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Review

# Histone modifications, DNA methylation, and Schizophrenia

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ABSTRACT

Studies have demonstrated that several schizophrenia candidate genes are especially susceptible to changes in transcriptional activity as a result of histone modifications and DNA methylation. Increased expression of epigenetic enzymes which generally reduce transcription have been reported in schizophrenia postmortem brain samples. An abnormal chromatin state leading to reduced candidate gene expression can be explained by aberrant coordination of epigenetic mechanisms in schizophrenia. Dynamic epigenetic processes are difficult to study using static measures such as postmortem brain samples. Therefore, we have developed a model using cultured peripheral blood mononuclear cells (PBMCs) capable of pharmacologically probing these processes in human subjects. This approach has revealed several promising findings indicating that schizophrenia subject PBMC chromatin may be less capable of responding to agents which normally 'open' chromatin. We suggest that the ability to appropriately modify chromatin structure may be a factor in treatment response. Several pharmacological approaches for targeting epigenetic processes are reviewed.



**Contents**



## 1. Background

For the past 40 years schizophrenia research has directed an intense and exciting effort to identify causative genes. However, peculiarities such as the noncomplete concordance between

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monozygotic twins, fluctuating disease course, sexual dimorphism, peaks of susceptibility, symptom changes coinciding with major hormonal rearrangements, and parent-of-origin effects are difficult to explain with a purely genetic approach (Petronis, 2004; Harlap et al., 2009).

The study of epigenetics has provided a platform whereby the impact of environmental factors (e.g., hormones, drugs of abuse, medications, infections, toxins, and diet), both within an individual and as passed through generations, can be measured and understood. Also, because epigenetic factors are pharmacologically alterable the potential for therapeutic interventions is profound.

## 2. Introduction to epigenetics

The nucleosome comprises one of the major components of epigenetic gene regulation. The nucleosome is composed of an octamer of histone proteins (a pair of H2a, H2b, H3 and H4 proteins) as well as approximately 146 base pairs of DNA. Examples of alterations to this unit include methylation of the DNA cytosine base and site specific amino acid histone protein modifications including methylation, acetylation, phosphorylation, ubiquitination, and sumolyation (Akbarian and Huang, 2009). These dynamic and often heritable changes have profound effects on the spatial and temporal regulation of gene expression by altering regulatory region availability to transcription factors (Egger et al., 2004).

One example of a covalent histone modification that increases promoter accessibility is the histone acetyltransferase (HAT) catalyzed acetylation of lysine amino acids on the N-terminal tails of histone 3 or 4 proteins (Turner, 2002). These histone modifications neutralize the proteins' positively charged N-terminal tails, making the negatively charged DNA molecule more available to the machinery of transcription (Hong et al., 1993). Conversely, the histone deacetylase (HDAC) catalyzed removal of acetyl groups condenses chromatin around gene promoters generally resulting in decreased gene expression (Peterson and Laniel, 2004).

Histone methylation can induce either a transcriptionally facilitative or repressive state depending on the amino acid residue being methylated. For example, while the methylation of the fourth lysine on histone 3 (H3K4) generally increases gene expression, methylation of lysines 9 (H3K9) or 27 (H3K27) of histone 3 generally produces the opposite effect (Peterson and Laniel, 2004).

DNA methylation provides another example of an epigenetic process that affects gene expression. The DNA methyltransferase (DNMT) family of enzymes methylate CpG dinucleotides using S-adenosylmethionine (SAM) as the methyl donor (Abdolmaleky et al., 2004). Generally, increased CpG methylation results in decreased gene expression (Szyf, 1996). Therefore, a CpG island (defined as a >500 bp region of DNA with >55% GC content (Takai and Jones, 2002)) within a gene's regulatory region makes it especially prone to epigenetic gene regulation.

