

Increased Brain-derived Neurotrophic Factor Exon IV Histone 3 Lysine 9 Dimethylation in Patients with Schizophrenia

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Abstract

Background: Studies have mentioned that mixed-lineage leukemia 1 (MLL1) and histone 3 lysine 4 trimethylation (H3K4me3) of brain-derived neurotrophic factor (BDNF) exon IV from the postmortem brain tissue of patients with schizophrenia are related to the psychopathology of schizophrenia. We intended to investigate the levels of *MLL1* messenger RNA (mRNA) and *BDNF* exon IV histone H3K9me2 and K27me3 in peripheral blood of patients with schizophrenia and healthy controls and to evaluate the relationships between aforementioned biomarkers and patients with/without clozapine treatment. **Methods:** During a one-year period, we recruited 36 patients with schizophrenia and 32 healthy controls. Symptom severity was assessed using the Positive and Negative Syndrome Scale (PANSS). We sampled 10 mL of peripheral blood from each participant to analyze the *MLL1* mRNA and *BDNF* exon IV H3K9me2 and K27me3 levels. **Results:** Significantly higher blood H3K9me2 ($p < 0.01$) levels were observed in patients with schizophrenia than those in healthy controls. However, no significant difference was found in H3K27me3 levels between patients with schizophrenia and controls. PANSS scores had significant correlations with H3K9me2 levels ($p < 0.01$). No significant differences were found in *MLL1* mRNA levels, H3K9me2 levels, and H3K27me3 levels between patients with clozapine treatment and nonclozapine treatment. **Conclusion:** Blood *BDNF* exon IV H3K9me2 levels may be involved in the psychopathology of schizophrenia. More knowledge is needed before we can develop it to be a biomarker for schizophrenia.

Key words: antipsychotic treatment, *H3K27me3*, *H3K9me2*, mixed-lineage leukemia 1
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Introduction

Schizophrenia is a complex and chronic mental illness with psychotic, affective, and cognitive symptoms which impair the patients' daily functions. Schizophrenia is also a multifactorial disorder that involves many neurotransmission pathways of the brain. Epigenetics is the structural adaptation of chromosomal regions, which may enhance or impair DNA transcription [1]. Multiple layers and players exist in epigenetics which are associated with human neurodevelopmental diseases [2]. In the recent years, studies had shown that epigenetic regulations such as DNA methylation and histone modification have contributed to the psychopathology of schizophrenia [3-8].

One of the more thoroughly studied candidate genes for schizophrenia is a brain-derived neurotrophic factor (BDNF) [9], which is involved in growth, survival, differentiation, and repair of neurons [10]. Another candidate gene for schizophrenia is mixed-lineage leukemia 1 (MLL1),

a chromatin-remodeling factor which plays an important rôle in neurogenesis and regulation of the epigenetic maintenance of the pattern of homeotic gene expression [11, 12]. In animal models and postmortem brain tissues, MLL1 is found to be possibly involved in the cortical dysfunction of schizophrenia [13-15]. MLL1 is also a histone 3 lysine-specific methyltransferase that is important in hippocampal synaptic plasticity and regulating the activation of genes downstream of nuclear factor-kappa B and through the mediation of tumor necrosis factor-alpha [16].

Histones form octameric protein complexes called nucleosomes, and the DNA strand coils around them [17]. The configuration of nucleosomes and their short-range interactions

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form an open (euchromatin) or condensed (heterochromatin) chromosomal configuration, either permitting or repressing gene transcription, respectively. Histone proteins can be modified with covalent additions of chemical groups to their *N*-terminal tails. These molecular tags regulate the specific location and tightness of coiling and thus the eventual chromatin configuration [18]. The most studied tags in relation to neurodevelopment are histone acetylations and methylations. Several proteins are responsible for these operations. An acetyl group to histone lysine side chains can be added by histone acetyltransferases or be removed by histone deacetylases (HDACs). Histone acetylation makes DNA more accessible for transcription and is usually enriched at enhancer elements and gene promoters to facilitate access of transcription factors [19]. Histones can also be methylated through histone methyltransferases (HMTs).

