Chapter 15

DNA methylation in bipolar disorder

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15.1 Introduction

15.1.1 DNA methylation

The discovery of potential biomarkers in the diagnosis and treatment of bipolar disorder has investigated the role of both inherited (e.g., genetic) and environmental (e.g., lifestyle, diet, etc.) differences. The study of epigenetics offers a distinct approach to analyzing the interface of genetics and environment. Epigenetics refers to changes in gene expression that are not due to changes in the underlying deoxyribonucleic acid (DNA) code itself. These include chromatin remodeling, ribonucleic acid-based (RNA) mechanisms such as noncoding RNA and DNA methylation. DNA methylation is the most commonly and well-studied form of epigenetic modification and refers to the addition of a methyl group to the 5′ position of a cytosine nucleotide (referred to as 5-methylcytosine). DNA methylation can occur at any cytosine throughout DNA, however, is particularly concentrated at C–G dinucleotides (CpG). Additionally, groups of CpG methylation can occur in different locations in the gene but are thought to be most important when they are highly concentrated (called CpG islands) at promoter and transcription start areas. Other DNA methylation, called CpH where H refers to adenine, cytosine, or thymine nucleotides, are not as readily since it is not known fully how much control they exert on DNA expression. Nevertheless, some evidence does suggest that CpH methylation, particularly in gene bodies within certain tissues, may have a role in controlling gene expression (Guo et al., 2014). DNA methylation occurs through an enzymatic process and demethylation occurs in both passive and active forms. The majority of DNA methylation research has concentrated on the aforementioned 5-methylcytosine, however, recent work has shown that other "alternative" marks that occur on the pathway of demethylation may play a critical role in gene regulation. These alternative marks include 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcyto-

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sine (5-caC). Research studies into these alternative marks are at their infancy, particularly in bipolar disorder research. This chapter be referring to 5-methylcytosine when we say "DNA methylation" unless stated otherwise.

15.1.2 Measurement of DNA methylation

DNA methylation can be measured in several forms (Gouil & Keniry, 2019). There are three primary categories of measurements: (1) global, (2) regional, and (3) single nucleotide. Global DNA methylation refers to a measurement of the entire genome's methylation status at a given time. This is generally expressed as a percentage of methylation or global methylation percentage. Several technologies exist for estimating global DNA methylation including mass spectrometry, restriction enzyme, enzyme-linked immunosorbent assay (ELISA), and sequencing of repetitive elements. Global methylation provides information of potential methylation changes occurring at a broad, genome level, however, it provides no information regarding region or gene-specific DNA methylation changes.

Regional methylation refers to measurements of DNA methylation across a specified number of nucleotides typically covering a targeted region of a gene like a CpG island or promoter. The primary method for measurement of regional DNA methylation is PCR-based assays (e.g., high resolution melting, methylation-specific quantitative PCR). Regional methylation measurements provide information on specific gene regions, however, it does not provide information at the single-nucleotide or individual CpG level.

Several technologies exist for the measurement of DNA methylation at the single-nucleotide and gene-specific level that includes Sanger sequencing, Illumina sequencing, pyrosequencing, single-molecule sequencing, and nanopore sequencing. Many sequencing-based strategies rely on manipulation of the DNA to "expose" the DNA methylation mark such as bisulfite treatment or immunoprecipitation. Bisulfite treatment is commonly and reliably performed with many commercial kits available. This process changes nonmethylated cytosine to uracil which is then part of the signal analysis during sequencing by measuring the ratio of converted cytosine to nonconverted cytosine. An additional method of single-nucleotide DNA methylation analysis is by array technology often used in genomic and transcriptomic studies. Commonly referred to as epigenome-wide association studies, the most common array is the Illumina HumanMethylation platform for the analysis of human DNA methylation. Originally developed to measure ~27,000 CpG sites (27 K array), the array has undergone a few rounds of updates and now measures ~800,000 sites (HumanMethylation EPIC array). DNA methylation analysis at the single-nucleotide level provides the advantage of individual cytosine information, which when performed by array or whole-genome approaches, can be utilized to assess regional and genomic location differences as well. These latter whole-genome approaches can be cost and bioinformatic intensive while

smaller-scale sequencing methods (i.e., bisulfite pyrosequencing) can require more assay design, optimization, and troubleshooting. The methods of DNA methylation measurement have grown over the past 10 years and can provide distinct or overlapping information. The choice of technology is dependent on the research design, available expertise and instrumentation, and funds.

