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REVIEW

Dissecting bipolar disorder complexity through epigenomic approach

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In recent years, numerous studies of gene regulation mechanisms have emerged in neuroscience. Epigenetic modifications, described as heritable but reversible changes, include DNA methylation, DNA hydroxymethylation, histone modifications and noncoding RNAs. The pathogenesis of psychiatric disorders, such as bipolar disorder, may be ascribed to a complex gene–environment interaction (G × E) model, linking the genome, environmental factors and epigenetic marks. Both the high complexity and the high heritability of bipolar disorder make it a compelling candidate for neurobiological analyses beyond DNA sequencing. Questions that are being raised in this review are the precise phenotype of the disorder in question, and also the trait versus state debate and how these concepts are being implemented in a variety of study designs.

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INTRODUCTION

Bipolar disorder (BD) is a chronic, disabling condition that is characterized by recurrent depressive, manic, mixed or hypomanic episodes. The majority of BD patients are either diagnosed with BD I (manic and/or mixed episodes) or BD II (depressed and/or hypomanic episodes).[1] The National Institute of Health conducted an international population-based study and found a 1.4% lifetime prevalence of BD,[2] reflecting the global burden of this chronic disorder.

Today's scientific consensus on the pathogenesis of affective disorders might be best described as genotype-dependent environmental influences on risk for an individual to be affected, although a precise model for the molecular mechanisms behind its interactions has not been established yet. The conventional gene–environment interaction (GxE) model does not specifically include epigenetic modifications, but they might represent the underlying mechanisms of the statistical interaction;[3] the importance of epigenetic regulations for complex traits disorders has been acknowledged.[4]

Finding a common definition for epigenetics has been a challenge for the scientific community for some time. In general, the term epigenetics is referred to as long-standing changes in gene expression that are regulated via transcriptional, post-transcriptional, translational and/or post-translational mechanisms (such as DNA methylation, DNA hydroxymethylation, histone modifications and noncoding RNAs for example), which does not entail any change in DNA sequence. The changes beyond DNA sequence can be maintained during the cell cycle (Table 1). A consensus about the question of a transgenerational transmission of epigenetic marks has not been reached yet,[5] but recent evidence supports this presumption for microRNA[6] and for DNA methylation.[7,8] The idea of heritable but reversible changes leads us to the question of how stable these epigenetic changes actually are. It is strongly debated whether these modifications in post-mortem brain tissue represent a stable disease-associated state or only snapshots of different moments in the course of time.[9] On the one hand, studies suggest that there are subtle differences in the epigenetic landscape of monozygotic twins, taken into account for phenotypical differences such as discordant diagnoses due to non-shared exposures.[10,11] This would set the methylation status further on the stable 'trait' end of the discussion. On the other hand, psychiatric drugs have been shown to influence methylation levels[12,13] and there is evidence suggesting that different mood episodes are associated with distinct epigenetic alterations[14,15]—which ultimately suggests that epigenetic modifications reflect a state rather than a trait. An evolutionary perspective proposes that only specific histone modifications might be stable and conserved between species, depending on factors such as, for example, clustered transcription factor binding sites or high GC content.[16] The majority of publications identify DNA methylation as a long-term and relatively stable epigenetic mark, in contrast to histone modifications that are thought to confer short-term and relatively flexible silencing of gene expression.[17,18]

Naturally, there are different approaches to address a complex disorder such as BD; the major part of scientists would approach it as a homogeneous research concept, searching for a trait marker for BD.[19–21] Others might divide it into two entities following the DSM-V classification system[1] and compare BD I and BD II.[22,23] Scientists searching for a 'state marker' will mainly focus on the diverging emotional states involving manic, mixed and depressive episodes.[14] Last but not least, research facilities with access to both bipolar and schizophrenic patient samples might focus on the psychosis-involving part of the disorder, combining both patient samples to one.[24–26]

Numerous studies about gene regulation mechanisms have been emerging in neuroscience over the past few years. In this review, we summarize the current knowledge about epigenetics in BD patients, including DNA methylation, DNA hydroxymethylation

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Table 1. Summary of epigenetic findings in bipolar disorder