Epigenetic modifications of *BDNF* gene have been investigated in the past. Decreased expression of BDNF and increased DNA methylation of promoters of BDNF exons IV and IX have been found in the frontal cortex and hippocampus of patients with schizophrenia [20, 21]. Our previous study reported that *BDNF* exon IV H3K4me3 and blood MLL1 are associated with the psychopathology of schizophrenia [22]. In the postmortem brains, H3K4me3 is associated with transcriptional activation and is frequently localized at sites of active promoters; levels of H3K4me3 at glutamate receptor gene promoters are positively correlated with the corresponding RNAs in human cerebellar and prefrontal cortices [23]. In contrast, tri- and dimethylated forms of histone H3 lysines 9 and 27 are associated with inactive or repressed gene promoters [24]. H3K9me2 and H3K27me3 have also been reported to be related to the psychopathology of schizophrenia and mental retardation [13, 25-27]. In the hippocampus of mice, H3K9me2 levels are increased 1 h after fear conditioning and decreased 24 h after context exposure alone and contextual fear conditioning; mice with *MLL* deficiency display deficits in contextual fear conditioning relative to wild-type animals; fear learning also triggers increases of H3K4me3 at *BDNF* promoter with altered DNA methylation and methyl-CpG-binding protein 2 (MeCP2) DNA binding [28]. MeCP2 is a CpG-binding protein that recruits HDACs to remove acetylation and repress gene transcription [29, 30] and can further enhance the repressive chromatin state by adding repressive H3K9me2 to HMT [31, 32]. Those data show that BDNF, MLL1, and histone modifications interact in a complex way.

Patients with schizophrenia are frequently treated with antipsychotic drugs, which can also affect epigenetic modifications of various genes [27, 33-35]. Clozapine, a second-generation (atypical) antipsychotic drug, increases the expression of *MLL1* messenger RNA (mRNA) [13]. In the cerebral cortex of mice, clozapine treatment increases glutamate decarboxylase (*GAD 1*) H3K4me3 levels and *MLL1* occupancy [36]. The first-generation (typical) antipsychotic drug haloperidol can rapidly induce phosphoacetylation of H3 in the mouse striatum [27, 37, 38]. In the brains of mice,

clozapine or sulpiride treatments, but not olanzapine or haloperidol, show dose-related increases in the cortical and striatal demethylation of hypermethylated reelin and glutamate decarboxylase 67 (*GAD67*) promoters [33]. Those studies show that antipsychotic drugs do affect different epigenetic mechanisms in various psychiatric disorders.

In this study, we intended to investigate the *MLL1* mRNA, *BDNF* exon IV H3K9me2, and H3K27me3 levels in peripheral blood of patients with schizophrenia and healthy controls and to find the relationships between these biological markers and clinical presentations. We also compared the blood levels of expression of *MLL1* mRNA, H3K9me2, and H3K27me3 between patients with/without clozapine treatment.

Methods

Study participants

From November 2013 to October 2014, we recruited patients with schizophrenia and healthy controls and evaluated them with a semi-structured interview based on *DSM-IV* criteria [39] and the Chinese Health Questionnaire-12 [40]. Study protocol was approved by the institutional review board of Chang Gung Memorial Hospital with the need of obtaining informed consents from study participants. The assessments of participants were done by the same senior psychiatrist.

We used the Positive and Negative Syndrome Scale (PANSS) to assess positive symptoms, negative symptoms, and general symptom severity of patients with schizophrenia [41, 42]. The antipsychotic drugs included clozapine 100–400 mg/day ($n = 15$), risperidone 3–6 mg/day ($n = 13$), olanzapine 10–20 mg/day ($n = 3$), paliperidone 12 mg/day ($n = 1$), aripiprazole 30 mg/day ($n = 1$), haloperidol 15 mg/day ($n = 1$), sulpiride 800 mg/day ($n = 1$), and trifluoperazine 10 mg/day ($n = 1$). Patients were allowed to have combined use of benzodiazepines (i.e., lorazepam 3 mg/day) or hypnotics (i.e., zolpidem 10 mg/day). Excluded from taking part in the study were participants with diseases, including cardiovascular, liver, and thyroid diseases, heavy smokers, or those with alcohol dependence.

