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The role of DNA methylation in the pathophysiology and treatment of bipolar disorder

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Abstract

Bipolar disorder (BD) is a multifactorial illness thought to result from an interaction between genetic susceptibility and environmental stimuli. Epigenetic mechanisms, including DNA methylation, can modulate gene expression in response to the environment, and therefore might account for part of the heritability reported for BD. This paper aims to review evidence of the potential role of DNA methylation in the pathophysiology and treatment of BD. In summary, several studies suggest that alterations in DNA methylation may play an important role in the dysregulation of gene expression in BD, and some actually suggest their potential use as biomarkers to improve diagnosis, prognosis, and assessment of response to treatment. This is also supported by reports of alterations in the levels of DNA methyltransferases in patients and in the mechanism of action of classical mood stabilizers. In this sense, targeting specific alterations in DNA methylation represents exciting new treatment possibilities for BD, and the 'plastic' characteristic of DNA methylation accounts for a promising possibility of restoring environment-induced modifications in patients.

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1. Introduction

Complex psychiatric disorders, including mood disorders such as major depressive disorder (MDD) and bipolar disorder (BD), have been shown to run in families and present considerable heritability (Dunn et al., 2015; Kerner, 2015). This is based on observations that first-degree relatives of patients with mood disorders have a higher risk to develop psychiatric disorders than the general population (Potash and DePaulo, 2000; Sullivan et al., 2000), and that monozygotic twins have higher concordance rates than dizygotic twins for these phenotypes (Rice et al., 2002; Smoller and Finn, 2003).

However, most of the studies that aimed at defining the genetic basis of these disorders have failed to accurately detect the same magnitude of heritability in the form of common genetic variants ('heritability gap'), which suggests that additional mechanisms are operative. Thus, the current model for BD views the disease as the result of the interaction between genetic susceptibility and environmental stimuli (Kerner, 2015). Accordingly, several environmental events have been reported to be associated with a higher incidence of BD, including childhood trauma and chronic stress (Brietzke et al., 2012). These external stimuli are thought to interact with a susceptible genotype in the pathogenesis of BD, which has been empirically shown by a few studies. For instance, patients who carry the Met allele at the brain-derived neurotrophic factor (*BDNF*) Val66Met polymorphism have been shown to be more likely to develop depressive episodes following stressful life events than Val allele homozygous (Hosang et al., 2010). In this same vein, a recent report has shown that pathogen exposure interacts with a single nucleotide polymorphism in the toll-like receptor 2 (*TLR2*) gene in the modulation of the risk of BD (Oliveira et al., 2016), and the same polymorphism has also been suggested to amplify the negative effects of childhood sexual abuse on age at onset of BD (Oliveira et al., 2015). Finally, interactions between early trauma and polymorphisms in or near genes coding for calcium channel activity-related proteins have also been suggested to have a potential effect on the development and manifestation of BD (Anand et al., 2015).

Such interactions suggest the action of so-called 'epigenetic mechanisms', which can modulate gene expression in response to the environment, and therefore might partly underlie the multifactorial heritability of BD. These mechanisms include the actions of microRNAs, long noncoding RNAs, and covalent modifications of histones and DNA, forming either repressive or permissive chromatin, thereby modulating gene expression (Breiling and Lyko, 2015; Kouzarides, 2007; Roundtree and He, 2015). DNA methylation is the most stable form of epigenetic alteration, and several studies suggested a critical role for this mechanism in BD (Li et al., 2015; Nestler et al., 2015). This paper aims to survey the literature to shed light on the potential role of DNA methylation in the pathophysiology and treatment of BD.

2. DNA methylation

DNA methylation in higher eukaryotes is the addition of a methyl (CH_3) group to the carbon-5 of cytosines in the DNA sequence, generating a modified nucleotide called 5-methylcytosine (5mC) (Figure 1) (Breiling and Lyko, 2015). Formation of 5mC has been classically involved in gene silencing and repressive chromatin (heterochromatin) (Schubeler, 2015), but recent evidence suggests additional functions for DNA methylation (Reddington et al., 2013). In vertebrates, DNA methylation occurs throughout the entire genome at cytosines of CpG dinucleotides. Even though these dinucleotides are underrepresented in the whole genome, they are frequently enriched around promoter regions, in so-called CpG islands (CGI) (Lo and Weksberg, 2014).

While CpG dinucleotides are commonly methylated across the genome, CGI regions are typically maintained in an unmethylated form (Lo and Weksberg, 2014). The higher CpG density of CGIs facilitates the DNA methylation-dependent control of promoter activity, in which methylation has been consistently linked to gene repression. In fact, methylation is responsible for several forms of epigenetic repression, such as imprinting, X chromosome inactivation, and silencing of repetitive DNA (Schubeler, 2015). Mechanistically, it can repress the activation of a promoter by either (a) sterically inhibiting the binding of transcription factors necessary for RNA polymerase II recruitment and initiation of transcription, or by (b) actively recruiting repressor proteins such as methyl CpG binding protein 2 (MeCP2) and histone deacetylases (HDAC), which will ultimately lead to the formation of heterochromatin (Breiling and Lyko, 2015; Klose and Bird, 2006). In fact, several proteins recognize and bind to methylated CpGs due to the presence of specific DNA-binding domains in their structures, such as the methyl-binding domain (MBD). At present, eleven proteins containing MBDs have been identified, of which the MeCP2 was the first described (Du et al., 2015). On the contrary, unmethylated CpGs are recognized by Cys-X-X-Cys (CXXC)-type zinc finger domains (where XX represent two any other amino acids), which are found in several proteins with functions related to DNA or chromatin modification (Frauer et al., 2011; Schubeler, 2015). These domains can target and recruit other proteins, such as the CXXC finger protein 1 (CFP1) or the histone demethylase KDM2A and KDM2B, to help maintain the unmethylated state of a particular cytosine (Schubeler, 2015).

Methylated cytosines are also prone to spontaneous deamination, yielding thymines and thus generating a C to T transition (Cortazar et al., 2011). The CG to TG transition is by far the most frequent single nucleotide polymorphism in the mammalian genomes; this is explained by the fact that deamination of unmethylated cytosines yields uracils, which can easily be detected by the repair machinery, while thymines are bona fide DNA nucleobases and repair of the mismatch is expected to have a 50% chance of leaving the thymine. This is also consistent with the higher density of CpGs in the typically unmethylated CGIs.

Of note, the role of DNA methylation in the cell is not confined to gene repression. Recent reports have suggested specific roles for DNA methylation in alternative splicing (Lev Maor et al., 2015) and transcription elongation (Wen and Tang, 2014), among other lesser understood mechanisms (Reddington et al., 2013; Wen and Tang, 2014). Moreover, in some

cases DNA methylation has been shown to actually enhance gene transcription (Bockmuhl et al., 2015). Specifically, methylation at the CpG island shore of the nuclear receptor subfamily 3, group C, member 1 (*Nr3c1*) gene, which encodes the glucocorticoid receptor, has been shown to disrupt the binding of a repressive protein, hindering its ability to recruit other repressive proteins that would otherwise induce post-translational modifications of histones and ultimately lead to a repressive chromatin (Bockmuhl et al., 2015). Such a mechanism is opposite to the known canonical repressive role of DNA methylation, and suggests that its effects are dependent on the location of the CpG. Accordingly, gene body methylation has also been recently shown to increase gene expression by a series of potential mechanisms, including the blocking of initiation of intragenic promoters, interfering with the activity of repetitive DNAs within the transcription unit (Maunakea et al., 2010), or forming an ordered structure within the nucleosome that might increase the rate of transcription either by elongation or splicing (Yang et al., 2014).