Findings	*Gene*	*Reference*
Human post-mortem brain studies		
DNA methylation		
Promoter hypermethylation	*Reelin*	87,88,90
Promoter hypermethylation	*SLC6A4*	32
Promoter hypermethylation in exon 1 to intron 1	*HCG9*	97
Promoter hypomethylation	*MB-COMT*	98
Higher promoter methylation level in psychotic BD compared with non-psychotic BD	*DTNBP1*	100
Promoter hypomethylation in mixed sample of BD and SZ	*ST6GALNAC1*	21
Histone modification		
Increased global histone H3 acetylation		58
Increased H3K4 trimethylation in BD and MDD		75
Peripheral blood		
DNA methylation		
Promoter hypermethylation	*SLC6A4*	32
Promoter hypomethylation	*PPIEL*	31
Higher promoter methylation in BD II compared with BD I	*BDNF exon I*	41
Promoter hypermethylation in BD II	*BDNF exon I*	22
Differential promoter methylation in BD I	*BDNF exon 3 and 5*	23
Promoter hypomethylation in BD compared with MDD	*BDNF exon I*	19
Promoter hypermethylation from exon 1 to intron 1	*HCG9*	97
Promoter hypomethylation in exon 11	*KCNQ3*	20
Promoter hypermethylation in a mixed sample of BD and SZ	*5HTR1A*	26
Promoter hypomethylation	*GPR24, ZNF659*	21
Histone modification		
Increased levels of acetylated histone 3	*H3K9/K14ac*	72
Saliva		
DNA methylation		
Promoter hypomethylation	*MB-COMT*	101
Higher promoter methylation level in psychotic BD compared with non-psychotic BD	*DTNBP1*	100

Abbreviations: BD, bipolar disorder; MDD, major depressive disorder; SZ, schizophrenia.

and histone modifications (Table 1). Questions that are being raised in this review are the precise phenotype of BD and its implications with psychosis and suicide, and also the trait versus state debate and how these concepts are being implemented in a variety of study designs. Furthermore, we briefly describe therapeutic interventions targeting epigenetic mechanisms—from well-established therapeutic drugs to potential agents.

EPIGENETIC MECHANISMS AND FINDINGS IN BD

DNA methylation

Methylation at the 5-position of cytosine is a well-studied epigenetic modification. DNA methylation occurs by transfer of a methyl group from *S*-adenosyl methionine to cytosine residues in the dinucleotide sequence CpG; the majority of the 28 million CpG dinucleotides are methylated. In most of the cases, the level of methylation correlates with the extent of gene inactivation. Early studies focused on CpG islands (CGI) representing DNA regions of a high CpG density, which were shown to be low or unmethylated. Recent work has shown that DNA methylation can also directly silence genes with non-CGI promoters.[27] In fact, regions with relatively low CpG density appear to be equally important. For example, in certain disease conditions, differentially methylated regions occur more frequently within CGI shores ($<$ 2kb flanking CGIs) or shelves ($<$ 2kb flanking outwards from a CpG shore) representing relatively low CpG density that flank traditional CGIs compared with within CGIs themselves.[28–30]

DNA methylation as a trait marker. Twin studies of monozygotic twins regarding discordant phenotypes such as mental health disorder are an excellent design to test genome-wide methods and reduce the noise to a minimum. A study with a sample of monozygotic twins discordant for BD performed methylation-sensitive representational difference analysis and found four regions of the genome to have significant alterations in methylation pattern.[31] Bisulfite sequencing revealed a significantly lower methylation level of peptidylprolyl isomerase E-like (*PPIEL*) gene in the affected twin. The authors suggest that this gene might be involved in specific neuronal functions, such as dopamine transmission or neuroendocrine systems, as it is highly expressed in the pituitary gland and the substantia nigra.[31]

Another twin study identified promoter hypermethylation of serotonin transporter gene *SLC6A4*, which seemed to be associated with BD in a sample of two monozygotic twins.[32] This finding led the authors to a case–control study in post-mortem brain samples, which found associations in the serotonergic system, connecting the S/S genotype of *HTTLPR* to a promoter hypermethylation of *SLC6A4* and finally to the downregulated mRNA on the level of gene expression.[32] No additional information about medication or current state of the disorder was given, which certainly constitutes a limitation to the findings.

