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Changes in DNA Sequence and Methylation Contribute to the Predisposition of Schizophrenia: Toward an Epigenetic Therapy

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<http://dx.doi.org/10.5772/65905>

Abstract

Schizophrenia has a heterogeneous and complex etiology that includes multiple candidate genes affected by a variety of mutational mechanisms including epigenetics, functional pathways, and environmental factors. This chapter mainly focuses on reviewing two sets of studies. The first one is whole-genome next-generation sequencing datasets involving monozygotic twins discordant for schizophrenia. The findings suggest that *de novo* sequence variations may underlie the discordance of monozygotic twins for schizophrenia. Second, whole-genome DNA methylation study suggesting the role of DNA methylation in the mechanisms of actions of antipsychotic drugs in treating the disorder as well as the manifestation of side effects such as metabolic disorders. Furthermore, we are reporting original research results using next-generation mitochondrial DNA sequence analysis of a pair of monozygotic twins discordant for schizophrenia as well as their mother. The chapter sheds light on the interplay between sequence variations and epigenetic signatures, including DNA methylation changes, in the etiology and pathophysiology of schizophrenia. Given the dynamic nature of methylation, it may be possible to develop a new treatment strategy for schizophrenia that is based on reversion of genomic methylation. This may involve environmental, dietary, and/or pharmaceutical approaches.

Keywords: schizophrenia, twins, sequence variation, DNA methylation

1. Introduction

The treatment of schizophrenia involves the suppression of hallucinations, delusions, agitation, and an array of behavioral problems that often accompany these symptoms [1]. When acute symptoms start to subside with antipsychotic drug treatment, psychotherapy and rehabilitation interventions can be undertaken. The heterogeneity of schizophrenia may account for concentration of the disease in some families, reduced concordance between monozygotic twins, and patient-specific causations. The identity of genes and pathways involved in schizophrenia and the mechanisms affecting them are forthcoming. This development has identified important insights including the fact that a relatively large number of genes affected in schizophrenia belong to relatively few critical pathways. This includes the Dopamine pathway that has provided the foundation for the development of primary treatment of the disease. There is an opportunity to focus on additional affected pathways for the treatment of a subset of patients. One of the mechanisms that may affect schizophrenia-related pathways is DNA methylation. This chapter will discuss primary molecular studies that support a threshold model for this complex disease, including complete genome sequences of monozygotic twins discordant (MZD). Specifically, we identify patient-specific genes that may be affected by a variety of mutational mechanisms, including DNA methylation. Here, the predisposition for the disease is realized on a threshold scale (**Figure 1**) via mutations involving a variety of mechanisms including sequence variations and copy number variations in nuclear genes as well as changes in genome-wide DNA methylation [2, 3]. The threshold model can only be tested on monozygotic twins discordant for the disease. Next, we will argue for the direct role of DNA methylation in schizophrenia using two sets of independent results; methylation differences between MZD twins and tissue-specific response of olanzapine (antipsychotic) treatment in rats *in vivo* [4, 5].

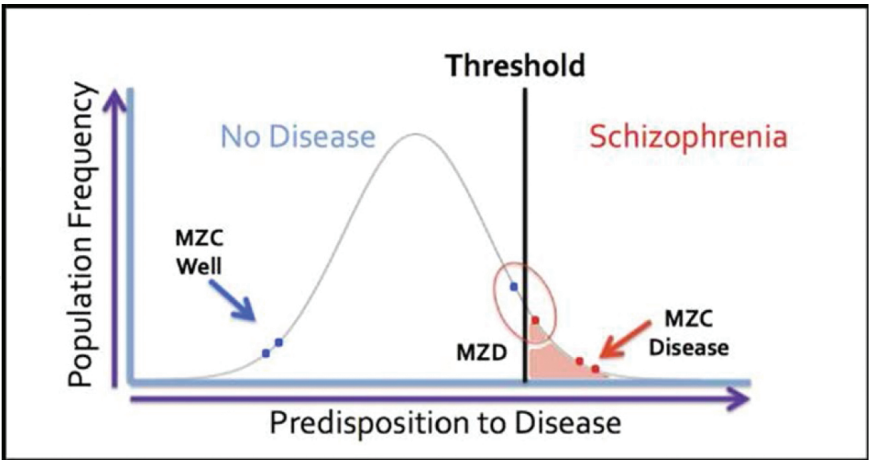


Figure 1. A threshold model for predisposition to schizophrenia in monozygotic twins discordant (MZD) for schizophrenia [2].