A specific group of enzymes called DNA methyltransferases (DNMTs) is responsible for catalyzing methylation of DNA and is known to be constitutively expressed (Lo and Weksberg, 2014). According to their roles and specificities they have been divided into (a) maintenance DNMTs, such as DNMT1, and (b) *de novo* DNMTs, which include DNMT3A, DNMT3B and DNMT3L (Sadakierska-Chudy et al., 2015). By recognizing hemimethylated DNA strands, DNMT1 faithfully maintains the pattern of DNA methylation across cell divisions, even though some patterns can be lost passively through imperfect maintenance (Schubeler, 2015). In contrast, active demethylation has been suggested to be initiated by the ten-eleven translocation (TET) family of proteins, including TET1, TET2, and TET3, by converting 5mC into 5-hydroxymethylcytosine (5hmC) (Figure 1) (Sadakierska-Chudy et al., 2015). Further oxidations catalyzed by TET enzymes leads to the formation of 5-formylcytosine (5fC) and 5-carboxylcytosine (5caC), which can then be efficiently removed by thymine-DNA glycosylase (TDG) (Schubeler, 2015). The orchestrated action of DNMTs, TETs and TDG is responsible for establishing and maintaining specific DNA methylation patterns in different cell types (Figure 2). Of note, particular roles for the oxidized forms of 5mC are only starting to be understood. The levels of 5hmC are very high in the central nervous system, which led to the hypothesis that hydroxyl methylation might play a role in the epigenetic control of neuronal function (Tognini et al., 2015). In addition, 5hmC is quite abundant in gene bodies and promoter regions of active genes, and evidence shows that MeCP2 can bind to it with an affinity comparable to 5mC (Spruijt et al., 2013). Of note, several other proteins have been shown to bind to 5hmC, 5fC and 5caC, including DNA glycosylases and DNA repair proteins, and each of them seems to recruit a distinct and dynamic set of proteins (Spruijt et al., 2013).

Apart from the studies of basic mechanisms, the translational analysis of DNA methylation in pathological conditions, such as BD, can be of great value by producing biomarkers of state, prognosis and treatment response, in addition to advancing our understanding of pathophysiological mechanisms. Moreover, the ready availability of genome-wide techniques has sparked intense research efforts to delineate 5mC patterns in health and disease. A particular challenge for these analyses is the cellular heterogeneity (different from genetic sequence, DNA methylation is cell specific and will only directly inform about the tissue being used in the analysis). The next sections will discuss findings reported for

alterations in DNA methylation and DNMTs in BD, followed by a review of the modulation of these mechanisms induced by mood stabilizers.

3. DNA methylation in BD

BD affects around 1% of the world's population and represents one of the leading causes of disability (Grande et al., 2016). It is a lifelong episodic illness with a variable course that is characterized by the occurrence of at least one manic episode (BD I) or one hypomanic and one major depressive episode (BD II). Specifically, manic or hypomanic episodes are states of elevated mood and increased motor drive that impair social or occupational functioning, and a comprehensive biological, social, and psychological approach is required for its treatment and investigation (Grande et al., 2016). Of note, no biomarker has yet been approved for its diagnosis or prognosis (Carvalho et al., 2016; Frey et al., 2013). Nevertheless, alterations in DNA methylation patterns in patients with BD have been extensively investigated for the past years, being considered a promising marker that could integrate both genotype and environmental effects. Genome-wide methylation as well as methylation of several specific candidate genes known to be linked to BD, such as brain-derived neurotrophic factor (*BDNF*) and the serotonin receptors, are discussed below and are summarized in Table 1.

3.1 *BDNF*

BDNF is one of the most extensively studied genes in BD, and its involvement with BD has been suggested by several studies (Wu et al., 2014). In particular, increased methylation of the *BDNF* promoter region (exon 1) was found in peripheral blood mononuclear cells from BD II patients, in which BDNF gene expression levels were also significantly downregulated when compared with controls (D'Addario et al., 2012; Dell'Osso et al., 2014). The authors also found higher levels of methylation at the *BDNF* gene in BD subjects on pharmacological treatment with mood stabilizers in combination with antidepressants (predominantly BD II) compared with those exclusively on mood-stabilizing agents (predominantly BD I). In another study, the degree of methylation differed between BD participants and controls for 11 of 36 CpG units analyzed in the promoters 3 and 5 of the *BDNF* gene (Strauss et al., 2013). Several of the significantly different CpGs overlapped with or were immediately adjacent to transcription factor binding sites in promoter 5, suggesting a possible role for DNA methylation in the modulation of gene expression in the *BDNF* gene. Interestingly, a recent study found a high correlation for *BDNF* methylation between post-mortem human peripheral and brain tissues, suggesting that peripheral DNA methylation of this gene can be used as a proxy for brain-specific alterations (Stenz et al., 2015). Accordingly, *BDNF* has been shown to be hypermethylated in post-mortem frontal cortex of patients compared to controls (Rao et al., 2012a), as well. Of note, one study found no differences in BDNF exon 1 methylation between BD patients and controls, which suggests that this marker is sample-specific and might not be generalizable to all populations (Carlberg et al., 2014). Nevertheless, DNA methylation alterations might be underlying the differences in BDNF expression that seem to play an important role in BD; thus targeting *BDNF* methylation might be beneficial for BD treatment.

As a limitation, it is important to note that BDNF expression is highly dependent on the promoter being modulated (Pruunsild et al., 2007), as well as on genetic polymorphisms that can interfere with its processing and function (Egan et al., 2003; Hong et al., 2011). Based on this, the study of *BDNF* methylation in BD will need to systematically take those features into account before we actually have an established picture of how it acts in determining clinical features in different populations. Moreover, even though most of the studies have focused on *BDNF* (which makes it look like the top ranked gene being modulated by methylation in BD), it is noteworthy that no genome-wide study found it as one of the top differentially methylated genes in BD, showing that the overemphasis on this gene is coming from candidate gene studies.

3.2 Serotonergic system

DNA methylation in genes related to the serotonergic system have also been consistently shown to be altered in BD. Particularly, DNA methylation in the promoter regions of the serotonin transporter and receptor genes, including the serotonin transporter (*5-HTT*) and the serotonin receptor 1A (*5-HTR1A*), were shown to be increased in leukocytes and post-mortem brain samples of BD patients, possibly explaining the decrease of their expression reported in patients (Abdolmaleky et al., 2014; Carrard et al., 2011). Interestingly, many studies have suggested that alterations in methylation levels may be stratified by specific DNA polymorphisms. For instance, the promoter region of the serotonin receptor 2A (*5-HTR2A*) gene was found to be hypermethylated at the −1438A/G polymorphic site, but hypomethylated at the T102C polymorphic site compared to controls (Ghadirivasfi et al., 2011). The T102C SNP is in perfect linkage disequilibrium with the −1438 SNP and has a role in gene expression (Polesskaya et al., 2006). Likewise, hypermethylation of the solute carrier family 6 (neurotransmitter transporter, serotonin) member 4 (*SLC6A4*) gene, which codes for the serotonin transporter, was significantly correlated with lower mRNA expression levels only in individuals with the two short alleles (S/S, known as risk genotype) of the gene (Sugawara et al., 2011). For the serotonin receptor 3A (*5-HT3AR*) gene, the methylation status has been shown to mediate the relationship between childhood maltreatment and clinical severity outcomes in adulthood of patients with BD and other psychiatric illnesses (Perroud et al., 2016). In summary, the findings related to serotonergic genes seem to be consistent and even correlate with important clinical parameters in patients. Along with *BDNF* gene, this system has been the most studied one in regards to methylation in BD, which might be attributed to the previously reported alterations in expression in patients and the known relevance of serotonergic receptors and transporters in BD. The stratification of some of the analyses by genotype adds a more complex level of interpretation to the analysis of DNA methylation, and suggests this approach to be taken into account in the study of other *loci*, as well.