A very recently published study assessing a sample of only BD patients draws the attention to the potassium voltage-gated channel gene *KCNQ3*,[20] which has been the focus of genetic linkage studies,[33,34] QTL-mapping[35] and genome-wide association studies[36] in BD patients. *KCNQ3* has been shown to be involved in the regulation of neuronal excitability by preventing hyperexcitability of neurons, thus increasing their responsiveness.[37] The CpG region of exon 11 upstream of the *KCNQ3* gene showed significantly lower methylation levels and correspondingly higher mRNA expression in BD patients compared with healthy controls.[20]

More recently, Perroud *et al.*[38] hypothesized that the *5-HT$_{3A}$R* (5-hydroxytryptamine 3A) methylation status would mediate the effect of childhood trauma on adult psychopathology such as BD, borderline personality disorder and attention deficit/hyperactivity disorder. Among various CpG sites, they were able to associate the methylation status of CpG2 III with the number of previous mood episodes, previous suicide attempts and the polymorphism in single-nucleotide polymorphism rs1062613, regardless of the underlying diagnosis. The authors admit that their results were mainly driven by borderline personality disorder subjects because of the high percentage of childhood maltreatment in this group of patients. The significance of these results for BD patients is certainly limited and not applicable to the question of a trait or state marker debate.

DNA methylation as a state marker. The lack of reliable peripheral blood markers in the psychiatric clinical setting accounts for the continuous attempts to correlate brain tissue findings with expression patterns in the peripheral blood. Peripheral DNA methylation is also an excellent source for studies searching for a trait marker in BD subjects, as peripheral sources can be accessed multiple times. Because of the role played by brain-derived neurotrophic factor (*BDNF*) in synaptic plasticity and stress response[39] and its intraindividual correlation between peripheral and post-mortem brain tissues,[40] it is a prominent candidate for methylation studies in affective disorder For example, Dell'Osso *et al.*[41] investigated alterations of *BDNF* exon I promoter methylation levels in blood samples of BD and major depressive disorder (MDD) patients. They compared BD I patients with BD II patients and found higher methylation levels in BD II patients. When MDD patients were compared with healthy subjects, they again found significantly higher levels of *BDNF* exon I promoter methylation in the MDD patients. Finally, they stratified for the mood state and showed that patients in a depressed state had higher methylation levels compared with patients in a manic/mixed state. D'Addario's[22] group was able to replicate Dell'Osso's results regarding a hypermethylation of the *BDNF* exon I promoter in BD II patients but not in BD I patients. Additional information regarding the pharmacological treatment was assessed too, demonstrating that the combination of antidepressant agents and mood stabilizers establish higher methylation levels than mood stabilizers only. Carlberg *et al.*'s[19] findings were also in line with those previously published and suggested that the altered methylation pattern between MDD and BD patients might be associated with the pharmacological treatment, rather than with the diagnosis itself—which again points to the conception of a flexible state marker. *BDNF* exon IV promoter methylation has been of interest in various psychiatric illnesses including MDD;[42] however, no data in BD patients are available to date, although preclinical data suggest an involvement of mood stabilizers in *BDNF* promoter IV methylation. Lithium treatment to hippocampal neurons induced *BDNF* gene expression, which was accompanied by *BDNF* exon IV hypomethylation.[43]

Another innovative approach focused on the global methylation levels and oxidative damage to the DNA of BD patients (measured through 5-methylcytosine and 8-OHdg (8-hydroxy-2'-deoxyguanosine) levels). Compared with the control group, BD patients had higher DNA levels of 8-OHdG.[15] A higher number of previous manic episodes could also predict higher 8-OHdG levels, which was interpreted as a marker for disease progression, although these levels could not be predicted by the number of depressive episodes. No difference in global methylation could be demonstrated.[15] One of the strengths of this study was that all subjects were in the wash-out phase of their medication and the phenotype effect of the disease was not confounded by the effect of the medication. Although the study might not have been designed to find a state marker, higher 8-OHdG levels in patients with previous manic episodes point in this direction.