Assessment of mixed-lineage leukemia 1 messenger RNA

We used a PAXgene Blood RNA Tube (Qiagen, Venlo, The Netherlands) to collect 2.5 ml of peripheral blood. Extraction was done using the PAXgene Blood RNA Kit. We reverse-transcribed total RNA (1 μ g) into cDNA with a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, California, USA). We performed real-time polymerase chain reaction (RT-PCR) of *MLL1* expression using SYBR Green (Applied Biosystems). We used 5 μ L of cDNA in a 0.5 μ M of each primer (final concentration) and 20 μ L of final volume. Primer pairs were ordered from Promega Biosciences (Fitchburg, Wisconsin, USA). We used the following PCR primers: 5'-AGA GTC CGA AGT CCC ACA AG-3' and 5'-AGC TGA ATT TCG GTC AGA GC-3' for MLL1. We did 45 cycles of quantitative PCR (Q-PCR) at 95°C for 5 s with a specific annealing

temperature of 60°C for 5 s and 72°C for 12 s. A 7500 Fast Real-Time PCR System (Applied Biosystems) was used. We examined the amplification specificity using a melting curve according to the manufacturer's instructions. We analyzed the results with the 7500 Fast Real-Time PCR System Software version 1.4.1 (Applied Biosystems, Foster, California, USA). In the same sample, the ratio of the RT-PCR product concentration to the glyceraldehyde-3-phosphate dehydrogenase concentration was calculated to show the gene expression level.

Chromatin immunoprecipitation

We obtained 10 ml of venous blood from each sample and analyzed levels of *BDNF* exon IV H3K9me2 (17-648, Millipore, Burlington, Massachusetts, USA) and H3K27me3 (17-622, Millipore, Burlington, Massachusetts, USA). With minor modifications in accordance with Upstate Biotechnology's protocol, we prepared one million cells for chromatin immunoprecipitation (ChIP) assay under each condition. The cells were treated for 10 min at room temperature to a final concentration of 1% formaldehyde. Glycine was added for 5 min at room temperature to a final concentration of 0.125 M. We washed and lysed the cells. DNA sonication was determined by agarose gel analysis to a size of 200–800 bp. The sonicated cell lysate was centrifuged at 13,000 × g for 10 min at 4°C. The supernatant containing the sheared chromatin was diluted in ChIP dilution buffer (0.01% sodium dodecyl sulfate [SDS], 1.1% Triton X-100, 1.2 mM ethylenediaminetetraacetic acid (EDTA), 16.7 mM Tris-HCl, pH 8.1, and 167 mM NaCl plus protease inhibitor) five-fold. After dilution, precleared with protein A/G magnetic beads (LSKMAGA02, Millipore, Burlington, Massachusetts, USA). Chromatin was immune-precipitated overnight with antibodies for H3K9me2 (17-648, Millipore, Burlington, Massachusetts, USA) and H3K27me3 (17-622, Millipore). The immune complex was collected using protein A/G magnetic beads (LSKMAGA02, Millipore) and rotated at 4°C for 1 h. Sequence wash the protein A/G magnetic beads (LSKMAGA02, Millipore, Burlington, Massachusetts, USA)/antibody/chromatin buffer with low salt buffer (0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris pH 8.0, 500 mM NaCl) 0.1% SDS, 1% Triton X-100, 2 mM EDTA, 20 mM Tris pH 8.0, 150 mM NaCl), LiCl buffer (0.25 M LiCl, 1% Igepal, 1% sodium deoxycholate, 1 mM EDTA, 10 mM Tris pH 8.0) and TE (10 mM Tris pH 8.0, 1 mM EDTA). The DNA was reverse cross-linked 4 h at 65°C. Protein was removed with protease K by incubation at 45°C for 1 h. The DNA was extracted with using ethanol and phenol-chloroform. The DNA from the ChIP sample and input was resuspended in 10 µl and 20 µl TE buffer, respectively. For Q-PCR analysis, all target gene fragments were calculated in IP DNA and "input" DNA (for normalization). Q-PCR was performed using the SYBR® Green and AB 7500 thermal cycler systems. The PCR conditions were 35 cycles at 95°C for 10 min, then at 94°C for 20 s, at 60°C for 32 s, and at 72°C for 30 s. Relative changes in gene expression from RT-PCR

experiments were analyzed using the $2^{-\Delta\Delta Ct}$ method, and the final relative expression was expressed as a value relative to the vehicle control.

Data analysis

Mean ± standard deviation was used for expression of all results. Pearson's correlations were calculated between PANSS scores, age, *MLL1* mRNA, H3K9me2, and H3K27me3 blood levels. Independent *t*-test was used to determine the significant differences between the blood levels of *MLL1* mRNA, H3K9me2, and H3K27me3 between healthy controls and patients with schizophrenia. We categorized patients with schizophrenia into clozapine treatment and nonclozapine treatment groups. The blood levels of *MLL1* mRNA, H3K9me2, and H3K27me3 between clozapine and nonclozapine groups were compared using the analysis of covariance (ANCOVA) with age adjustment.