3.3 *GAD1*

Methylation analyses of genes beyond the neurotrophic and serotonergic system include, for instance, the glutamate decarboxylase 1 (GAD1). Abnormal expression of this gene has been reported in schizophrenia and BD, and there are diagnosis- and circuit-specific DNA methylation changes at a subset of GAD1 regulatory network genes in the hippocampus in these disorders (Ruzicka et al., 2015). These genes have been shown to participate in

chromatin regulation and cell cycle control, supporting the concept that the established GABAergic dysfunction in these disorders is related to disruption of GABAergic interneuron physiology at specific circuit locations within the human hippocampus.

3.4 *HCG9*

Studies in two brain tissue cohorts have reported lower DNA methylation in BD patients compared with controls at an extended HLA complex group 9 (HCG9) region (Kaminsky et al., 2012; Pal et al., 2016), which seems to depend on age and DNA sequence variation. Particularly, a hypomethylation of this gene has also been shown to occur in white blood cells and in the germline, suggesting a potential use of this alteration as a biomarker of disease (Kaminsky et al., 2012). The authors of the latter study have argued that the mildly predictive value found for this marker may be accounted for the cellular heterogeneity in the white blood cells and brain tissue DNA, which seems to be an issue in most of the DNA methylation studies. Interestingly, given that a hypomethylation of *HCG9* was also found in the sperm of BD patients, it is possible to speculate that this methylation pattern is transmitted to the offspring and may account for the familial risk of BD shown by this population (Kaminsky et al., 2012).

3.5 *MB-COMT*

Alterations have also been reported at the gene coding for the membrane-bound catechol-O-methyltransferase (MB-COMT), which is involved in the degradation of synaptic dopamine in the brain and is one of the most investigated genes in psychiatry (Abdolmaleky et al., 2006; Nohesara et al., 2011). Particularly in the postmortem frontal lobe, a hypomethylation of its promoter in patients was also accompanied by a reduction in the transcript levels (Abdolmaleky et al., 2006), which could lead to an increase in the rate of dopamine degradation and thereby to a hypodopaminergic state in the frontal lobe. Of particular relevance, a later study by the same group also showed that the MB-COMT hypomethylation can be detected in DNA derived from saliva, suggesting it as a potential epigenetic biomarker of disease (Nohesara et al., 2011).

3.6 *FKBP5*

A study by Fries et al. (2015) has assessed the patterns of methylation at the FK-506 binding protein 5 (*FKBP5*) gene in patients with BD, unaffected siblings, and healthy controls as a means to explore mechanisms associated with the glucocorticoid receptor resistance found in patients. FKBP5 is a negative modulator of the glucocorticoid receptor, and the methylation of specific intronic regions of its gene has been shown to modulate the receptor activity by interfering with the FKBP5 ultra-short feedback loop (Fries et al., 2015; Klengel et al., 2013). Patients showed an increased methylation at introns 7 and 2 of *FKBP5* gene, and negative correlations between the number of previous manic episodes and the methylation status at two CpG sites were also reported. These epigenetic alterations might be partly responsible for the glucocorticoid receptor resistance presented by patients, ultimately leading to an impaired hypothalamus-pituitary-adrenal axis activity and stress resilience.

3.7 *RELN*

Reelin (RELN) is an extracellular matrix protease responsible for normal lamination of the brain during embryogenesis, and it has been shown to be involved in cell signaling and synaptic plasticity in adults (Fatemi, 2011). A significant reduction of the expression of *RELN* has been consistently reported in postmortem brain samples from BD patients, which supports its connection to the illness (Fatemi et al., 2000; Guidotti et al., 2000; Ovadia and Shifman, 2011). Interestingly, *RELN* has been shown to be epigenetically regulated by DNA methylation (Guidotti et al., 2016), and the down-regulation of *RELN* expression in neurons from BD patients has been associated with an overexpression of DNMT1 and DNMT3a (Veldic et al., 2007), suggesting that a hypermethylation of the gene might be responsible for its decreased expression. One study has examined methylation levels of the *RELN* gene in postmortem forebrains from patients as a means to further explore the regulation of RELN expression in BD (Tamura et al., 2007). Even though their results found no differences in the average levels of DNA methylation among groups, patients with BD did not show a correlation between age and *RELN* methylation levels, which was seen in healthy individuals. These results have not been replicated yet, but suggest that an epigenetic aberration from the normal DNA methylation control of *RELN* may confer susceptibility for the disorder (Tamura et al., 2007).

3.8 *DTNBP1*

The dystrobrevin binding protein 1 (DTNBP1), also known as dysbindin, has been suggested to be involved in glutamatergic neurotransmission by influencing exocytotic glutamate release (Domschke et al., 2011). Due to the role of glutamate in BD and psychosis, several studies have been assessing the potential role of genetic variations in the *DTNBP1* gene in these disorders (Corvin et al., 2008; Joo et al., 2007; Yun et al., 2008). Accordingly, a hypermethylation of CpG sites upstream of this gene has been found in the brain of female BD patients (Mill et al., 2008), whereas a hypomethylation of its promoter has been shown in patients, especially in those who were under drug treatment (Abdolmaleky et al., 2015). Of note, methylation status was significantly lower in non-psychotic patients compared to psychotic BD or schizophrenic patients, and a correlation was found between the extent of antipsychotic drug use and DTNBP1 expression in the brains of BD patients (Abdolmaleky et al., 2015). This suggests that the overall reduction in methylation seen in BD patients might be due to drug treatment effects. The same study also found a hypermethylation of *DTNBP1* promoter in BD with psychotic depression compared to other BD patients, suggesting it as a marker of psychotic phenotype. Interestingly, even though it hasn't been assessed in the saliva of BD patients, *DTNBP1* methylation might also represent a biomarker of illness (in this case, of psychosis, since a hypermethylation was reported in saliva from schizophrenic patients) (Abdolmaleky et al., 2015).

3.9 *KCNQ3*

Previous studies have hypothesized that the dysregulated neuronal hyperexcitability in BD might be accounted for altered function of specific voltage-gated potassium channels called M-channels. These channels are heterodimers comprised of the Kv7.2 and Kv7.3 proteins, which are encoded by the potassium channel, voltage gated KQT-like subfamily Q, members

2 and 3 (KCNQ2 and KCNQ3) genes, respectively (Kaminsky et al., 2015). In a series of experiments Kaminsky et al. (2015) demonstrated that postmortem prefrontal cortex of patients with BD present significantly lower methylation of CpGs located in the exon 11 of *KCNQ3* gene, which was significantly correlated with mRNA levels. Interestingly, the authors were also able to show that mood stabilizers increase the methylation of this region in rats. By doing so, it was hypothesized that these drugs might restore the ion channel dysfunction and channelopathy that is seen in BD (Kaminsky et al., 2015).

3.10 *PPIEL*

After a comprehensive scan of DNA methylation in lymphoblastoid cells, the putatitve promoter region of the peptidylprolyl isomerase E-like (PPIEL) was shown to be globally hypomethylated in a patient with BD compared to his unaffected co-twin (Kuratomi et al., 2008). The methylation status was significantly correlated with *PPIEL* expression, and the hypomethylation of *PPIEL* was also replicated in an independent sample of patients with BD (Kuratomi et al., 2008). Even though the role of the PPIEL protein is still unknown, it is believed that it might be involved in specific neuronal function due to its peptidyl-prolyl cis-trans isomerase (PPI) domain. Future studies are now required to validate this finding and to identify the relevance of this alteration in BD.

3.11 *FAM63B*

The family with sequence similarity 63, member B (FAM63B) gene was found to be one of the top ranked *loci* identified in an epigenome-wide association study (EWAS) with schizophrenic patients (Aberg et al., 2014). Because of the high degree of comorbidity found between schizophrenia and BD, the methylation status of *FAM63B* has been recently assessed in whole blood from patients with BD (Starnawska et al., 2016), and the results suggest that patients present a lower methylation at this gene compared to controls. As was the case for several of the findings reviewed here, variations in cell composition might be contributing to the variation in *FAM63B* methylation. In addition, even though the biological function of this gene is still not know, it is suggested to play a role in circadian clock (Starnawska et al., 2016), which is interesting considering its known link with psychiatric disorders (Karatsoreos, 2014).