15.1.3 Sample source for DNA methylation analysis

Like work on the transcriptome evaluating gene expression, the choice of sample or tissue for analysis of DNA methylation can have important implications on analysis and interpretation since methylation is cell-type specific. This is particularly important in the context of DNA methylation analysis in bipolar disorder where often the tissue of interest, the brain, is not available for analysis (Bakulski et al., 2016). This means that clinically accessible sample sources like blood and saliva are often used. This has advantages and disadvantages in biomarker research but often it is assumed to reflect some process of the brain or disease itself. Some work has looked at correlation between various epigenetic signatures across tissues (e.g., Roadmap Epigenomic Project support by the NIH, etc.) which is a further level on information required to validate peripheral DNA methylation biomarkers. Novel strategies have also been pursued in bipolar disorder and other psychiatric disorders including the use of postmortem brain samples which again, have their advantages and disadvantages. Finally, some approaches to measure DNA methylation in the periphery such as the blood can use statistical methods to control for cell type and thus give a more clear representation of the DNA methylation being measured through the removal of the confounding effect of multiple cell types present in the blood (Kaushal et al., 2017). Work on all levels will be required to develop DNA methylation biomarkers that truly represent the disease and/or treatment but are clinically accessible for future implementation at the bedside or in the clinic.

15.1.4 Role of DNA methylation in bipolar disorder

Given the heritability estimates of bipolar disorder, DNA methylation, which theoretically captures the interaction of genes and the environment, is a useful strategy in the discovery of biomarkers that predict disease risk, disease outcome, and treatment response in bipolar disorder. The investigation of DNA methylation in bipolar disorder has been extensive and diverse with nearly 100 peer-reviewed studies reported on this topic. This chapter will highlight findings in bipolar disorder DNA methylation biomarker research and provide a listing of studies within tables that can be further pursued based on one's interests in regard to: (1) diagnosis, (2) treatment response, and (3) animal/preclinical model studies.

15.2 DNA methylation and bipolar disorder diagnosis

Most of the work in DNA methylation in bipolar disorder has looked at the role of DNA methylation in the development of the illness. This has primarily proceeded by case-control studies of peripheral blood, saliva, or postmortem brain samples in bipolar disorder patients compared to healthy controls. The three main epigenetic approaches, aligning with our description above, have been used to assess global methylation, whole-genome or epigenome-wide methylation and gene-specific, targeted methylation (Tables 15.1 and 15.2).

15.2.1 Global methylation studies of bipolar disorder

The analysis of global methylation status based on bipolar diagnosis has been reported in several studies that used peripheral blood as their DNA source. Five studies identified a decrease in global methylation while one study identified no difference in global methylation (Ceylan et al., 2018; Huzayyin et al., 2014; Li et al., 2018; Li et al., 2019a; Murata et al., 2020; Soeiro-de-Souza et al., 2013) in bipolar disorder patients versus healthy controls. Additionally, one of the studies that found a decrease in global methylation in bipolar disorder patients identified this at two surrogate sites in their analysis while they found an increase at another site (Li et al., 2019a). The reason for this discrepancy could be that this was the only study to evaluate global methylation using Alu repetitive elements which are less commonly utilized markers of global methylation. The other studies used either ELISA or LINE-1 repetitive elements as a marker of global methylation. These studies seem to point to decreased global methylation in the peripheral blood of bipolar disorder patients versus healthy controls. Future work linking global methylation to bipolar disorder symptomatology could increase its potential utility as a biomarker.

15.2.2 Whole-genome analyses

In addition to global methylation, several whole-genome methylation analyses have investigated methylation differences between bipolar disorder patients and healthy controls (Table 15.2). These studies were performed on blood and brain samples in a case-control design and the most common technology used genome-wide technology was the Illumina HumanMethylation arrays followed by methylation DNA immunoprecipitation (MeDIP) or bisulfite treatment paired with genome sequencing. MeDIP utilizes immunoprecipitation of methylated cytosine for enrichment followed by sequencing in contrast to bisulfite treatment which treats and sequences the entire DNA to estimate methylation fractions. In the studies utilizing postmortem brain samples from bipolar disorder patients and healthy controls numerous gene methylation dif-

TABLE 15.1 **Studies of bipolar disorder disease associations with global and gene-specific methylation.**

Gene	Main findings	Technology used	Citation	Gene	Main findings	Technology used	Citation
ALOX12	ND	qPCR	Rao et al. (2012)	IL-1B	ND	NGS	Duffy et al. (2019)
ARHGAP26	ND	PSQ	Sugawara et al. (2018)	IL-6	ND	NGS	Duffy et al. (2019)
ARNTL	↑	NGS	Bengesser et al. (2019)	ITGB5	ND	PSQ	Chagnon et al. (2020)
BDNF	↑ ↓ ND	PSQ, qPCR, Mass Spec, NGS	Rao et al. (2012), Duffy et al. (2019), Carlberg et al. (2014), Dell'Osso et al. (2014), Nassan et al. (2020), Stenz et al. (2015), Strauss et al. (2013)	KITLG	↓	450 K	He et al. (2018)
CACNA1C	↑	Mass Spec	Starnawska et al. (2016a)	NR3C1	↑	NGS	Duffy et al. (2019)
COMT	↓	qPCR	Abdolmaleky et al. (2006), Nohesara et al. (2011)	NF–KB	ND	qPCR	Rao et al. (2012)
COX-2	↓	qPCR	Rao et al. (2012)	OXTR	↓	PSQ	Rubin et al. (2016)
CREB	ND	qPCR	Rao et al. (2012)	P450 Epoxygenase	ND	qPCR	Rao et al. (2012)
CTAGE11P	ND	PSQ	Sugawara et al. (2018)	PDYN	↑	qPCR	D'Addario et al. (2018)