The repressive processes of histone deacetylation, H3K9 or H3K27 methylation and DNA methylation have been found to cooperate in reducing gene expression. This occurs both through direct and indirect means. The direct recruitment of both HDACs and HMTs by DNMTs (Bachman et al., 2001; Fuks et al., 2000, 2001, 2003a; Robertson et al., 2000; Rountree et al., 2000) and DNMTs by HMTs (Epsztejn-Litman et al., 2008) have been reported. In addition, indirect interactions, whereby methyl-CpG binding domain proteins (MBDs), such as MeCP2, lead to increased histone methylation (Fuks et al., 2003b) and histone deacetylation have been documented (Jones et al., 1998). HP1, a repressor protein which is associated with H3K9 methylation (Jacobs and Khorasanizadeh, 2002; Wallace and Orr-Weaver, 2005; Lachner et al., 2001), is able to both independently condense chromatin and recruit DNMTs (Lehnertz et al., 2003; Fuks et al., 2003a) (Fig. 1A).

## 3. Epigenetics and schizophrenia

### 3.1. GABAergic system, schizophrenia, and epigenetics

The evidence for epigenetic abnormalities being operant in schizophrenia in part comes from the study of schizophrenia candidate gene regulation, including GAD67 and reelin. A decrease in GAD67, an enzyme that catalyzes decarboxylation of glutamate to form GABA in chandelier type GABA interneurons, is thought to lead to asynchronized cortical activity and the working memory deficits observed in schizophrenia (Lewis et al., 2005). Reelin most likely plays a role in synaptic plasticity learning and memory formation. A deficit in reelin likely accounts for the decreased numbers of dendritic spines in postmortem brains of schizophrenia subjects (Guidotti et al., 2005).

Among the most consistent findings in all of schizophrenia research is the downregulation of these genes (Akbarian et al., 1995; Guidotti et al., 2000; Fatemi et al., 2005; Akbarian and Huang, 2006; Hashimoto et al., 2008). The promoters of both reelin and GAD67 reside within CpG islands making them especially prone to regulation through DNA methylation. In cultured NT2 neuronal precursor cells decreased expression of the reelin gene has been associated with increased promoter CpG island methylation (Chen et al., 2002), and decreased acetylated histone levels (Mitchell et al., 2005). In addition, cultured cell and animal model experiments have shown that DNMT inhibitors (Kundakovic et al., 2007), knocking down the expression of DNMT1 (Noh et al., 2005), and HDAC inhibitors result in increased reelin and GAD67 expression (Chen et al., 2002; Kundakovic et al., 2009).

### 3.2. One-carbon metabolism abnormalities

In the 1960s and 1970s clinical researchers performed studies in which schizophrenia patients were treated with L-methionine. Their hypothesis was that methionine, which gets converted to SAM in the body, would serve as a methyl donor to dopamine, thereby hastening its inactivation (Antun et al., 1971; Berlet et al., 1965). Surprisingly, 79% of patients treated with methionine experienced a worsening of symptoms (Wyatt et al., 1971).

Costa et al. (2002) hypothesized that the methionine-induced worsening of symptoms was because methionine was being converted into SAM, leading to increased DNA methylation and decreased transcription of schizophrenia GABAergic candidate genes, such as reelin and GAD67. Since then data from experiments in which mice were administered L-methionine have supported this hypothesis (Tremolizzo et al., 2002; Dong et al., 2005, 2007). Interestingly, L-methionine treated mice exhibited many of the endophenotypes associated with schizophrenia, including: being less socially interactive, less habituation to an intruder, faster ppi, and impaired attention (Tremolizzo et al., 2002, 2005). Administration of the HDAC inhibitor and mood stabilizer, valproic acid (VPA), following L-methionine treatment, resulted in gene expression normalization and corrected several of the behavioral manifestations caused by L-methionine treatment (Tremolizzo et al., 2005).

Subsequent findings from several postmortem experiments have supported the increased DNA methylation hypothesis. Increased levels of SAM (Guidotti et al., 2007), and increased DNA methylation at the reelin promoter (Abdolmaleky et al., 2005; Grayson et al., 2005), SOX10 promoter (Iwamoto et al., 2005), and in female subjects at the MARLIN-1, Pbx1/Meis1, DTNBP1, and HCG9 promoters (Mill et al., 2008) have been reported in schizophrenia patient samples. As expected promoter hypermethylation of the