3.12 *RGS4*

A recent study has looked for the effects of genotype and methylation status of the regulator of G protein signaling 4 (RGS4) gene in postmortem dorsolateral prefrontal cortex from patients with BD, schizophrenia, and healthy controls (Ding et al., 2016), following up on previous evidence of reduced expression of RGS4 in the brain of schizophrenic patients (Mirnics et al., 2001). Of note, a trend toward lower $RGS_{4\text{-}2}$ mRNA expression has been reported in the brain of BD patients (Ding and Hegde, 2009), as well as a decrease in the mRNA ratio of $RGS_{4\text{-}2}$ to $RGS_{4\text{-}3}$ and $RGS_{4\text{-}2}$ to $RGS_{4\text{-}5}$ compared to controls (which are different splice variants of the gene). Contrary to the initial hypothesis, no evidence of hypermethylation of *RGS4* was found in patients compared to controls, suggesting that DNA methylation may not play a role in the decrease of RGS4 expression in the brain of BD patients.

3.13 Global methylation

Global methylation patterns have also been assessed in bipolar patients, and were shown to be significantly influenced by insulin resistance, second-generation antipsychotics use, and smoking (Burghardt et al., 2015). In an earlier study, no differences between bipolar patients and controls were found in leukocytes (Bromberg et al., 2009). However, a marked global hypermethylation has been reported in postmortem frontal cortex from affected individuals compared to controls (Rao et al., 2012a), while two other studies have reported a decrease in global methylation in transformed lymphoblasts (Huzayyin et al., 2014) and in whole blood from BD subjects (Soeiro-de-Souza et al., 2013). These discrepancies might be accounted for not only by the different populations studied, but also by the different methods used, which have been shown to vary significantly (Lisanti et al., 2013): three studies have assessed global methylation by ELISA (Huzayyin et al., 2014; Rao et al., 2012b; Soeiro-de-Souza et al., 2013), while others have used luminometric methylation assay (Burghardt et al., 2015) or cytokine-extension assay (Bromberg et al., 2009). Of note, the overall clinical relevance of alterations in global methylation still need to be determined.

3.14 Genome-wide methylation studies

Genome-wide methylation differences have also been analyzed in blood (Dempster et al., 2011; Houtepen et al., 2016; Li et al., 2015; Sabunciyan et al., 2015; Walker et al., 2016) and in different post-mortem brain regions, such as the BA9 (Zhao et al., 2015), frontal cortex (Mill et al., 2008; Xiao et al., 2014), anterior cingulate (Xiao et al., 2014), and cerebellum (Chen et al., 2014) from BD patients and controls.

Six out of the 9 published genome-wide methylation studies in BD have been performed in blood (with one paper analyzing both blood and postmortem brain (Dempster et al., 2011)), and they suggest that several differentially methylated regions (DMRs) can be found in the periphery of BD patients. Moreover, four studies have analyzed genome-wide methylation changes in postmortem brain tissues, and they suggest that differences between groups (for example, cases and controls) strongly depend on the brain region (Xiao et al., 2014). As in the case in the blood analysis, the altered cellular heterogeneity between distinct regions might be playing a role in the methylome differences. Interestingly, in the frontal cortex and anterior cingulate, only a few differentially methylated regions overlapped with promoters, whereas a greater proportion occurred in introns and intergenic regions (Xiao et al., 2014). A similar pattern was found in another study, where a high percent of DMRs was found in introns and repeat elements (Zhao et al., 2015) in the brain region BA9. In this particular study, hypermethylated DMRs significantly overlapped with long intergenic non-coding RNAs (lincRNAs), while hypomethylated DMRs significantly overlapped with introns, as well as with short and long interspersed nuclear elements. Interestingly, many of the intronic DMRs overlapped with microRNAs, suggesting a role for them in DMR-induced gene expression changes in the BA9 region of patients of BD (Zhao et al., 2015). By using different datasets, a highly significant correlation between methylation and gene expression has been reported in the cerebellum, as well (Chen et al., 2014). Collectively, these studies suggest that alterations in DNA methylation may play an important role in the dysregulation of gene expression in BD (either directly or indirectly by microRNAs or lincRNAS), and a

few studies have actually suggested their potential to be used as biomarkers to improve diagnosis and to better understand the pathophysiology of this illness.

So far the inconsistency between studies and lack of replicated findings warrant more investigation of the topic. Specifically, the inconsistencies are coming not only from the different tissues analyzed (blood, prefrontal cortex, anterior cingulate, cerebellum, and sperm), but also due to methodological variations in the studies: three studies have used methylated DNA immunoprecipitation (MeDIP-seq) followed by next generation sequencing (Li et al., 2015; Xiao et al., 2014; Zhao et al., 2015), two studies have used the Illumina Infinium HumanMethylation27 beadchip (Chen et al., 2014; Dempster et al., 2011), three studies have used the Illumina Infinium HumanMethylation450 beadchip (Houtepen et al., 2016; Sabunciyan et al., 2015; Walker et al., 2016), and one used CpG-island microarrays (Mill et al., 2008) for the assessment of methylation changes. Of note, even though MeDIP-seq and microarray-based analyses have been shown to present a good positive correlation, MeDIP-seq allows a wider interrogation of methylation regions of the genome, including thousands of non-RefSeq genes and repetitive elements that are not assessed in microarrays (Clark et al., 2012). Moreover, different studies use distinct software algorithms in the data analysis, even when analyzing data from a similar method. That includes, for instance, different preprocessing methods (quality control and data filtering), distinct stringency levels for the identification of DMRs (from false discovery rate < 0.1 following multiple testing correction (Walker et al., 2016) to $p < 0.05$ without adjustment (Sabunciyan et al., 2015) for the analysis of the HumanMethylation450 chip, for example), and controlling or not for differences in cellular composition.

4. The role of DNMTs in BD

Based on several studies reporting alterations in DNA methylation patterns in BD patients, it is reasonable to assume that DNMTs might be altered and may even be taken as future targets for the treatment of this disorder. However, so far only a few studies have been performed to examine the role of these enzymes – particularly of DNMT1 – in BD (Table 2), and when taken together their results are still inconclusive.

The methyl donor S-adenosyl methionine (SAM) was shown to be increased by about two-fold in the prefrontal cortex in schizophrenia and BD, which was also associated with an overexpression of DNMT1 mRNA in Brodmann's area 9 GABAergic neurons (Guidotti et al., 2007). This increase of DNMT1 expression was also reported by other studies (Dong et al., 2015; Guidotti et al., 2013; Veldic et al., 2005; Veldic et al., 2007). In addition, Dong et al. (2015) has shown that higher expression of DNMT1 and TET1 enzymes was associated with an increase in the DNMT1 binding to the promoters of *GAD1*, *RELN* and *BDNF,* as well as with downregulation of these genes in the brains of schizophrenia and BD patients (Dong et al., 2015). These data are consistent with the hypothesis that the down-regulation of specific GABAergic and glutamatergic genes in schizophrenia and BD patients may be mediated, at least in part, by a brain region- and promoter-specific DNMT1 action.

A role for DNMT1 in the treatment of BD has been further evidenced by the inhibitory effect of at least some antidepressants in DNMT1 enzymatic activity (Gassen et al., 2015a;

Zimmermann et al., 2012). Of note, this inhibitory action appears to depend on FKBP51, a regulator of the glucocorticoid receptor (Wochnik et al., 2005) which has been implicated in antidepressant response (Binder et al., 2004; Gassen et al., 2015b; Gassen et al., 2014).