We will also discuss three facets of schizophrenia and their implications in the development of any strategy for amelioration: (i) the role of *de novo* sequence variations (nuclear and mtDNA)

in the etiology and treatment of psychiatric disorders, including schizophrenia; (ii) the involvement of DNA methylation in the development of psychiatric disorders, particularly schizophrenia; and (iii) the interplay between DNA sequence variation and DNA methylation. The insights covered will be incorporated into the development of strategies toward personalized medicine for the treatment of psychiatric disorders.

2. Role of sequence variations in the etiology and treatment of psychosis

A growing body of evidence suggests the significance of genetic variants in the etiology of mental health disorders, including schizophrenia. For example, the Psychiatric Genomics Consortium identified several SNPs that are associated with major psychiatric disorders including schizophrenia [6], which included *CACNA1C* variants that have been previously associated with autism [7]. Also, angiotensin-converting enzyme (ACE) gene insertion/deletion polymorphism was reported to be associated with schizophrenia susceptibility as well as the severity of schizophrenia depressive symptoms in a Chinese population [8]. According to the schizophrenia-working group of the Psychiatric Genetic Consortium, the expression of the C4 gene was affected by SNPs in the gene resulting in putative synapse elimination in schizophrenia patients [9]. It has recently been reported that 15 of 48 schizophrenia cases were found to carry rare or novel missense coding variants in four signaling genes studied. These findings suggest that single genes harboring *de novo* mutations in individuals with psychosis as compared to healthy controls may play a critical role in influencing the phenotypes of psychosis and hence may be potential targets for developing treatment strategies. A study from our lab reported that copy number variants (CNVs) in monozygotic twins discordant for schizophrenia could be an important underlying factor in the discordance of the twins for the disease [3]. The findings identified several CNVs and genes, in four of the six twin pairs studied, that were previously implicated in mental health disorders. These findings suggest a role for CNVs in the discordance of twins for schizophrenia.

Similarly, unpublished studies from our lab on complete genomes of two pairs of monozygotic twins discordant for schizophrenia showed multiple individual sequence-specific differences between cotwins. The observed differences included small nucleotide changes (single nucleotide variation, block substitutions, and small indels), copy number variations, and structural variations that were unique to either the affected or healthy cotwins. Also, by comparing the sequence differences between cotwins with that of their parents, it was possible to identify *de novo* variants. The study revealed several genes and gene-networks that may have predisposed the affected cotwins to the disease corroborating the fact that *de novo* variations between cotwins may be an underlying factor for their discordance to the disease.

Due to the fact that there has been no single gene identified that is responsible for causing the disorder, it is imperative that future research be focused on the polygenetic nature of schizophrenia, as well as the networks and pathways relevant to neurodevelopment and function. A recent study identified genes and pathways associated with psychosis in 22q11.2 deletion syndrome subjects [10]. The study revealed specific pathways affected in 22q11 deletion

Syndrome carriers with psychosis and autistic spectrum disorders. Expression changes associated with psychosis symptoms in 22q11 deletion syndrome was also associated with pathways involved in transcriptional regulation. In addition, schizophrenia was reported to be the only psychiatric disorder observed at a higher rate in 22q11 deletion syndrome compared to other neurodevelopmental disorders [11, 12].

The 22q11.2 deletion represents one of the most established genetic risk factors for the development of psychiatric disorders. Our lab also reported the effect of DNA methylation in the promoter regions of genes located in the microdeletion region on chromosome 22. Accordingly, from a set of genes located in the 22q11.2 microdeletion region that has been previously implicated in psychosis, 29 genes showed increased DNA methylation in their promoters, following olanzapine treatment [13]. In that study, the effect of the antipsychotic drug was revealed through significantly increased ($p < 0.01$) DNA methylation of genes affecting several networks including neurological disease, inflammatory disease, inflammatory response, cancer, tumor morphology, and cell death and survival.