DNA hydroxymethylation

Hydroxymethylation (5hmc) is another mechanism of epigenetic modification. Similar to cytosine methylation (5mc), hydroxymethylation adds a hydroxymethyl group to the C5 position. Hydroxymethylation is highly enriched at promoters and in intragenic regions but is largely absent from non-gene regions.[44] Among various tissues, hydroxymethylation is highly abundant in the brain.[45,46] How hydroxymethylation is associated with cytosine methylation and consequent gene expression is not completely understood yet, but there seems to be a dynamic balance between cytosine methylation and hydroxymethylation.[47] In this regard, several hypotheses have been proposed. For example, hydroxymethylation is implicated in demethylation[48] and might be a necessary intermediary for methylation—allowing the promoter sites to be prepared for activation.[49] Another model places hydroxymethylation in a correlation with gene expression, depending on the methylation level.[50] Hydroxymethylation could also be understood as a factor trying to overcompensate for the repressing effect of hypermethylation by increasing gene transcription.[51] The pathophysiological role of hydroxymethylation, especially with regard to neuropsychiatric disorders, has only very recently been introduced,[46,52,53] although its consistency across peripheral tissues has been questioned.[54] Most of the studies of hydroxymethylation have been carried out in embryonic stem cells[51,55] or undifferentiated *in vitro* cells.[56] Scola *et al.*[56] investigated the effect of decreased mitochondrial complex I activity—an established neurobiological finding in BD—on methylation and hydroxymethylation levels in *in vitro* cortical neuronal rat cells. They found increased methylation and hydroxymethylation (measured by 5hCH), which was successfully prevented by pretreatment with lithium.[56]

Histone modifications

Post-translational histone modifications are reversible chromatin rearrangements that have an effect on transcription without affecting the DNA sequence. Histones can be acetylated/deactylated,[57] phosphorylated/dephosphorylated,[58] methylated/demethylated,[59] ubiquitinated/deubiquitinated[60] and sumoylated/desumolyated[61] to regulate gene transcription. The nomenclature of histone modifications indicates which histone tail (H1–4) and which amino acid (R for arginine, K for lysine) is being modified. Additionally, lysines can be monomethylated (me), dimethylated (me2) or trimethylated (me3); the abbreviation Ac is used to refer to the acetyl state. H3K9me3, for example, a mark associated with repressive heterochromatin, stands for a trimethylation of lysine 9 on histone tail 3.[62]

Histone deacetylation inactivates gene transcription by changing the chromatin structure, and histone deacetylase (HDAC) inhibitors have been discussed as therapeutic targets in the field of cognition and behavior.[57] At this point, 11 different HDACs have been discovered to interact with chromatin.[63] In BD patients, HDAC4 mRNA showed increased expression pattern in a depressed state compared with healthy controls, whereas HDAC6 and HDAC 8 were decreased—suggesting a complex expression pattern overall.[64] HDACs also seem to interact with other proteins than histones. Histone acetylation of cAMP response element-binding protein increased its transcription,[65] which is thought to be involved in the pathophysiology of BD.[66] Epigenetic interactions such as those between histone modifications and methylation state need to be closely examined and revisited.[67] Another family of deacetylases, sirtuins, also target histone marks: the gene expression of sirtuin 1–7 (ref. 68) has been investigated in mood disorder patients. One study found state-dependent alterations in sirtuin 1, 2 and 6 in peripheral blood cells of BD and MDD patients.[69] Duong *et al.*[70] aimed to identify mitochondrial complex 1 dysfunction in a BD post-mortem brain sample, but no significant alteration could be determined for *Sirt*-3.[70]

A post-mortem brain investigation compared the levels of acetylated histone 3 (H3K9/K14ac) between a mixed patient sample (BD and schizophrenia (SZ)) and controls, targeting psychosis candidate gene promoters; acetylation levels of the mixed patients sample differed significantly to the controls.[71] Another post-mortem study showed increased global histone H3 acetylation in BD subjects compared with age-matched controls.[58] *In vitro* experiments as well as *in vivo* follow-ups of the same sample showed how HDAC inhibitors increased the levels of H3K9/K14ac.[72]

H3K4 trimethylation is another highly characterized histone modification,[73] whose mechanism of action is thought to be the opening up of chromatin and thereby facilitating promoter binding and leading to the initiation of transcription.[74] H3K4 trimethylation has been studied in synapsin genes (*SYN1*, *SYN2* and *SYN3*) in post-mortem brain samples of BD and MDD patients. This study showed increased H3K4 trimethylation with a distinct synapsin profile for each group.[75]

The first, above-mentioned acetylation study[71] featured a state marker approach, whereas the second mentioned acetylation study[58] as well as the trimethylation study[75] focused on comparing patients with diagnoses—qualifying them for a trait marker approach.