Of particular interest, the expression of DNMT1 seems to be mood state-dependent, since the levels of DNMT1 mRNA have been shown to be decreased in the depressive but not in the euthymic state of MDD and BD (Higuchi et al., 2011). Altogether, these few studies support the hypothesis that DNMTs, particularly DNMT1, may play a role in BD, possibly being taken as targets for future novel treatments. Indeed, as reviewed in the next section, currently available medications for the treatment of these disorders are able to alter DNA methylation, and it is likely that they accomplish this by directly or indirectly modulating DNMT expression or activity.

5. The effects of mood stabilizers on DNA methylation

Pharmacological treatment of BD typically includes mood stabilizers (e.g. lithium, valproate, lamotrigine, and carbamazepine), antidepressants and atypical antipsychotics (Jann, 2014). In this section we will focus on mood stabilizers, which are the main class of medications used in BD. Although their mechanisms of action have been studied by different groups, most of the information currently available focuses on the acute and subchronic effects of these drugs. In other words, the current model for evaluating a drug's effect focuses on synaptic signaling, receptor/transporter regulation, intracellular signaling and posttranslational modification, while its effects on gene expression and neuroplasticity appear less investigated. While the acute effects are important to decipher the initial events, it is also important to evaluate the long-term molecular events that parallel symptoms relieve. Long-term changes in DNA methylation are attractive read-outs for potential long-term effects of medications' effects. In this section we will attempt to structure the current understanding of DNA methylation and the use of mood stabilizers in BD (Table 3).

Lithium is the most commonly prescribed mood stabilizer and the foundation of treatment of BD (Jann, 2014), yet the specific mechanisms by which it exerts its effects are incompletely understood. It is known that lithium increases inhibitory (GABA) neurotransmission and decreases excitatory (dopamine and glutamate) neurotransmission (Malhi et al., 2013), but the long-term effects of lithium on gene expression are only recently being evaluated. It is believed that lithium plays a role in inositol and glycogen synthase kinase-3β (GSK-3β) signaling leading to the downregulation of Dnmt3a2, thereby inducing a reduction in DNA methylation and increasing gene expression at specific *loci* (Lee et al., 2015). Indeed, lithium treatment resulted in hypomethylation of *Igf2* (which encodes a protein involved in cell proliferation, differentiation and survival), *Igf2r*, and *H19* (regulation of cell proliferation) in mouse embryonic stem cells (Popkie et al., 2010), as well as of the *BDNF* gene in peripheral blood mononuclear cells (PBMCs) from patients (D'Addario et al., 2012; Dell'Osso et al., 2014) and in rat hippocampal neurons (Dwivedi and Zhang, 2014). In addition, global 5mC levels were reduced by lithium in transformed lymphoblasts from relatives from BD patients (Huzayyin et al., 2014). Interestingly, lithium did not show any effect on global methylation in BD patients, which remained decreased when compared to controls even after treatment (Huzayyin et al., 2014).

Valproate is an anticonvulsant typically prescribed for epilepsy that has been shown to be effective in controlling impulsive behavior, as is observed in BD patients. As seen with lithium, the exact mechanisms of action of valproate are still unknown but they have been linked to the blockade of voltage-dependent sodium channels, as well as potentiation of inhibitory GABAergic transmission (Lee et al., 2015; Phiel et al., 2001). In addition, valproate has been shown to be a potent inhibitor of HDACs (Phiel et al., 2001), which are believed to have a direct influence on DNA methylation (Dobosy and Selker, 2001). In line, Milutinovic et al. found that valproate induced histone acetylation and activated DNA demethylation in the same gene systems in HEK293 cells, suggesting that valproate might trigger demethylation of genes through histone acetylation (Milutinovic et al., 2007). Indeed, in human PBMCs, treatment with valproate led to a significant reduction of ~24% in *BDNF* promoter methylation (D'Addario et al., 2012; Dell'Osso et al., 2014), whereas it was also shown to decrease the methylation of *p21* (Aizawa and Yamamuro, 2015), *RELN* (Dong et al., 2008), and glutamate type I transporter (*GLT-1*) genes (Perisic et al., 2010), as well as induce a significantly altered methylation signature in BD patients (Houtepen et al., 2016).

Regarding other mood stabilizers, carbamazapine has been shown to induce hypermethylation of 64 genes and hypomethylation of 14 genes in a neuroblastoma cell line (Asai et al., 2013), while lamotrigine induced no changes in global DNA methylation in another study (Perisic et al., 2010). Altogether, these findings suggest that the effects of mood stabilizers on DNA methylation are drug-specific and still warrant substantial analysis in different samples, even though common effects on the methylation of a specific group of genes have been reported (Asai et al., 2013). Similar effects have also been suggested to take place in the mechanism of action of antidepressants (Menke and Binder, 2014) and antipsychotics (Guidotti and Grayson, 2014; Houtepen et al., 2016), which further corroborates the hypothesis that the reversal of symptoms induced by BD treatment involves the modulation of DNA methylation and thereby alteration of gene expression.

6. Perspectives

In summary, the findings reviewed in this paper indicate the occurrence of alterations in DNA methylation in patients with BD compared to healthy controls. Some studies take the analysis further and also suggest a role for this mechanism in determining specific clinical features, response to treatment, and prognosis. Some genes, such as *BDNF* and those encoding serotonin transporters, have been extensively analyzed in BD, which is not surprising considering that previous studies had already shown alterations in their expression. In fact, most of the studies have analyzed DNA methylation as a potential modulator of gene expression, which is true for most cases but might not be the case in others. The nuances and possibilities of DNA methylation, as discussed earlier, are still beginning to be understood.

6.1 The clinical utility of DNA methylation

The enthusiasm about the potential benefits of more detailed information on methylation profiles is leading to the initiation of more extended clinical trials with BD patients. Moreover, as DNA methylation appears to be determined by genetic polymorphisms as well,

this information should also be taken into account in studies assessing methylation patterns. In other words, it is reasonable to assume that the combination of genotype and DNA methylation determines the ability to respond to a particular medication, as well as other clinical features. By understanding the effects of such alterations in patients, we might be able to identify distinct groups of patients according to methylation markers and appropriately treat them in a more tailored and targeted way. Of note, from the evidence gathered in this review, no methylation marker identified so far seems to have enough specificity and sensitivity to be taken as a clinically useful marker of diagnosis or treatment prediction of BD at this point, and no such approach is currently being used in clinical routines. However, this review strongly suggests that DNA methylation plays an important role in the pathophysiology of BD, and it is likely that specific markers will be clinically useful in the near future. For that to occur, however, clinical studies need to start adding DNA methylation as one of their outcomes, and longitudinal studies are warranted. This is particularly relevant when considering the potential role of DNA methylation in the risk of BD, which is supported by several theoretical frameworks but not by strong empirical evidence.

6.2 Peripheral methylation as a proxy of the brain

Of note, most of the studies have analyzed DNA methylation in peripheral tissues of patients with BD. There are in fact some reports showing concordances in methylation patterns between blood and brain, suggesting the utility of peripheral blood in human epigenetic studies (Davies et al., 2012). Specifically, Horvarth et al. was able to find a correlation of around $r = 0.9$ across the 2 tissues (Horvath et al., 2012), and DNA methylation changes in ten genes identified in the brain of schizophrenic and BD patients have also been confirmed in peripheral blood samples (Xiao et al., 2014). In addition, methylation in peripheral blood has been correlated with brain volume in healthy individuals and schizophrenia patients (Liu et al., 2015), and it has also been correlated with symptoms of major psychiatric disorders, including schizophrenia, depression and BD (Liu et al., 2014; Sabunciyan et al., 2015).