An increased number of studies suggest that rare genetic variations play an important role in the genetic etiology of schizophrenia. However, the existence of rare genetic variations may not always lead to the predisposition of schizophrenia. For example, a rare missense variation in *UCL13B* was identified by whole-exome sequencing, which was present in five of six schizophrenia-affected individuals but not in eight unaffected individuals [14]. In a follow-up case-control study of two independent Japanese populations, there was no significant association between this missense variation and schizophrenia [14].

Chr	Position	Reference allele	Sample allele	Variation type	Gene regions	Gene symbol	Sample call quality	Sample read depth in G/H/F	Translation impact
M	8701	A	G	SNV	Exonic	MT-ATP6	255	600/680/611	Missense
M	8860	A	G	SNV	Exonic	MT-ATP6	255	336/367/322	Missense
M	10819	A	G	SNV	Exonic	MT-ND4	255	729/714/667	Synonymous
M	10873	T	C	SNV	Exonic	MT-ND4	255	613/662/573	Synonymous
M	11719	G	A	SNV	Exonic	MT-ND4	255	544/652/562	Synonymous

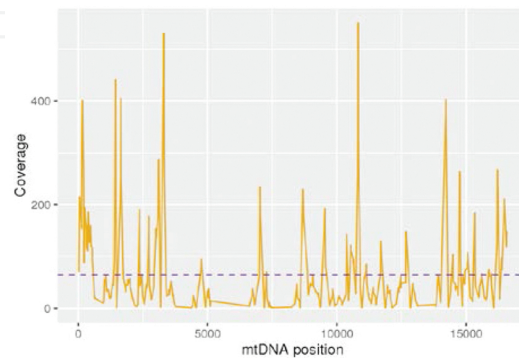
Note: G/H/F: stands for healthy cotwin, affected cotwin, and their mom, respectively; Chr: chromosome.

Table 1. Mitochondrial single nucleotide variations detected in the discordant twins as well as their mother.

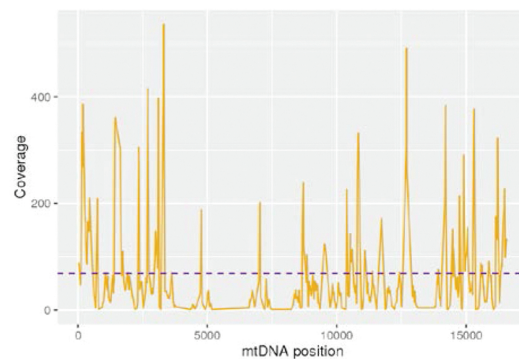
In addition to our analysis of nuclear DNA sequence variation, we have also conducted mitochondrial DNA sequence analysis involving a pair of monozygotic twins discordant for schizophrenia and their mother. Ingenuity Variant Analysis (Ingenuity System Inc, CA, USA) identified no difference in the sequence variations between the discordant twins as well as their mother. All of the biologically relevant variations detected were single nucleotide variations (SNVs) in exonic regions of the *MT-ATP6* and *MT-ND4* genes (**Table 1**). The translational

impact of the variation found in *MT-ATP6* was predicted to be *missense* while that of *MT-ND4* was predicted to be *synonymous*. All of the single nucleotide variations found in the mitochondria were detected in both of the twins and their mother. As we will discuss in the subsequent sections, despite the identification of these biologically relevant sequence variations in all samples, their interaction with epigenetic signatures including DNA methylation may differ between individuals and lead to differences in susceptibility to disease.