All study data were computed using the Statistical Package for the Social Sciences software version 19 for Windows (SPSS, Inc., Chicago, Illinois, USA). The differences between groups were considered significant if *p*-values were smaller than 0.05.

Results

We recruited 68 participants for this study. Among 68 participants, 36 participants were patients with schizophrenia while 32 of them were healthy controls. Some demographic data, *MLL1* mRNA levels, and total PANSS score had previously been published [22]. The ages of the patients were 38.7 ± 9.3 years, with body mass index (BMI) of 25.3 ± 4.5 kg/m². The ages of the controls were 33.6 ± 5.9 years, with BMI of 22.3 ± 3.0 kg/m². There were 11 men and 25 women in the schizophrenia group and 10 men and 22 women in the controls. The illness durations for the patients were 13.7 ± 6.9 years.

The mean blood levels of *BDNF* exon IV H3K9me2 were 3.46 ± 3.16 relative expressions and 1.66 ± 1.76 relative expressions in patients with schizophrenia and healthy controls, respectively. Higher blood H3K9me2 levels were noted in patients with schizophrenia than in healthy controls using independent *t*-test ($t = 2.849$, $p < 0.01$). The Pearson's coefficients between H3K9me2 level and age and total PANSS score were 0.098 (not significant) and 0.331 ($p < 0.01$), respectively.

For *BDNF* exon IV H3K27me3, the mean levels were 1.89 ± 1.36 relative expressions and 1.49 ± 1.40 relative

Table 1. Brain-derived neurotrophic factor exon IV H3K9me2 and H3K27me3 blood levels (relative expression)

	Schizophrenia patients ($n=36$)	Controls ($n=32$)
H3K9me2 levels	3.46 ± 3.16	1.66 ± 1.76**
H3K27me3 levels	1.89 ± 1.36	1.49 ± 1.40

* $p < 0.05$; ** $p < 0.01$.

BDNF, brain-derived neurotrophic factor; H3K, histone 3 lysine

Table 2. Demographic data and blood lymphocyte mixed-lineage leukemia 1 mRNA and brain-derived neurotrophic factor exon IV H3K9me2 and H3K27me3 levels (relative expression) in patients with clozapine treatment and nonclozapine treatment

	Age (years)	BMI (kg/m ²)	Duration of illness (years)	PANSS score	<i>MLL1</i> mRNA blood levels	H3K9me2 blood levels	H3K27me3 blood levels
Clozapine (<i>n</i> = 15)	38.5 ± 7.7	23.9 ± 3.4	16.7 ± 6.9	52.7 ± 17.3	0.58 ± 0.36	3.80 ± 3.58	2.31 ± 1.50
Nonclozapine (<i>n</i> = 21)	38.8 ± 10.5	26.4 ± 4.9	11.6 ± 6.2	49.5 ± 19.7	0.84 ± 0.66	3.22 ± 2.89	1.59 ± 1.19

p* < 0.05; *p* < 0.01. No significant differences were found in all comparisons of items between patients with and without clozapine treatment using the ANCOVA with age adjustment. ANCOVA, analysis of covariance; BDNF, brain-derived neurotrophic factor; BMI, body mass index; PANSS, Positive and Negative Syndrome Scale; H3K, histone 3 lysine; *MLL1*, mixed-lineage leukemia 1

expressions in patients with schizophrenia and healthy controls, respectively. There were no significant differences between patients with schizophrenia and healthy controls by independent *t*-test (*t* = 1.189, not significant). The Pearson's coefficients between H3K27me3 level and age and total PANSS score were -0.036 (not significant) and 0.121 (not significant), respectively. Those data are summarized in Table 1.

Using ANCOVA with age adjustment, there were no significant differences in *MLL1* (*F* = 0.300, not significant), H3K9me2 (*F* = 0.007, not significant), and H3K27me3 (*F* = 0.632, not significant) between patients with clozapine treatment and nonclozapine treatment (Table 2).

