, three (*Gls, Psd* and *Psd2*) were affected in hippocampus: one (*Nr1*) was affected in cerebellum, and two (*Nr2b* and *Glud1*) in the liver. Interestingly, a deficit of brain γ-aminobutyric acid (GABA)ergic function in schizophrenia patients has been linked to the downregulation of GABAergic genes [67]. The cadherin pathway was also affected by antipsychotic-induced DNA methylation in a rat model [36]. These adhesion molecules that constitute a super-family of transmembrane receptors mediate Ca^{2+}-dependent cell-to-cell communication [68]. The organization of these genes allows differential expression including gene-specific DNA methylation and differential splicing to facilitate expression of specific cadherin(s) in different cells and cell types [69]. Taken together, the results offer novel insights into the role of DNA methylation in altering expression of genes involved in nondopamine pathways potentially affecting the pathophysiology of psychosis. It is likely that additional pathways critical in psychosis will be identified in the future. There is every reason to hypothesize that at least some of these genes will involve alterations regulated by DNA methylation.

Conclusion

DNA methylation may play an important role in psychiatric disorders. Emerging evidence shows that antipsychotic drugs that are used to treat such disorders may also involve alterations in DNA methylation. This conclusion is based on the results from two distinct, yet overlapping, projects from our lab. First, our study on olanzapine treated rats showed that olanzapine-induced DNA methylation affects psychosis relevant pathways, including the dopamine, ephrin, GABAergic, cholinergic and cadherin pathways. However, an increase in promoter DNA methylation and GABAergic gene expression downregulation has been detected in the post-mortem brain of psychosis patients [70,71]. Also, Reelin promoter hypermethylation and its reduced mRNA expression has been reported [72]. This argues that antipsychotic-induced methylation (whether direct or indirect) may underlie the amelioration of psychotic symptoms as well as account for certain adverse effects including metabolic syndrome through upregulation or downregulation of relevant genes and thus have the potential to lead to an increase or decrease in impairment. As expected, the observed methylation changes were cell type specific and offered novel insight into the mechanism of action of antipsychotics [36]. However, the study did not assess DNA hydroxymethylation, which would be of future research interest. Second, the study on monozygotic twins discordant for schizophrenia, which included patients exposed to antipsychotic drugs, has uncovered many of the same pathways and networks, particularly relating to the dopamine pathway and GABAergic functions. These network functions have previously been linked to schizophrenia and were found both in our olanzapine-treated rats and in schizophrenia-affected twins when compared with their unaffected co-twins. Overall, the collective understanding of DNA methylation in psychosis demonstrates that methylation changes in the genome likely play a role in disease etiology. In addition, antipsychotic-induced changes in DNA methylation suggest that antipsychotic use could result

in methylation changes as a consequence of treatment. Thus, it remains the challenge of future research to tease apart the specific roles of methylation in the etiology of the disease, in response to drugs, and also in developing effective therapeutic strategies involving reversal of methylation. The results have provided a reminder that clinical research should no longer be about nature versus nurture, but instead about the complex interplay of nature and nurture. Also, the implications of these findings need to be carefully examined. They may help develop early diagnosis as well as methylation based grouping of patients to overcome heterogeneity. We note that DNA methylation is reversible and that this may allow for targeted methylation changes in the treatment of neurodevelopmental disorders.

Future perspective

Future epigenetic studies are sure to contribute a wave of Epigenome-Wide Association Studies (EWAS) results. It is critical to pair any EWAS data with a better understanding of the theoretical backdrop of epigenetic changes. This will include a better understanding of epigenetic response to treatment and other influences including environmental factors. Moving forward, a database of epigenetic profiles in healthy controls, in patients on antipsychotics and in patients without medication will allow researchers to begin to unravel some of these questions. For example, recent studies have begun to unravel involvement of aberrant DNA methylation changes involving medication-free [31] and drug naïve patients [50]. Methylome-wide studies also suggest that the methylation status of blood reflects those in the brain, supporting the use of blood as a surrogate tissue [73]. The timing of epigenetic change is crucial to ascertaining the role of epigenetics in complex traits such as psychosis, which may need further attention.

The inherent dynamic and responsive nature of the methylation landscape makes it both a candidate for the explanation of disease discordance in monozygotic twins for a number of phenotypes yet also presents an experimental challenge given its complexity and the vast number of factors that are now known to affect such changes. The question moving forward remains: What epigenetic changes being identified in cases are causal toward disease? Technological advances, increased sample sizes, model organism studies and an increased understanding of the timing and origin of epigenomic mutations will help us uncover the answers to some of these questions, which in turn will allow for a better understanding of the complex phenomenon of methylopathy. Finally, molecules will have to be identified that will help correct such aberrations and ameliorate the disorder.

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No writing assistance was utilized in the production of this manuscript.

Executive summary

The etiology of psychiatric disorders involves DNA methylation

- Studies on brain and blood from patients with schizophrenia show differences in DNA methylation. This includes differences in monozygotic twins discordant for schizophrenia.
- We argue that methylation plays a critical role during neurodevelopment. Any aberration may contribute to the development of psychosis.
- Antipsychotic drugs that are often used to treat psychosis may function via their effect on DNA methylation.

Methylation changes in psychosis affect multiple pathways

- Alterations in DNA methylation may mediate the efficacy as well as side effects of antipsychotic drugs accomplished by a variety of unrelated pathways.
- The dopamine pathway and genes involved in glutamatergic neurotransmission are hypothesized to play a major role in psychosis related disorders.
- The dopaminergic pathway may serve as a prominent framework for the treatment of psychosis.
- Nondopaminergic pathways such as GABAergic, glutamatergic and cholinergic pathways likely also play a significant role.
- Other pathways, some still unknown, may be important in selected patients including families.

Future perspective

- There is a need to fully understand the involvement of DNA methylation in neurodevelopmental disorders.
- This understanding will be critical in the development of novel corrective measures currently not available in psychosis, which remain a societal burden.

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