A – Healthy co-twin



B – Schizophrenia-affected co-twin



C – Mother of the twins

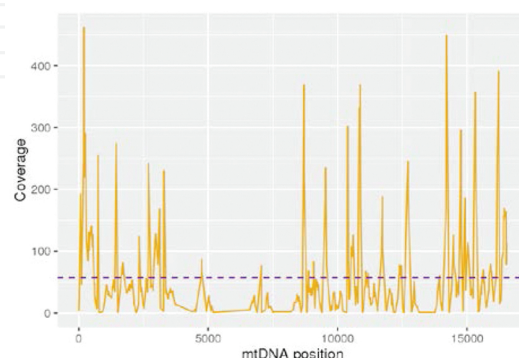


Figure 2. The dotted line in purple shows the mean sequencing coverage of the mitochondrial genome for each sample. (A) Healthy cotwin. (B) Schizophrenia-affected cotwin. (C) Mother of the twins.

Position	Mutation	Locus
10398	A>G	MT-ND3
10819	A>G	MT-ND4
10873	T>C	MT-ND4
11719	G>A	MT-ND4
12705	C>T	MT-ND5
14212	T>C	MT-CYB
1438	A>G	MT-RNR1
14766	C>T	MT-CYB
14905	G>A	MT-CYB
150	C>T	MT-DLOOP2
152	T>C	MT-DLOOP2
15301	G>A	MT-CYB
15326	A>G	MT-CYB
16172	T>C	MT-DLOOP1
16183	A>C	MT-DLOOP1
16189	T>C	MT-DLOOP1
16223	C>T	MT-DLOOP1
1647	T>C	MT-TV
16519	T>C	MT-DLOOP1
195	T>C	MT-DLOOP2
2352	T>C	MT-RNR2
263	A>G	MT-DLOOP2
2706	A>G	MT-RNR2
3316	G>A	MT-ND1
4769	A>G	MT-ND2
7028	C>T	MT-C01
73	A>G	MT-DLOOP2
750	A>G	MT-RNR1
8701	A>G	MT-ATP6
8860	A>G	MT-ATP6
9540	T>C	MT-C03

Table 2. Homoplasmies detected in the discordant twins and their mother.

We used mtDNA-server [15] to identify heteroplasmies. All sites with a log likelihood ratio (LLR) of ≥ 5 were considered as heteroplasmic sites. This analysis did not identify any heteroplasmies in our samples. Among other reasons, the sequencing coverage may have affected our ability to detect heteroplasmies in the present samples (**Figure 2**). Coverage of $\geq 10\times$ fold per strand is required on both the forward and reverse strand to accurately identify heteroplasmic sites [15]. We believe that better coverage would more accurately identify heteroplasmies that may have a role in the etiology of schizophrenia. Furthermore, analyzing larger samples may help investigate heteroplasmies and their association with schizophrenia; as it is possible that each patient could signify a specific etiology and pathophysiological manifestation of the disease via his/her unique genetic makeup and epigenomic signature. In another study, novel and rare nonsynonymous mutations were identified in mtDNA genes (*ND6*, *ATP6*, *CYTB*, and *ND2*) in subjects with psychiatric disorders [16]. The authors also reported mtDNA heteroplasmy at a locus that was known to be associated with schizophrenia (*T16519C*). The homoplasmies detected in the aforementioned sample of a pair of twins discordant for schizophrenia and their mother are presented in **Table 2**.

3. The role of DNA methylation in the development of psychosis

DNA methylation represents a core epigenetic mechanism that involves the covalent binding of a methyl group to the 5-carbon position of cytosine, often leading to altered gene expression [17]. DNA methylation is influenced by stochastic events, including exposure to a variety of environmental factors, such as drug treatment [18, 19]. Epigenetic mechanisms, including DNA methylation, regulate normal cognition, neurodevelopment, and function. In addition, DNA sequence variations only explain a small proportion of the heritability of the disease. The remaining heritability, often referred to as missing heritability, could be, at least partially, explained by epigenetic changes. Interestingly, a number of animal model studies of neurodevelopmental disorders signified that reversing the underlying molecular deficits could lead to substantial improvements in function giving hope to effective treatments even starting in adulthood [20]. These points highlight the need to further investigate the role of epigenetic signatures in the etiology and treatment of psychiatric disorders, including schizophrenia.