Discussion

The most important finding in this study (Table 1) was that patients with schizophrenia had significantly higher *BDNF* exon IV H3K9me2 levels than healthy controls in peripheral blood (3.46 ± 3.16 vs. 1.66 ± 1.76 relative expressions, *p* < 0.01). H3K9me2 represses gene promoters [24], so it can potentially lower *BDNF* levels, as patients with schizophrenia have been found to have lower levels of serum *BDNF* [43]. There are only a handful of studies specifically investigating the roles of H3K9 methylations. H3K9me2 serves as a docking site for HP1 and other heterochromatin-associated proteins and is thought to be important for heterochromatin formation [44, 45]. H3K9me2 represses gene promoters by recruiting DNA methyltransferase enzymes. Those DNA methyltransferase enzymes catalyze methylation of CpG dinucleotides, leading to recruitment of methyl-CpG-binding proteins, and form repressive chromatin-remodeling complexes [46]. In both postmortem parietal cortices and lymphocyte samples, a diagnosis of schizophrenia is a significant predictor for increased H3K9me2 levels [47]. A family history of schizophrenia, longer durations of illness, and worsening of specific symptoms are associated with increased HMT mRNA expression [47]. In the lymphocytes of patients with schizophrenia, significantly increased baseline levels of H3K9me2 are observed than those in healthy controls. The same study has also been found a negative correlation between age at onset of illness and levels of H3K9me2 [48]. Our finding is in line with both of those studies. Several other studies investigated the roles of H3K9 acetylation, frequently associated with a more expressive chromatin state. Using

cultured lymphocytes from patients with schizophrenia and healthy controls, lower acetylation levels of H3K9K14 are found in lymphocytes from patients with schizophrenia, and treating the lymphocytes with HDAC inhibitor trichostatin A induces a smaller increase of H3K9K14 acetylation level in the schizophrenia group [49]. In rats suffering from chronic restraint stress, the downregulation of total and exon IV *BDNF* mRNA levels and a decrease in levels of acetylated H3K9K14, as well as an increase in MeCP2 binding at *BDNF* promoter IV, have been reported [50]. The aforementioned changes from chronic restraint stress in rats can be prevented by administration of olanzapine, another second-generation antipsychotic drug [50]. The levels of acetylated H3K9K14 at the promoter regions of eight schizophrenia-related genes in the postmortem prefrontal cortices from patients with schizophrenia and bipolar disorder and healthy controls are correlated with gene expression levels of glutamate decarboxylase 1 (*GAD1*), 5-hydroxytryptamine receptor 2C, translocase of outer mitochondrial membrane 70 homolog A, and protein phosphatase 1E [27]. This study also pointed out that when compared with age-matched controls, significant hypoacetylation of H3K9K14 is detected in young patients with schizophrenia [27]. In the peripheral blood, patients with schizophrenia have remarkably lower baseline levels of acetylated H3K9K14, compared to patients with bipolar disorder [51]. Taken together, the methylation and acetylation statuses of H3K9 are involved in many aspects of schizophrenia.

Blood H3K27me3 is repressive like H3K9me2 [24]. H3K27me3 represses DNA transcription through interacting with Polycomb-group silencing proteins [52, 53]. But, in our study, no significant difference was found between patients with schizophrenia and healthy controls (Table 2). There have been few studies on H3K27. In the prefrontal cortices of patients with schizophrenia, lower methylation levels of eight CpG sites of *GAD1* gene have been found in repressive chromatin region (H3K27me3), compared to those of controls. The methylation levels of *GAD1* do not differ between patients and controls in the open chromatin (H3K4me3). Those findings suggest that histone modification also affects DNA methylation [54].

In our study (Table 2), we found no significant differences in blood levels of *MLL1* mRNA, H3K9me2, or H3K27me3 between clozapine and nonclozapine groups. There are few data on clozapine and repressive histone modifications, but