SUICIDE IN BD

BD has the highest lifetime risk of suicide within all psychiatric disorders.[76] About 50% of BD patients attempt suicide at least once in their life.[77] Very few articles have been published to date that focus on the epigenetics of suicide in BD patients. Most of the neurobiological studies carried out so far focus on genetic polymorphisms associated with suicide in BD. Among them, associations with serotonin transporter polymorphisms,[78] genetic variations in apoptotic regulatory genes such as forkhead box O3a[79] and polymorphisms of *BDNF*[80] were drawn. Gene expression studies showed increased platelet serotonin 2A receptors in suicidal patients with the biggest effect size in suicidal bipolar depression versus normal controls.[81] Dracheva *et al.*[82] found altered splicing activity of the 5-hydroxytryptamine 2C receptor in the dorsolateral prefrontal cortex (PFC) of suicidal patients; the effect was independent of their given diagnosis (BD versus SZ).

Post-mortem brain tissue from suicide completers is used for most of current epigenetic studies, not explicitly referring to any diagnosis. For example, Fiori and Turecki[83] had a sample combining MDD patients, substance dependent patients and suicide completers. They found a downregulation of the expression of polyamine regulatory gene spermine *N*1-acetyltransferase 1, which was negatively correlated with CpG methylation in suicide completers. In a similar study with suicide completers, they showed upregulation of another polyamine regulation gene, ornithine decarboxylase antizyme 1, which was associated with increased histone 3 lysine 4 trimethylation in the upstream area.[84]

Dwivedi *et al.*[85] found epigenetic alterations of the neurotrophin receptors in the PFC and the hippocampus of suicide subjects: phosphorylation of all tropomyosin receptor kinase receptors was decreased in the hippocampus but not in the PFC; the decreased phosphorylation was noted only for tropomyosin receptor kinases A and B. Increased expression ratios of corresponding mRNA were also observed in the PFC and hippocampus. These changes were interpreted as part of apoptotic programming. Maussion *et al.*[86] analyzed methylation patterns, and their results point to a hypermethylation of the tropomyosin receptor kinase B-T1 transcript in the 3′-untranslated region in the frontal cortex of suicide completers, again with no specific diagnoses indicated.

PSYCHOSIS IN BD AS A SHARED TRAIT

Many studies investigate both psychotic bipolar patients and schizophrenia patients and refer to the mixed sample of these patients as patients affected by psychotic illnesses.[24–26] These studies are not explicitly trying to define either a trait or state marker, but one could argue that the mixed samples are pointing towards a common trait for psychotic illness; apart from this, only one of the cited studies qualified for the trait marker search by including patients with current psychotic episodes.

At the turn of the century, the very first studies of epigenetics associated with BD focused on downregulated reelin (*RELN*) and glutamate decarboxylase *(GAD)67* gene expression in human cortical brain tissue.[87–89] *GAD67* is one of the two decarboxylases that synthesize GABA, whereas *RELN* is an extracellular matrix protein that is preferentially synthesized and secreted by GABAergic interneurons. It was suggested that downregulation of both *RELN* and *GAD67* genes were associated with hypermethylation of their respective promoter CGIs. Interestingly, hypermethylation of these genes were correlated with increased expression of DNA methyltransferase 1 (DNMT1) in cortical GABAergic interneurons.[90–92] Since these studies, *RELN* and *GAD67* have been investigated extensively showing consistent hypermethylation of the promoter and corresponding mRNA downregulation in the BD and SZ patient population.[47,88,93–96]

Given the extent of previous studies, the serotonergic axis is certainly one of the candidates to look for alterations in methylation patterns. Carrard *et al.*[26] found a hypermethylation of the serotonin 1A receptor gene promoter in a sample of SZ and BD patients; the downregulation of the corresponding mRNA was already well established.