With this in mind, our lab has performed two sets of studies on DNA methylation in schizophrenia. The first study focused on two pairs of monozygotic twins discordant for schizophrenia and their parents to investigate differences in genome-wide DNA methylation using a NimbleGen Methylation Promoter Microarray. Since monozygotic twins share nearly identical DNA, the study represents an ideal design to investigate the role of DNA methylation in the etiology of the disease. The genomic DNA was processed at ArrayStar (Rockville, MD, USA). Pair files were analyzed with the tiling workflow in Partek Genomics Suite version 6.6 (St. Louis, Missouri, USA). Details of the methodology have been previously described [4]. As a result, differentially methylated regions (DMRs) were identified between discordant monozygotic twins. Some of the DMRs were shared with parents of the discordant twins while others represented *de novo* methylation changes [4]. The study also reported that 27 genes were affected by DMR changes that were commonly detected in the schizophrenia-affected member

of the two discordant monozygotic pairs of unrelated families. Many of these genes were found to be a part of the histone coding gene family, which has been previously linked to the causation of schizophrenia [21–23]. Moreover, the identified genes affected by DMRs were linked to specific networks including “cell death and survival” and “cellular movement and immune cell trafficking” [4]. Interestingly, those genes and their networks have been previously associated with the etiology of schizophrenia. The findings of this particular study corroborated the notion that DNA methylation may play a critical role in the discordance of monozygotic twins for schizophrenia. The results also shed light on the relevance of gene-specific DNA methylation changes and on the involvement of multiple genes harboring methylation changes across specific pathways in the discordance of monozygotic twins for schizophrenia.

The second study comprised an animal model experiment investigating genome-wide DNA methylation changes following the administration of a therapeutic dose of olanzapine in rats *in vivo*. Hippocampus and cerebellum brain regions were used and liver was included as a nonbrain tissue [5]. As a result, our study revealed that DNA methylation is not only involved in the etiology of mental health disorders, but also may be the underlying mechanism by which antipsychotic drugs function in treating the disorder. This was supported by a number of pathways significantly influenced by methylation changes. These included “nervous system development and function, tissue morphology, cellular assembly and organization,” (**Figure 3**). These findings suggested that an increase or decrease in DNA methylation of specific gene promoters, following olanzapine treatment, might decrease or increase transcriptional efficiency [37, 38], specifically in the hippocampus. The hippocampus is viewed as one of the primary sites associated with psychotic symptoms [7, 24, 25]. We also reported that the *dopamine-DARPP32 feedback in cAMP signaling* pathway ($p < 1.6E-3$) was the most significant pathway identified in the hippocampus region of the olanzapine-treated rat brain. Neurons in the midbrain release dopamine, which modulates *cAMP* (cyclic adenosine 3,5-monophosphate) production by activating dopamine receptors [1]. These results may suggest that antipsychotic effects of olanzapine involve alterations in gene-specific methylation that would lead to dysregulation of genes involved in the dopamine *DARPP32 feedback in cAMP signaling* pathway. This includes several differentially methylated genes such as *Drd1/5* and *Nos1*. It is an established fact that dopamine blockade leads to the progressive treatment of psychosis while its disturbance leads to the manifestation of psychosis [26]. And, all currently used antipsychotics block postsynaptic D2 receptors [27].

Schizophrenia patients either partially respond to antipsychotic drugs or do not respond at all [28]. This may be due to several factors, and one possibility is the delay in the onset of therapeutic actions partly or fully influenced by downstream effects, such as altered transcription [29, 30]. As such, differentially methylated genes involved in the dopamine-signaling pathway may stop or reduce transcription and gene expression [17, 29, 30].