there is more on transcription-activating histone modifications, such as H3K4me3 and H3 acetylations. In the cerebral cortex of mice, clozapine treatment can increase *Gad1* H3K4me3 and MLL1 occupancy but not haloperidol treatment [13, 36]. Chronic clozapine treatment in mice decreases the capabilities of metabotropic glutamate 2/3 receptor agonist to activate G-proteins in the frontal cortex, through the effect of HDAC2, as HDAC2 knockout mice are immune to that effect [55]. Chronic restraint stress in rats can decrease total and exon IV *BDNF* mRNA levels and H3 acetylation levels, and those abnormalities can be prevented by olanzapine, another atypical antipsychotic, but not haloperidol [50]. The effects of different antipsychotics on histone modifications warrant further investigation, but there have been few studies so far. It is worth noting that valproate, a mood stabilizer frequently used to augment the effect of antipsychotic drugs to treat patients with schizophrenia [56], has been found to be a HDAC inhibitor and is more widely investigated. Stem cells treated with valproate have increased BDNF mRNA expression at various time points [57]. In the lymphocytes isolated from patients with schizophrenia and bipolar disorder, valproate treatment induces a 383% increase in *GAD67* mRNA, an 89% increase in total H3K9K14 acetylation levels, and a 482% increase in H3K9K14 acetylation attachment to the *GAD67* promoter [51]. In the lymphocytes of 11 patients with schizophrenia and 4 patients with bipolar disorder, a 4-week valproate treatment can increase *GAD67* mRNA expression although no remarkable change has been found in H3K9K14 acetylation levels [51]. The application for knowledge in epigenetics can provide a new route to developing new therapeutic approaches to psychiatric disorders [58].

Study limitations

The readers are warned against over-interpret our study results because this study has four limitations:

- The sample size could be bigger.
- The nonclozapine group consists of several antipsychotic drugs, and each of them could have different effects on the histone modifications.
- The levels of blood BDNF mRNA and protein were not included. Since those could represent the final outcomes of epigenetic modifications, their inclusion would make interpretation of our data easier.
- If funding is permitted, we could have verified the primary results in cell cultures or animal models. The verifications could strengthen the results of the investigation.

Summary

Blood BDNF exon IV H3K9me2 levels may be involved in the psychopathology of schizophrenia. More knowledge is needed before we can develop it to be a biomarker for schizophrenia.

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Conflicts of Interest

There are no conflicts of interest.

References

1. Bird A: Perceptions of epigenetics. *Nature* 2007; 447: 396-8.
2. LaSalle JM, Powell WT, Yasui DH: Epigenetic layers and players underlying neurodevelopment. *Trends Neurosci* 2013; 36: 460-70.
3. Akbarian S: Epigenetics of Schizophrenia. Behavioral Neurobiology of Schizophrenia and its Treatment. Berlin, Germany: Springer Berlin Heidelberg, 2010. p. 611-28.
4. Peter CJ, Akbarian S: Balancing histone methylation activities in psychiatric disorders. *Trends Mol Med* 2011; 17: 372-9.
5. Puckett RE, Lubin FD: Epigenetic mechanisms in experience-driven memory formation and behavior. *Epigenomics* 2011; 3: 649-64.
6. Tamminga CA, Holcomb HH: Phenotype of schizophrenia: a review and formulation. *Mol Psychiatry* 2005; 10: 27-39.
7. Tremolizzo L, Carboni G, Ruzicka WB, et al.: An epigenetic mouse model for molecular and behavioral neuropathologies related to schizophrenia vulnerability. *Proc Natl Acad Sci U S A* 2002; 99: 17095-100.
8. Tsankova N, Renthal W, Kumar A, et al.: Epigenetic regulation in psychiatric disorders. *Nat Rev Neurosci* 2007; 8: 355-67.
9. Thompson Ray M, Weickert CS, Wyatt E, et al.: Decreased BDNF, trkB-TK+ and GAD67 mRNA expression in the hippocampus of individuals with schizophrenia and mood disorders. *J Psychiatry Neurosci* 2011; 36: 195-203.
10. Lewin GR, Barde YA: Physiology of the neurotrophins. *Annu Rev Neurosci* 1996; 19: 289-317.
11. Lim DA, Huang YC, Swigut T, et al.: Chromatin remodelling factor mll1 is essential for neurogenesis from postnatal neural stem cells. *Nature* 2009; 458: 529-33.
12. Schuettengruber B, Chourrout D, Vervoort M, et al.: Genome regulation by polycomb and trithorax proteins. *Cell* 2007; 128: 735-45.
13. Akbarian S, Huang HS: Epigenetic regulation in human brain-focus on histone lysine methylation. *Biol Psychiatry* 2009; 65: 198-203.
14. Kim SY, Levenson JM, Korsmeyer S, et al.: Developmental regulation of eed complex composition governs a switch in global histone modification in brain. *J Biol Chem* 2007; 282: 9962-72.
15. Wynder C, Hakimi MA, Epstein JA, et al.: Recruitment of MLL by HMG-domain protein iBRAF promotes neural differentiation. *Nat Cell Biol* 2005; 7: 1113-7.
16. Wang X, Zhu K, Li S, et al.: MLL1, a H3K4 methyltransferase, regulates the TNF α -stimulated activation of genes downstream of NF- κ B. *J Cell Sci* 2012; 125: 4058-66.
17. Luger K, Mäder AW, Richmond RK, et al.: Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature* 1997; 389: 251-60.
18. Jenuwein T, Allis CD: Translating the histone code. *Science* 2001; 293: 1074-80.
19. Wang Z, Zang C, Rosenfeld JA, et al.: Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet* 2008; 40: 897-903.
20. Gavin DP, Akbarian S: Epigenetic and post-transcriptional dysregulation of gene expression in schizophrenia and related disease. *Neurobiol Dis* 2012; 46: 255-62.
21. Ikegame T, Bundo M, Murata Y, et al.: DNA methylation of the BDNF gene and its relevance to psychiatric disorders. *J Hum Genet* 2013; 58: 434-8.
22. Huang TL, Lin CC, Chen RF, et al.: Higher blood MLL1 mRNA and BDNF promoter IV on histone H3K4me3 levels in patients with schizophrenia. *Psychiatry Res* 2016; 243: 207-9.
23. Stadler F, Kolb G, Rubusch L, et al.: Histone methylation at gene promoters is associated with developmental regulation and region-specific expression of ionotropic and metabotropic glutamate receptors in human brain. *J Neurochem* 2005; 94: 324-36.