However, the studies on this topic are still controversial, and some findings indicate that most DNA methylation markers in peripheral blood do not reliably predict brain DNA methylation status (Walton et al., 2016). Only a specific subset of peripheral data was shown to proxy methylation status of brain tissue, which suggests that studies on peripheral methylation should ideally restrict their analysis to these markers (Walton et al., 2016). In addition to peripheral blood, similar patterns of methylation have been observed in saliva and post-mortem tissue in two studies (Ghadirivasfi et al., 2011; Nohesara et al., 2011). Interestingly, saliva and blood methylation have been shown to present a positive correlation overall, although DNA methylation in saliva seems to be more similar to patterns of specific brain regions than methylation in blood (Smith et al., 2015). Taking these limitations into account and being aware that results from peripheral tissues should be interpreted with caution, we believe that the assessment of peripheral methylation can be valuable for psychiatric epigenetics, particularly for BD, mostly for allowing its use in longitudinal studies in living subjects and for potentially providing biomarkers of illness.

6.3 Candidate gene *vs.* genome-wide methylation studies

So far, the majority of published studies have chosen to analyze the methylation of specific genes rather than assess methylation in a genome-wide fashion. Today, psychiatric consortiums with significantly large sample sizes are being formed, allowing for genome-wide studies with later validation and replication for the identification of biologically significant DNA methylation changes between patients and controls. Different BD populations have been analyzed by these techniques, but the results between them are still not consistent or concordant enough. This might be due to the lack of control for genotype, differences in the tissue being analyzed, sample heterogeneity, as well as still insufficient sample sizes. The variability between studies has also been pointed out by a recent systematic review (Teroganova et al., 2016) of findings in peripheral tissues (blood and saliva), especially in regards to the different methods used for the assessment of DNA methylation, DNA extraction, and lack of control for potential effects of clinical variables (such as diet, exercise, smoking, and ethnicity).

6.4 Non-canonical roles of DNA methylation

Several studies have been reshaping the traditional view of DNA methylation as simply a repressor of gene expression and are unraveling novel functions and features of this epigenetic alteration (Kulis et al., 2013; Reddington et al., 2013), including the regulation of alternative intragenic promoters, regulation of intragenic non-coding RNAs and transposable elements, and RNA processing (reviewed by Kulis et al., 2013). By doing so, the role of DNA methylation in BD might eventually become clearer and ultimately help tackle specific pathways for its treatment. For instance, the recently suggested effects of DNA methylation on alternative splicing mechanisms likely contribute to the splicing alterations reported in BD patients, where a dysfunctional gene splicing is being considered as a pathophysiological hallmark of the disorder (Glatt et al., 2009; Glatt et al., 2011). In addition, correlations of DNA methylation markers with gene expression and transcripts profiles, as well as with dynamic cell functions, will lead to more groundbreaking and certainly more relevant results to be followed-up. Of note, even though analyses in cancers have revealed that differences in DNA methylation only result in gene expression changes in a low percentage of genes (Simmer et al., 2012), this does not seem to be the consensus in neuronal cells, where strong correlations are seen (especially with methylation *loci* around the transcription start site and gene bodies) (Kozlenkov et al., 2016). These discrepancies suggest that the extent to which DNA methylation modulates gene expression is also dependent on the tissue being analyzed, in addition to other still unknown factors that might link the association between these two events.

Of particular relevance, DNA methylation is now beginning to be seen as a dynamic process, as well. A growing body of evidence suggests that it can be modified by neuronal activity and thus play a key role in dynamic processes, such as learning and memory formation (Lister and Mukamel, 2015). This is especially important when considering the highly dynamic and phasic nature of the course of BD, and suggests that some methylation markers might parallel changes in mood, as well.

6.5 DNA methylation in the risk of BD

Even though the hypothesis that methylation has functional effects on the risk to develop BD is supported by preliminary findings in patients and preclinical models, only recently have researchers started to actually measure the so-called ‘non genetic intergenerational transmission’ of DNA methylation patterns over generations (Weber-Stadlbauer et al., 2016; Yehuda et al., 2015), which will in fact demonstrate whether the risk shown by populations at familial risk of BD can be modulated by such alterations. Specifically, part of the ‘heritability gap’ might be certainly accounted for by environmental events, but recent preclinical studies with the ‘cross-fostering’ approach (that eliminates the ‘environment factor’) are also suggesting that stress-related DNA methylation markers can be transmitted by non-genetic intergenerational transmission, as well (Klengel et al., 2016a, 2016b). In this sense, it is possible that such markers may account for part of the familial risk of BD proposed by family and twin studies (when in combination with multiple genetic markers of small effect and the effects of environmental triggers), although this still needs to be empirically assessed. Of note, there are no published studies (neither cross-sectional nor longitudinal) on DNA methylation in populations at high risk of BD, including offspring and siblings, even though preliminary findings suggest that they might share altered DNA methylation patterns compared to healthy controls (Fries et al., 2016).

6.6 Targeting DNA methylation in BD

The use of methylation inhibitors is promising in regards to the possibility of permanently reversing several alterations in DNA methylation that have been consistently shown in patients. In fact, preclinical studies in animal models of relevance for BD show promising results (Sales et al., 2011; Sales and Joca, 2016), and studies have shown that targeting DNMT is a crucial part of the mechanism of action of antidepressants and the resolution of depressive symptoms (Gassen et al., 2015a; Zimmermann et al., 2012). In this sense, several DNMT inhibitors have been identified and are being tested in different medical conditions, especially cancers (Erdmann et al., 2015). Of note, it is known that DNMTs are targeted to specific sequences and that adaptor proteins are required for it (Szyf, 2011), suggesting that targeting those proteins might also be relevant in a methylation-modifying therapy.

At present, however, the lack of specificity of these inhibitors is still a major issue. Because methylation is known to determine and maintain the different cell phenotypes within an organism, the potential carcinogenic property of these drugs needs to be considered. In fact, a screening for methylation alterations has actually been proposed for the assessment of carcinogenicity of drugs in addition to the tradition mutagenesis screening (Szyf, 2011). Moreover, because methylation has been shown to play a role in learning and long-term memory storage, one could also argue that a generalized demethylation could affect those individual characteristics, as well. In summary, the rationale for using such methylation inhibitors, albeit very promising in light of the full range of alterations reviewed in this article, may still depend on our ability to target methylation at specific *loci* rather than simply inhibiting non-specific enzymes.

7. Conclusions

In conclusion, the literature reviewed here strongly indicates an important role for DNA methylation in the pathophysiology and treatment of BD. However, its potential in the risk of developing the disorder still needs to be investigated. Of particular interest, targeting specific alterations in DNA methylation represents exciting new treatment possibilities for BD, and the 'plastic' characteristic of DNA methylation accounts for a promising possibility of restoring environment-induced modifications in patients.

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Highlights

- DNA methylation is a stable epigenetic modification that can alter gene expression
- DNA methylation might partly account for BD heritability
- Several genome-wide and gene-specific methylation patterns have been found in BD
- Bipolar patients have been shown to present alterations in DNMTs, particularly DNMT1
- Mood stabilizers have been shown to alter DNA methylation in several genes

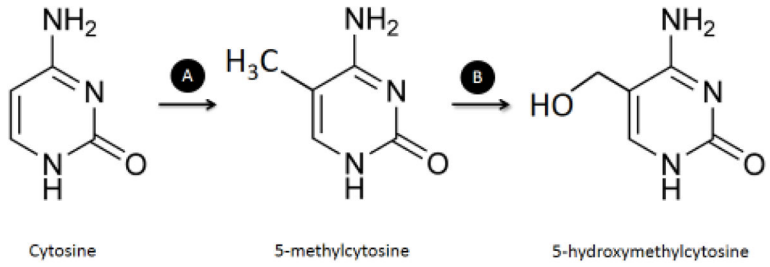

Figure 1.

Catalysis of DNA methylation and hydroxylation. A) Cytosine at CpG dinucleotides can be methylated at carbon-5 by DNMTs, which use SAM as methyl-carrier, generating a modified nucleotide called 5-methylcytosine (5mC). B) Possibly as first step of an active demethylation process, 5mC can be oxidized by TET enzymes to 5-hydroxymethylcytosines (5hmC), whose exact function is still unknown. DNMT – DNA methyltransferase; TET – ten-eleven translocation.