Significant hypomethylation in two CpG sites of the *FAM63B* gene in bipolar disorder patients have been recently reported [31]. Their findings plus previous hypomethylation results reported in another study involving schizophrenia patients suggest that *FAM63B* may be a common risk gene for both disorders. Although the authors reported correlation in methylation levels at the two sites, they did not find significant association of DNA methylation with

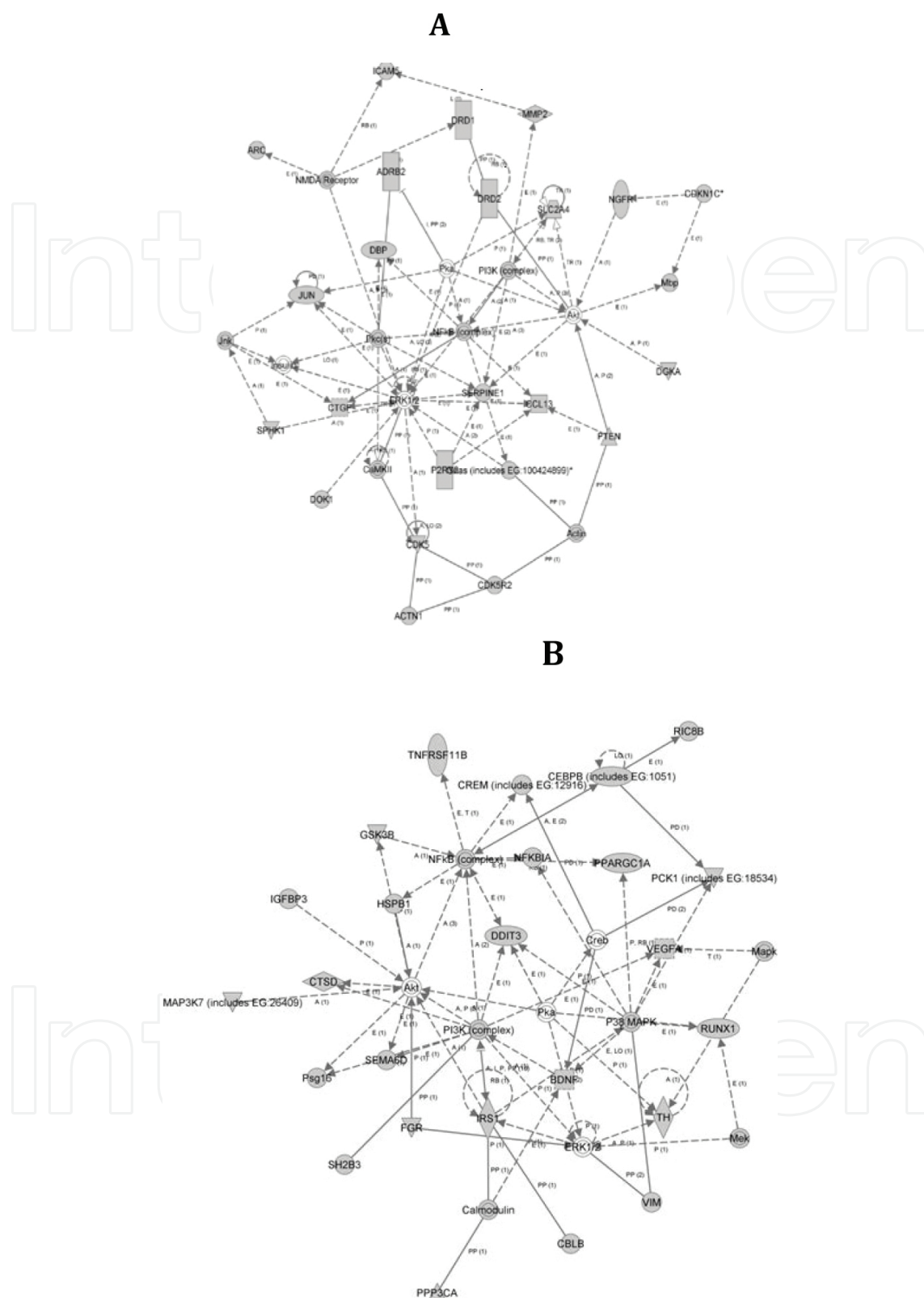


Figure 3. (A) Nervous system development and function, tissue morphology, cellular assembly and organization. (B) Metabolic disease, tissue morphology, endocrine system disorders. Genes shaded in gray were affected by changes in promoter methylation [5].

nearby SNPs, which may further corroborate the biological significance of identifying epigenetic signatures in addition to conducting genome-wide association analyses. On their own, association analyses will not always reveal risk variants or genes due to their limitations such as low statistical power or the presence of genotyping error as well as the detection of false positives.