24. Barski A, Cuddapah S, Cui K, et al.: High-resolution profiling of histone methylations in the human genome. *Cell* 2007; 129: 823-37.
25. Adegbola A, Gao H, Sommer S, et al.: A novel mutation in JARID1C/SMCX in a patient with autism spectrum disorder (ASD). *Am J Med Genet A* 2008; 146A: 505-11.
26. Iwase S, Lan F, Bayliss P, et al.: The X-linked mental retardation gene SMCX/JARID1C defines a family of histone H3 lysine 4 demethylases. *Cell* 2007; 128: 1077-88.
27. Tang B, Dean B, Thomas EA: Disease- and age-related changes in histone acetylation at gene promoters in psychiatric disorders. *Transl Psychiatry* 2011; 1: e64.
28. Gupta S, Kim SY, Artis S, et al.: Histone methylation regulates memory formation. *J Neurosci* 2010; 30: 3589-99.
29. Nan X, Ng HH, Johnson CA, et al.: Transcriptional repression by the methyl-cpG-binding protein meCP2 involves a histone deacetylase complex. *Nature* 1998; 393: 386-9.
30. Jones PL, Veenstra GJ, Wade PA, et al.: Methylated DNA and meCP2 recruit histone deacetylase to repress transcription. *Nat Genet* 1998; 19: 187-91.
31. Moore LD, Le T, Fan G: DNA methylation and its basic function. *Neuropsychopharmacology* 2013; 38: 23-38.
32. Fuks F, Hurd PJ, Deplus R, et al.: The DNA methyltransferases associate with HP1 and the SUV39H1 histone methyltransferase. *Nucleic Acids Res* 2003; 31: 2305-12.
33. Dong E, Nelson M, Grayson DR, et al.: Clozapine and sulpiride but not haloperidol or olanzapine activate brain DNA demethylation. *Proc Natl Acad Sci U S A* 2008; 105: 13614-9.
34. Grayson DR, Kundakovic M, Sharma RP: Is there a future for histone deacetylase inhibitors in the pharmacotherapy of psychiatric disorders? *Mol Pharmacol* 2010; 77: 126-35.
35. Narayan P, Draganow M: Pharmacology of epigenetics in brain disorders. *Br J Pharmacol* 2010; 159: 285-303.
36. Huang HS, Matevosian A, Whittle C, et al.: Prefrontal dysfunction in schizophrenia involves mixed-lineage leukemia 1-regulated histone methylation at GABAergic gene promoters. *J Neurosci* 2007; 27: 11254-62.
37. Li J, Guo Y, Schroeder FA, et al.: Dopamine D2-like antagonists induce chromatin remodeling in striatal neurons through cyclic AMP-protein kinase A and NMDA receptor signaling. *J Neurochem* 2004; 90: 1117-31.
38. Zhao C, Wang F, Pun FW, et al.: Epigenetic regulation on GABRB2 isoforms expression: developmental variations and disruptions in psychotic disorders. *Schizophr Res* 2012; 134: 260-6.
39. First MB, Spitzer RL, Gibbon M, et al.: *Structured Clinical Interview for DSM-IV Axis I Disorders, Clinician Version (SCID-CV)*. Washington, D.C.: American Psychiatric Press, Inc., 1996.
40. Chong MY, Wilkinson G: Validation of 30- and 12-item versions of the Chinese Health Questionnaire (CHQ) in patients admitted for general health screening. *Psychol Med* 1989; 19: 495-505.
41. Kay SR, Fiszbein A, Opler LA: The positive and negative syndrome scale (PANSS) for schizophrenia. *Schizophr Bull* 1987; 13: 261-76.