Choosing a multitissue approach will always provide an additional benefit to the conducted study. Kaminsky *et al.*[97] analyzed brain tissues, white blood cells and germ cells of 1000 BD patients based on their previous findings in major psychosis patients (mixed sample of BD and SZ patients), which included significant alterations in human leukocyte antigen genes. They found consistent alteration patterns across tissues and were able to establish a logistic regression model based on the covariates age, a single-nucleotide polymorphism (rs1128306) and the DNA methylation pattern at CpGs 5–8 to predict whether a sample was a BD patient or part of the healthy control sample. Gene ontology analyses suggest that the expression of human leukocyte antigen complex group 9 (*HCG9*) is involved with immune system-related functions such as inflammation and regulation of B-cell-based immunological tolerance.[97]

Abdolmaleky *et al.*[98–100] conducted various epigenetic studies in BD and SZ subjects. In 2006 they analyzed the methylation of 115 post-mortem frontal cortex samples and showed hypomethylation of catechol-O-methyltransferase (*MB-COMT*) in both BD and SZ subjects.[98] Nohesara *et al.*[101] replicated these findings in saliva samples of BD and SZ patients and found that the hyperexpression of *MB-COMT* was even more prominent in drug-free patients.[101] Recently, they compared the dystrobrevin binding protein 1 (*DTNBP1*) promoter methylation in BD patients with and without a psychotic episode in saliva and post-mortem brain tissues. They found that psychotic BD patients had higher methylation rates than BD patients with no current psychotic episode.[100] The *DTNBP1* gene has been the focus of various genome-wide association studies. Both in BD and in SZ patients, genotypic and haplotypic associations between the phenotype and *DTNBP1* have been established.[102–104] It has been suggested that *DTNBP1* may have a role in the AMPA receptor complexes, which binds glutamate as an agonist.[105] These findings suggest that genetic variants of the *DTNBP1* gene confer to the susceptibility of disorder with psychotic features, whereas the actual risk of having a psychotic episode is bound to the

methylation status of the *DTNBP1* promoter, which interferes with the excitatory glutamate transmission system.

Dempster *et al.*[21] studied genome-wide methylation in 22 twin pairs, which were discordant for BD or SZ and found interesting results. According to the diagnostic groups, different results were found: the promoter region of sialyltransferase gene *ST6GALNAC1*, which has an important role in protein metabolism, showed the most significant difference between the psychosis-associated twins and the healthy twins, and the G-protein-coupled receptor 24 (*GPR24*) promoter gene, which interacts with the energy metabolism, showed the most significant change in methylation levels between bipolar twins and their healthy twins. Interestingly, there were also CpG sites that ranked highly in both groups (SZ and BD), but displaying opposite changes in methylation pattern. For example, zinc-finger protein 659 (*ZNF659*) was significantly hypomethylated in BD twins but hypermethylated in SZ twins, whereas in the mixed patients sample it did not even appear in the list of the 100 highest ranked CpG sites as the hyper- and hypomethylation leveled each other out. This study supports the claim that changes in methylation levels should at least be investigated in a disorder-specific manner.

Therapeutic interventions by epigenetic modifications

Therapeutic interventions by epigenetic modifications range from the discovery of the epigenetic mechanism of well-implemented mood stabilizers to substances that are currently being developed or that show promising results in preclinical trials.[106] Exciting developments are occurring around EpiEffectors, engineered transcription factors such as transcription activator-like effectors or zinc-finger-proteins, which have been designed to bind at specific loci in the genome. These newly introduced chromatin changes have only been tested in animal models so far, but show promising results in the field of neuroscience.[107,108] However, there is a debate about the ability to target the appropriate cell type and cause systemic instead of specific alterations. A more thorough understanding of the recent developments of EpiEffectors is provided in a recent review by Kungulovski and Jeltsch.[109]

DNMT inhibitors. DNMTs such as DNMT3A and DNMT3b are involved in the process of *de novo* methylation, whereas DNMT1 adds a methyl group to hemimethylated strands. There is evidence supporting that antidepressants might cause reduced DNMT1 activity by the histone methyltransferase G9a.[110] Sales *et al.*[111] supported this finding by systemic inhibition of DNMT1 in a rodent model, resulting in antidepressant-like effects. However, an *in vitro* study suggested that antidepressant treatment did not change the expression levels of DNMT1.[110]