Different brain regions as well as a variety of cell types are known to have different epigenetic signatures, and depending on the subpopulations analyzed, specific cell types may even show different epigenetic signatures within their own subpopulation [32, 33]. These reported differences in epigenetic signatures of different brain regions and our observations of significant differences in DNA methylation patterns of hippocampus and cerebellum in a rat model study, reflect the possibility that these epigenetic signatures may play a role in regulating gene expression and thereby causing psychiatric disorders including schizophrenia.

Several CpGs have been reported to show significant differences in DNA methylation levels in psychosis cases [34]. These results shed light on the significance of epigenetic signatures in the causes and treatment of mental health disorders. Our previous studies revealed brain tissue-specific DNA methylation changes [5]. As a result, scientists are now advising for caution when interpreting the findings of DNA methylation differences in schizophrenia affected and healthy subjects using peripheral tissues, including blood samples [35].

As we noted in our previous reports, olanzapine caused an increase or a decrease in methylation of genes previously implicated in schizophrenia, which may reflect the fact that olanzapine could result in the recovery of psychiatric symptoms via mechanisms involving DNA methylation. Among the genes that showed a decrease in methylation in hippocampus is *Map6*, which is implicated in schizophrenia [36], and involved in molecular transport, nervous system development, and function [5]. This implies that methylation may serve an intermediary role whose actual effect is realized through gene expression.

Apart from the involvement of DNA methylation in the treatment of schizophrenia via antipsychotic administration, methylation changes may also affect genes and pathways that reflect the side effects of the drugs. In a genome-wide assessment, our studies showed methylation changes in several genes and pathways that may alter metabolomics leading to the efficacy as well as side effects of olanzapine. The side effects were reflected by significant increases in body weight gain and a pathway affecting metabolic disorders. Interestingly, genetic variations in various genes including *BDNF* have been implicated in antipsychotic-induced weight gain [37]. However, the relationship between sequence variations and methylation changes in leading to the predisposition of individuals to the disease, their role in the efficacy of antipsychotic treatment, and also their role in the side effects of the drugs remains to be investigated.

It is an established fact that genomic imprinting is an epigenetic phenomenon by which certain genes are expressed in a parent-of-origin specific manner [38]. Also, X-chromosome inactivation invariably involves epigenetic phenomenon [39, 40]. The genomic distributions of epimutations play an important role in their effects on the disorder. In particular, we

would like to emphasize that not all observed epigenetic changes in the genome play a role in the regulation of gene expression. Although most epimutations located in nonpromoter regions do not often lead to changes in gene expression, epigenetic signatures in the promoter regions are often associated with regulation of gene expressions [29, 41]. Interestingly, long-lasting alterations in DNA methylation and their effect on neurodevelopmental disorders have been reported [42]. However, changes in DNA methylation needs to be interpreted with caution, as methylation and its function are context-dependent [43]. More importantly, effects of epimutations on neurodevelopmental disorders are tissue-specific, cell-type, and organ specific [44, 45]. Interestingly, our lab reported tissue-specific changes in promoter DNA methylation of several psychosis related genes using hippocampus, cerebellum, and liver samples [5].