42. Kay SR, Opler LA, Lindenmayer JP: Reliability and validity of the positive and negative syndrome scale for schizophrenics. *Psychiatry Res* 1988; 23: 99-110.
43. Cui H, Jin Y, Wang J, et al.: Serum brain-derived neurotrophic factor (BDNF) levels in schizophrenia: a systematic review. *Shanghai Arch Psychiatry* 2012; 24: 250-61.
44. Grewal SI, Jia S: Heterochromatin revisited. *Nat Rev Genet* 2007; 8: 35-46.
45. Horn PJ, Peterson CL: Heterochromatin assembly: a new twist on an old model. *Chromosome Res* 2006; 14: 83-94.
46. Fuks F: DNA methylation and histone modifications: teaming up to silence genes. *Curr Opin Genet Dev* 2005; 15: 490-5.
47. Chase KA, Gavin DP, Guidotti A, et al.: Histone methylation at H3K9: evidence for a restrictive epigenome in schizophrenia. *Schizophr Res* 2013; 149: 15-20.
48. Gavin DP, Rosen C, Chase K, et al.: Dimethylated lysine 9 of histone 3 is elevated in schizophrenia and exhibits a divergent response to histone deacetylase inhibitors in lymphocyte cultures. *J Psychiatry Neurosci* 2009; 34: 232-7.
49. Gavin DP, Kartan S, Chase K, et al.: Reduced baseline acetylated histone 3 levels, and a blunted response to HDAC inhibition in lymphocyte cultures from schizophrenia subjects. *Schizophr Res* 2008; 103: 330-2.
50. Seo MK, Kim YH, McIntyre RS, et al.: Effects of antipsychotic drugs on the epigenetic modification of brain-derived neurotrophic factor gene expression in the hippocampi of chronic restraint stress rats. *Neural Plast* 2018; 2018: 2682037.
51. Gavin DP, Kartan S, Chase K, et al.: Histone deacetylase inhibitors and candidate gene expression: an *in vivo* and *in vitro* approach to studying chromatin remodeling in a clinical population. *J Psychiatr Res* 2009; 43: 870-6.
52. Cao R, Wang L, Wang H, et al.: Role of histone H3 lysine 27 methylation in polycomb-group silencing. *Science* 2002; 298: 1039-43.
53. Wang L, Brown JL, Cao R, et al.: Hierarchical recruitment of polycomb group silencing complexes. *Mol Cell* 2004; 14: 637-46.
54. Huang HS, Akbarian S: GAD1 mRNA expression and DNA methylation in prefrontal cortex of subjects with schizophrenia. *PLoS One* 2007; 2: e809.
55. de la Fuente Revenga M, Ibi D, Cuddy T, et al.: Correction: chronic clozapine treatment restrains via HDAC2 the performance of mGlu2 receptor agonism in a rodent model of antipsychotic activity. *Neuropsychopharmacology* 2019; 44: 455-6.
56. Casey DE, Daniel DG, Wassef AA, et al.: Effect of divalproex combined with olanzapine or risperidone in patients with an acute exacerbation of schizophrenia. *Neuropsychopharmacology* 2003; 28: 182-92.
57. Pandamooz S, Salehi MS, Safari A, et al.: Enhancing the expression of neurotrophic factors in epidermal neural crest stem cells by valproic acid: a potential candidate for combinatorial treatment. *Neurosci Lett* 2019; 704: 8-14.
58. Ganguly S, Seth S: A translational perspective on histone acetylation modulators in psychiatric disorders. *Psychopharmacology (Berl)* 2018; 235: 1867-73.