HDAC inhibitors. HDAC inhibitors do not only inhibit the removal of acetyl groups as their name suggests. The effects of HDAC inhibitors on DNA demethylation have also been discovered over the past years.[12] *De novo* methylator DNMT3B[112] or deoxygenase TET1[113] might be involved in the mechanism of DNA methylation by HDAC inhibitors. In particular, the role of valproic acid (2-propylpentanoic acid) as an HDAC inhibitor has been investigated with regard to BD.[114–116] Valproate accelerates the demethylation of previously hypermethylated *RELN* promoter in the frontal cortex of adult mice.[90] Scola *et al.*[56] found methylation and hydroxymethylation levels of frontal cortex cells to be immune to a manipulated increase through retinone, if the cells were pre-treated with lithium. Yasuda *et al.*[117] showed that not only HDAC inhibitors such as lithium and valproate operate through the activation of *BDNF* exon IV promoter but butyrate sodium (sodium butanoate) and trichostatin A (7-[4-(dimethylamino)phenyl]-*N*-hydroxy-4,6-dimethyl-7-oxohepta-2,4-dienamide) also use the same pathway. *BDNF* transcription may be a key target for the effects of mood stabilizers.[118]

HDAC inhibitors such as sirtinol (2-[(2-hydroxynaphthalen-1-ylmethylene)amino]-*N*-(1-phenethyl)benzamide) or MS-275 (pyridin-3-ylmethyl *N*-[[4-[(2-aminophenyl)carbamoyl]phenyl]methyl] carbamate) are also investigated as potential antidepressants in a rodent model.[119] To date, no clinical studies have been conducted to evaluate the effect of newly designed HDAC inhibitors in MDD or BD patients.[120]

Histone methyltransferase inhibitors. Histone methyltransferase inhibitors have not yet been associated with the neuropsychiatric field,[121] but the recent development of small-molecule histone methyltransferase inhibitors as a class of anticancer agents[122] makes it likely for them to be considered a future therapeutic target in psychiatric disorders.

Methyl donors. DNA hypomethylation can be corrected by methyl donors. L-Methylfolate has been tested as an adjunctive therapy in many clinical trials,[123–127] proving to be safe and effective in MDD patients. Another methyl donor, *S*-adenosyl methionine ((2*S*)-2-amino-4-[[(2*S*,3*S*,4*R*,5*R*)-5-(6-aminopurin-9-yl)-3,4-dihydroxyoxolan-2-yl]methyl-methylsulfonio]butanoate), has been shown to restore normal gene expression in neuroblastoma cells.[128] It has also been demonstrated that methionine administration increases the methylation levels of *GAD67* and *RELN* with a consequent downregulation of the corresponding mRNAs.[88] Clinical studies of *S*-adenosyl methionine were conducted in the late 1990s,[129] but the substance was never introduced to treatment guidelines because of its suggested instability and costs. Recent studies are re-evaluating its feasibility as a therapeutic target in the neuropsychiatric field.[130,131]

CONCLUSION AND FUTURE PERSPECTIVES

In this review, we compiled epigenetic studies in BD that were conducted over the past 10 to 15 years, summarizing the results and implications. We also attempted to draw lines between the outcomes and compare different study designs to each other. Methylation, being one of the most studied epigenetic mechanisms, was discussed from various angles: data ranges from exploratory methylation studies in monozygotic twins to the search for methylation patterns as peripheral markers, and the measurement of global methylation levels in bipolar patients. A limitation in methylation research is the fact that most studies depend on patients who receive pharmacological treatment, although these drugs may influence methylation levels.[19,132] The same is true for age-associated methylation changes, although this variable is not as difficult to control for.[99,133] Genome-wide approaches in big samples or in monozygotic twin studies should be favored as study designs to broaden the horizons and avoid 'candidate' CpG sites. Another major limitation of previous studies is the consistency of alterations in methylation patterns across multiple tissues. On account of feasibility, most studies use peripheral blood cells as a proxy for expression or methylation levels in the brain.[134] More data are needed to support consistent methylation alterations across different tissues, as previously carried out for *BDNF*,[40] or in a multitissue study by Kaminsky *et al.*[97] Further analyses should also focus on specific cell types.[135]

DNA hydroxymethylation has just been discovered with regard to epigenetic modifications. It is likely to have an important role within the network of methylation and transcription, not to mention its abundance in brain tissue compared with other tissues.[52] Further research is needed to specify its role in epigenetic modifications and elucidate its association with affective disorders.