Overall, the results from our lab and elsewhere suggest that aberrant DNA methylation in a set of candidate genes may be involved in mental disorders, including schizophrenia [46]. Also, it may explain the therapeutic efficacy, side effects, and individual specificity of responses to antipsychotic treatments. They may result from changes in tissue-specific DNA methylation in a set of genes [5, 13, 47, 48]. However, DNA methylation changes in mental health patients and their effects on the expression of psychosis relevant genes as well as the development of psychotic symptoms require attention in future studies. Also, effects of DNA methylation on the expression of specific sets of genes leading to the development of schizophrenia, requires further investigation. To this effect, the use of endophenotypes (intermediate phenotypes that are quantifiable traits of the disease) may help facilitate the investigation of the underlying biological basis of schizophrenia. The United States Food and Drug Administration has accepted endophenotypes as therapeutic treatment targets [49]. Further, our study based on two sets of studies (a rat model investigating effects of olanzapine on DNA methylation of brain regions, and monozygotic twins discordant for schizophrenia) revealed genes and gene networks commonly affected in the two sets of studies. The findings reflect the fact that a considerable portion of the observed methylation changes are likely to be caused by antipsychotic drugs in both studies [50]. Also, it is likely that some of the methylation changes seen can be attributed to the underlying factors that predispose patients to the disorder. Further studies are still needed to confirm the role of methylation changes in the etiology and pathophysiology of the disease.

4. Interplay between DNA sequence variation and DNA methylation

The role of DNA methylation in gene regulation and the interplay between genomic sequence variations and various environmental features as well as their implications on disease phenotypes remains to be investigated. However, studies support the role of DNA methylation in regulatory interactions influencing gene expression [51]. It is also established that there is an interplay between sequence variation, DNA methylation, and gene expression [52]. A recent study has highlighted the fact that mutated CpG sites (CpG-SNPs) could play a critical role in the cause and treatment of the disease [34].

Our lab recently identified several genes that differ between the affected and unaffected members of two monozygotic twin pairs discordant for schizophrenia. The differences were assessed using a genome-wide methylation promoter array and complete genome sequences. The results shed light on a number of facts. First, monozygotic twins differ in both DNA methylation as well as *de novo* sequence variations. Antipsychotic drugs could have caused the observed DNA methylation changes as was evidenced in our rat model study discussed above. Also, there is a possibility that DNA methylation changes could be caused by *de novo* events. It has been reported that differences in genetic components underlie the differences in DNA methylation profiles observed between individuals [53]. Second, some genes that were affected by differential promoter methylation between discordant monozygotic twins also harbor a number of different types of sequence variations [2]. Some of the variations may represent *de novo* sequence variations. Third, a number of the observed changes are located in known candidate genes for schizophrenia. Thus, the genes harboring promoter DNA methylation changes could contribute to neuropsychiatric disorders, including schizophrenia. Moreover, when the DNA sequence differences were analyzed independently, additional previously reported candidate genes of schizophrenia were identified (unpublished data) suggesting that these findings may reflect that many of the previously identified candidate genes of schizophrenia can be revealed by differences in DNA sequence. These findings also highlight the patient-specific nature of these differences. Any sequence variation or promoter DNA methylation between a patient and their healthy cotwin was considered as a potential predisposing factor for the disease. Overall, the findings to date argue that it is not only sequence variation but also their interactions with chromatin structure and other epigenetic signatures which regulate disease outcomes. Therefore, future studies need to focus on investigating the interactions of sequence variation, including nuclear and mitochondrial DNA sequence variations, with epigenetic signatures, in subjects with psychosis and healthy controls.

Studies in the past support the notion that DNA methylation may play a critical role in the therapeutic efficacy of olanzapine. For example, findings suggest that DNA methylation changes in the promoter regions of several genes including genes located in the 22q11.2 microdeletion region and the cadherin/protocadherin genes impact the response of olanzapine treatment [47]. These impacts have been revealed through the identified pathways that have been previously implicated in psychosis.

In conclusion, the results from our lab and elsewhere corroborated the fact that various types of *de novo* sequence, including copy number variants and their interactions with epigenetic signatures, may underlie the etiology of schizophrenia and also may hold the key to discovery of drug targets in developing personalized medicine for psychosis. Epigenetic changes, DNA methylation in particular, may play a critical role in the therapeutic efficacy of antipsychotic drugs. Overall, the known functions of genes affected by olanzapine-induced DNA methylation changes suggest that DNA methylation differences may underlie the amelioration of psychosis symptoms as well as account for certain adverse effects of drugs used to treat the disorder.

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