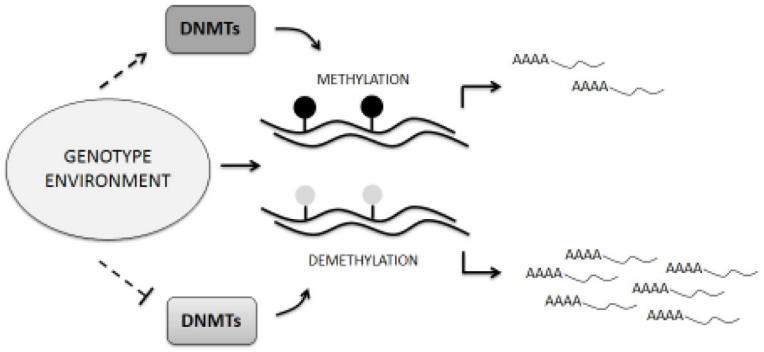

Figure 2.

Typical dynamics of DNA methylation. DNA methylation patterns are determined by a combination of genetic background and environmental exposure. By means of modulating the activity of DNMTs, CpG dinucleotides will undergo methylation or (passive) demethylation of promoters, gene bodies and/or gene regulatory regions. Hypermethylation of CGIs in promoter regions have been traditionally associated with decreased gene expression, whereas their hypomethylation has been linked to increased gene expression. DNMT – DNA methyltransferase; CGI – CpG island.

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Table 1

Alterations in DNA methylation in bipolar disorder

Gene	Cell/tissue	Method	Main findings	Reference
5-HTR1A	Leukocytes	HRM	Hypermethylation of *5HTR1A* gene.	Carrard et al., 2011
5-HTR2A	Saliva	MSP and bisulfite sequencing	Hypomethylation of the 5-*HTR2A* gene at the T102C polymorphic region.	Ghadirivasfi et al., 2011
5-HTR2A	Postmortem frontal lobe	MSP	Hypermethylation of the 5-*HTR2A* promoter region around −1438A/G polymorphic region. Hypomethylation of the T102C region in patients.	Abdolmaleki et al., 2011
5-HT3AR	Peripheral blood	Bisulfite pyrosequencing	Methylation of two CpGs mediated the effects of childhood maltreatment on the severity of the disorder in adulthood.	Perroud et al., 2016
5-HTT	Postmortem frontal lobe	MSP	Tendency of a hypermethylation of *5HTT* in antipsychotic-free BD patients.	Abdolmaleki et al., 2014
BDNF	Peripheral blood mononuclear cells	Bisulfite sequencing	Hypermethylation of *BDNF* in BD II compared to BD I. Hypermethylation in depressed patients compared with manic/mixed patients. Lower methylation levels in patients on lithium and valproate.	Dell'Osso et al., 2014
BDNF	Peripheral blood mononuclear cells	MSP	*BDNF* exon I methylation was increased in MDD subjects compared to BD patients and controls. Increased methylation associated with antidepressant use.	Carlbert et al., 2014
BDNF	Venous blood	MassARRAY platform	Different methylation degree between BD and controls for 11 of 36 CpG units.	Strauss et al., 2013
BDNF	Peripheral blood mononuclear cells	MSP	Increased *BDNF* methylation in BD II (but not type I). Negative correlation between methylation and gene expression.	D'Addario et al., 2012
Chromosome X	Buccal mucosa and peripheral leukocytes	Restriction enzymes analysis	Discordant twins for BD are more discordant for the methylation of chromosome X, especially compared to concordant twins for BD.	Rosa et al., 2008
DTNBP1	Saliva	Bisulfite sequencing	Higher *DTNBP1* promoter methylation in BD patients with psychotic depression compared to other BD patients.	Abdolmaleky et al., 2015
FAM63B	Whole peripheral blood	iPLEX assay	Hypomethylation of two CpG sites in the exon 9 of the *FAM63B* gene.	Starnawska et al., 2016
FKBP5	Peripheral blood mononuclear cells	Bisulfite pyrosequencing	Increased *FKBP5* methylation.	Fries et al., 2014

Gene	Cell/tissue	Method	Main findings	Reference
GAD1 regulatory network genes	Post-mortem hippocampus	Bisulfite sequencing	Diagnosis- and circuit-specific methylation changes at a subset of *GAD1* regulatory network genes.	Ruzicka et al., 2015
Genome-wide methylation	Post-mortem brain region BA9	MeDIP-seq	16,599 differentially methylated regions in BD compared to controls (6,836 hypermethylated and 9,763 hypomethylated).	Zhao et al., 2015
Genome-wide methylation; *CYP11A1*	Blood	Illumina HumanMethylation450 BeadChip and pyrosequencing	Hypomethylation at the *CYP11A1* gene in manic patients. Association of this methylation with inflammatory markers.	Sabunciyan et al., 2015
Genome-wide methylation	Blood	Illumina HumanMethylation450 BeadChip	Altered methylation patterns in BD patients in use of quetiapine and valproic acid after adjusting for drug-related changes on cell type composition.	Houtepen et al., 2016
Genome-wide methylation	Blood	Illumina HumanMethylation450 BeadChip	Altered methylation in carriers of a haplotype linked to BD and MDD, including genes related to neurodevelopment and ion channel activity (such as *FANCI*).	Walker et al., 2016
Genome-wide methylation	Blood	MeDIP-Seq	Thousands of differentially methylation regions located preferentially in promoters 3′-UTRs and 5′-UTRs of genes.	Li et al., 2015
Genome-wide methylation	Frontal cortex and anterior cingulate	MeDIP-seq	Several differentially methylated regions in BD compared to controls. Different distributions of methylation across the genome between the two brain regions.	Xiao et al., 2014
Genome-wide methylation	Cerebellum	Illumina Infinium HumanMethylation27	CpG gene pairs were found to significantly correlate with the differential expression and methylation of *PIK3R1*, *BTN3A3*, *NHLH1*, and *SLC16A7* genes.	Chen et al., 2014
Genome-wide methylation	Peripheral blood and postmortem brain	Illumina Infinium HumanMethylation27	Hypomethylation of the promoter region of the *ST6GALNAC1* gene in affected twins with psychosis and in brains from patients.	Dempster et al., 2011
Genome-wide methylation	Postmortem frontal cortex and germline	CpG-island microarrays	Specific differences in methylation between males and females. No difference between controls and patients in the methylome of germlines.	Mill et al., 2008
Global methylation	Whole blood	ELISA-based assay	Lower 5hmC levels in BD patients, but no difference in global 5mC levels compared to controls.	Soeiro-de-Souza et al., 2013
Global methylation	Leukocyte	Luminometric Methylation Assay	Methylation in BD patients stable on medication was significantly influenced by insulin resistance, second-generation antipsychotic use and smoking.	Burghardt, 2015
Global methylation	Transformed lymphoblasts	ELISA	Decreased methylation in BD subjects and their relatives compared to controls	Huzayyin et al., 2014