The emergence of microRNA into neuroscience only dates several years back and very few post-mortem brain studies have been conducted with regard to BD.[136–138] These studies analyzed mixed patient samples, which do not contribute to the

consistency of the results after all. More research is needed to discuss the role and relevance of microRNA in the field of epigenetics.

When discussing the neurobiology of psychiatric disorder, particularly in BD, the state versus trait question is a prominent one. 'State', a stable characteristic, corresponds to BD as a diagnosis in this context, whereas 'trait' refers to a temporary characteristic corresponding to a depressive, manic or mixed episode. Most studies do not differentiate between patients having a manic or depressive episode, thus searching for a trait marker for BD. Interestingly, in methylation studies in particular, a different mental state could be shown as linked to significant alterations in DNA methylation—be it a different state within the same person, before and after medication, or different episodes within a sample of BD patients. Given the limited number of studies conducted regarding hydroxymethylation, histone modifications and small noncoding RNA in BD, this claim cannot be generalized to epigenetic modifications. Thus, more studies are needed with a focus on epigenetic modifications aside from DNA methylation and on state markers instead of diagnoses.

The definition of a bipolar patient sample, as well as the inclusion and exclusion criteria, may influence the outcome of the samples. Especially the grouping of BD with schizophrenia patients is a prevalent scientific practice, although homogeneity of this mixed sample is questioned. The significant difference in methylation patterns between psychotic BD patients and BD patients without any psychotic features[139] suggest that BD, which of course is not always associated with psychosis, should be investigated as a singular disease entity rather than within a mixed sample. Another study, supporting this paradigm, shows divergent methylation tendencies of BD versus SZ patients within the same CpG sites.[21] Given that BD patients have the highest risk of attempting suicide in a lifetime among psychiatric disorders, the epigenetics of BD in the context of suicide need to be investigated. Most current studies aim to find distinct epigenetic patterns for suicidality, independent of underlying psychiatric disorders[84,140]—most likely reflecting the clinical demand for a ubiquitous biomarker.

Maintenance of DNA methylation and histone modifications are critical for the development and proper functioning of the brain. Any aberrations in these modifications can lead to changes in normal brain functioning and in the development of disease phenotype. From the studies mentioned above, it appears that epigenetic modifications in critical genes that are involved in various physiological functions in the brain may have a role in the etiology of BD. Interestingly, some of the epigenetic modifications in genes that occur in BD are also common with SZ, suggesting the involvement of shared trait such as psychosis. Given the complex nature of BD, further studies are needed to dissect the precise role of epigenetic modification in the etiology of this disease phenotype. For example, it will be interesting to know whether there are shared or independent epigenetic changes that may coordinately be having a role in depressed and manic phases of BD. Similarly, the brain regions that are critical in BD need to be studied in a greater detail and in a coherent manner to examine how histone acetylation, methylation and other chromatin modifications exert their effects on gene regulation that may influence the development of BD. Regarding psychiatric disorders from a neurobiological standpoint gives us the rare chance to question rigid diagnoses and to be more precise in terms of phenotypes. A symptom- or state-based approach might help reduce the complexity of neurobiological functioning, especially when it comes to a highly heterogeneous clinical picture such as in BD.

The potential reversibility of epigenetic processes has made them interesting for therapeutic approaches. While HDAC and DNMT inhibitors show promising results in preclinical studies, methyl donors made a comeback to clinical trials, owing to recent scientific contributions to their neurobiological mechanism. A well-known shortcoming of drugs targeting epigenetic modifications is their inability to precisely target a specific cell type. Reservations should be held regarding the global inhibition of HDAC, as it has been shown that the expression pattern of the 11 known HDACs is complex and not necessarily directed in the same way.[64] The recent emergence of EpiEffectors surely has the potential of introducing new target-specific psychopharmacological drugs, but a more comprehensive understanding of chromatin biology is needed to make progress in epigenetic editing.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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