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Gene	Cell/tissue	Method	Main findings	Reference
Global methylation	Leukocytes	Cytosine-extension assay	No diferences between patients and controls.	Bromberg et al., 2009
Global methylation, *COX-2*, *BDNF*, debrin-like protein	Postmortem frontal cortex	MSP and ELISA (for global methylation)	Global hypermethylation; *COX-2* hypomethylation; *BDNF* hypermethylation; debrin-like protein gene hypermethylation.	Rao et al., 2012
HCG9	Postmortem prefrontal cortex	Bisulfite pyrosequencing	Significant differences between patients and controls detected in CpG modifications.	Pal et al., 2016
HCG9	Postmortem brain tissues, peripheral blood cells, and sperm	Bisulfite pyrosequencing	Low *HCG9* methylation in patients.	Kaminsky et al., 2012
KCNQ3	Postmortem prefrontal cortex	Bisulfite pyrosequencing	Lower methylation of the *KCNQ3* exon 11 in BD.	Kaminsky et al., 2015
MB-COMT	Saliva	MSP	Hypomethylation of the *MB-COMT* promoter.	Nohesara et al., 2011
MB-COMT	Postmortem frontal lobe	MSP and bisulfite sequencing	Hypomethylation of the *MB-COMT* promoter.	Abdolmaleky et al., 2006
RELN	Postmortem forebrain	Restriction enzymes analysis and PCR	No correlation between *RELN* methylation and age (which was shown in controls).	Tamura et al., 2007
RGS4	Dorsolateral prefrontal cortex	Bisulfite sequencing	No alteration in the methylation status of CpG islands of the *RGS4* gene.	Ding et al., 2016
SLC6A4	Lymphoblastoic cell lines and postmortem prefrontal cortex	Bisulfite sequencing	Hypermethylation of the *SLC6A4* gene.	Sugawara et al., 2011
Upstream regions of *SMS* and *PPIEL*	Transformed lymphoblastoid cells	MS-RDA	Aberrant methylation in upstream regions of the *SMS* and *PPIEL* genes.	Kuratomi et al., 2008

5mC – 5-methylcytosine; 5hmC – 5-hydroxymethylcytosine; 5-HT3AR – serotonin receptor 3A; 5HTR1A – serotonin receptor 1A; 5HTR2A – serotonin receptor 2A; 5-HTT – serotonin transporter; BD – bipolar disorder; BDNF – brain-derived neurotrophic factor; BTN3A3 - butyrophilin, subfamily 3, member A3; COX-2 - cyclooxygenase-2; CYP11A1 - Cytochrome P450, Family 11, Subfamily A, Polypeptide 1; DTNBP1 - dystrobrevin binding protein 1; ELISA – enzyme-linked immunosorbent assay; FAM63B - family with sequence similarity 63, member B; FANCI - Fanconi anemia, complementation group I; FKBP5 – FK506-binding protein 5; GAD1 - glutamate decarboxylase 1; HCG9 - HLA Complex Group 9; HRM – high-resolution melting; KCNQ3 - potassium channel, voltage gated KQT-like subfamily Q, member 3; MB-COMT – membrane-bound catechol-O-methyltransferase; MDD – major depressive disorder; MeDIP - Methylated DNA immunoprecipitation; MSP – methylation-specific PCR; MS-RDA – methylation-sensitive representational difference analysis; NHLH1 - nescient helix loop helix 1; PIK3R1 - phosphoinositide-3-kinase, regulatory subunit 1; PPIEL - peptidylprolyl isomerase E-like; RGS4 - regulator of G protein signaling 4; RELN - reelin; SLC16A7 - solute carrier family 1 (neurotransmitter transporter, serotonin) member 7; SLC6A4 - solute carrier family 6 (neurotransmitter transporter, serotonin) member 4; SMS – spermine synthase.

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Table 2

Alterations in DNMT expression reported in BD

Cell/tissue	Findings	References
Post-mortem prefrontal cortex (BA9) and cerebellar tissue	Increased binding of DNMT1 to GABAergic and glutamatergic promoters in the cortex but not in the cerebellum in BD and schizophrenia. Increased binding of DNMT1 positively correlated with increased binding of MBD2.	Dong et al, 2015
Post-mortem prefrontal cortex	Increase in DNMT1 mRNA-positive neurons in psychotic patients (including BD).	Guidotti et al., 2013
Post-mortem brain	Co-variations of DNMT1 and DNMT3B with GABRB2 expression in BD samples.	Zhao et al., 2012
Peripheral blood mononuclear cells	Reduction in the mRNA expression of DNMT1 in bipolar depression, but not in euthimia.	Higuchi et al., 2011
BA9 and GABAergic neurons	Increased S-adenosyl methionine and DNMT1 mRNA expression in patients with BD and schizophrenia.	Guidotti et al., 2007
BA9 and GABAergic neurons	Increased DNMT1 mRNA and protein levels in patients with BD and psychosis.	Veldic et al., 2005
GABAergic caudate nucleus and putamen neurons	No increase of reelin and GAD67 mRNA expression after DNMT1 overexpression in neurons from patients with BD (which occurs in cells from schizophrenic patients).	Veldic et al., 2007

BA9 - brodmann area 9; BD - bipolar disorder; DNMT - DNA methyltransferase; GABRB2 - gamma-aminobutyric acid (GABA) A receptor, beta 2; GAD67 - glutamic acid decarboxylase 67; MBD2 - methyl-CpG binding domain protein 2; MDD – major depressive disorder.

Table 3

Effects of mood stabilizers on DNA methylation

Drug	Gene	Disease/model	Method	Main effects	Reference
Lithium	5mC and 5hmC	Rat primary cortical neurons	Immunocytochemis try	Prevention of rotenone-induced increase in DNA methylation.	Scola et al., 2014
Lithium	*BDNF*	PBMCs from BD patients	MSP	Reduction of *BDNF* methylation.	D'Addario et al., 2012
Lithium	*BDNF*	PBMCs from BD patients	MSP	Reduction of *BDNF* methylation.	Dell'Osso et al., 2014
Lithium	*Bdnf*	Rat hippocampal neurons	MSP	Hypomethylation of *Bdnf* exon IV promoter.	Dwivedi and Zhang, 2014
Lithium	Genome-wide methylation	SK-N-SH cells	Infinium HumanMethylation 27	Hypermethylation of 345 genes and hypomethylation of 138 genes.	Asai et al., 2013
Lithium	Global methylation	Lymphoblasts from BD patients	ELISA	No effect on global methylation in patients (it remained decreased compared to controls after treatment). Rescue of methylation levels in relatives.	Huzayyin et al., 2014
Lithium	*Igf2, Igf2r, H19*	Mouse ESCs	Bisulfite sequencing	Hypomethylation of imprinted control regions via Dnmr3a2.	Popkie et al., 2010
Valproate	*BDNF*	PBMCs from BD patients	MSP	Reduction of *BDNF* methylation.	D'Addario et al., 2012
Valproate	*BDNF*	PBMCs from BD patients	MSP	Reduction of *BDNF* methylation.	Dell'Osso et al., 2014
Valproate	Genome-wide methylation	SK-N-SH cells	Infinium HumanMethylation 27	Hypermethylation of 64 genes and hypomethylation of 36 genes.	Asai et al., 2013
Valproate	Global methylation	Primary rat astreocyte	LUMA	Global hypomethylation.	Perisic et al., 2010
Valproate	*Glt-1*	Primary rat cortical astrocyte	Bisulfite sequencing	Demethylation of *Glt-1* promoter.	Perisic et al., 2010
Valproate	*p21*	Mouse hippocampal cells	PCR and restriction enzymes analysis	Demethylation of the distal region of *p21*.	Aizawa and Yamamuro, 2015
Valproate	*RELN*	Mouse striatum (frontal cortex)	5-methyl CpG ChIP	Decrease of *RELN* methylation after treatment to increase DNMT activity.	Dong et al., 2008
Carbamazepine	Genome-wide methylation	SK-N-SH cells	Infinium HumanMethylation 27	Hypermethylation of 64 genes and hypomethylation of 14 genes.	Asai et al., 2013
Lamotrigine	Global methylation	Primary rat cortical astrocyte	LUMA	No changes in DNA methylation.	Perisic et al.,

BD – bipolar disorder; Igf2 - insulin-like growth factor 2; Igf2r - insulin-like growth factor 2 receptor; HTR2A – serotonin receptor 2A; BDNF – brain-derived neurotrophic factor; 5mC – 5-methylcytosine; 5-hmC – 5-hydroxymethylcytosine; SLC6A4 - solute carrier family 6 (neurotransmitter transporter, serotonin) member 4; RELN - reelin; Glt-1 - glutamate type I transporter; LUMA - luminometric methylation analysis; MSP – methylation-specific PCR; PBMC – peripheral blood mononuclear cells; ChIP – chromatin immunoprecipitation.