Gene	Main findings	Technology used	Citation	Gene	Main findings	Technology used	Citation
DBNL	↑	qPCR	Rao et al. (2012)	PPIEL-1	↓	PSQ	Kuratomi et al. (2008)
DTNBP1	↑	27 K, qPCR	Abdolmaleky et al. (2015)	SLC1A2	↑	PCR	Jia et al. (2017)
FAM63B	↓	PSQ, Mass Spec	Sugawara et al. (2018), Starnawska et al. (2016b)	SMS	↑	PSQ	Kuratomi et al. (2008)
FKBP5	↑ ↓	PSQ	Fries et al. (2014), Saito et al. (2020)	SPCS3	ND, SNPc	PSQ	Chagnon et al. (2020)
FZD3	ND	PSQ	Chagnon et al. (2020)	SYN1	ND	Mass Spec	Cruceanu et al. (2016)
GAD-1 network[a]	↓ ND	450 K	Ruzicka, Subburaju, and Benes (2015)	SYN2	↓	Mass Spec	Cruceanu et al. (2016)
Global	↑	Repetitive elements, ELISA, Mass spec	Li et al. (2019a), Huzayyin et al. (2014), Ceylan et al. (2018), Soeiro-de-Souza et al. (2013), Li et al. (2018), Murata et al. (2020)	SYN3	ND	Mass Spec	Cruceanu et al. (2016)
GR-1F	ND	PSQ	Schür et al. (2018)	SYNE1	SNPc	450 K	Li et al. (2019b)
HCG9	↑ ↓	NGS, PSQ	Kaminsky et al. (2012), Pal et al. (2016)	SYP	ND	qPCR	Rao et al. (2012)
HT2RA	↓	NGS, qPCR	Ghadirivasfi et al. (2011)	TBC1D22A	↑	PSQ	Sugawara et al. (2018)
HTR3AR	↓	PSQ	Perroud et al. (2016)				

Table gives alphabetical listing of gene studied, the primary finding of methylation with bipolar disorder (increased, decreased, or no difference found), and the study's citation. The "Global" line refers to all studies of global methylation regardless of technology used to measure global methylation.*Abbreviations*: *ALOX12*, arachidonate 12-lipoxygenase, 12S type; *ARHGAP26*, rho GTPase activating protein 26; *ARNTL*, aryl hydrocarbon receptor nuclear translocator like; *BDNF*, brain-derived neurotrophic factor; *CACNA1*, calcium voltage-gated channel subunit alpha1 C; *COMT*, catechol-*O*-methyltransferase; *COX-2*, prostaglandin-endoperoxide synthase 2; *CREB*, CREB binding protein; *CTAGE11P*, CTAGE family member 11, pseudogene; *DBNL*, drebrin like; *DTNBP1*, dystrobrevin binding protein 1; *FAM63B*, family with sequence similarity 63 member B; *FKBP5*, FK506 binding protein 5; *FZD3*, frizzled class receptor 3; *GAD-1*, glutamate decarboxylase 1; *GR-1F*, nuclear receptor subfamily 3 group C member 1; *HCG9*, HLA complex group 9; *HT2RA*, 5-hydroxytryptamine receptor 2A; *HTR3AR*, serotonin receptor 3A; *IL-1B*, interleukin 1 beta; *IL-6*, interleukin 6; *ITGB5*, integrin subunit beta 5; *NGS*, next generation sequencing; *KITLG*, KIT ligand; *NR3C1*, nuclear receptor subfamily 3 group C member 1; *NF-KB*, nuclear factor kappa B; *OXTR*, oxytocin receptor; *P450 epoxygenase*, cytochrome P450; *PDYN*, prodynorphin; *PPIEL-1*, peptidylprolyl isomerase E-like pseudogene; *PSQ*, pyrosequencing; *SLC1A2*, solute carrier family 1 member 2; *SMS*, spermine synthase; *SNPc*, correlation with SNP in same gene; *SPCS3*, signal peptidase complex subunit 3; *SYN1*, synapsin I; *SYN2*, synapsin II; *SYN3*, synapsin III; *SYNE1*, spectrin repeat containing nuclear envelope protein 1; *SYP*, synaptophysin; *qPCR*, quantitative polymerase chain reaction; *TBC1D22A*, TBC1 domain family member 22A.
[a] *GAD1-network genes included 27 genes of interest.*

ferences have been identified. For example, Xiao et al. performed a genome-wide methylation analysis using MeDIP on seven frontal cortex samples from bipolar patients and six frontal cortex samples from healthy controls (Xiao et al., 2014). In bipolar disorder patients, one area of the cortex revealed more hypomethylated genes (BA9) while another region (BA24) revealed more hypermethylated genes. Some notable differentially methylated regions they identified were in the following genes: protein phosphatase 3 catalytic subunit gamma (*PPP3CC*), DNA methyltransferase 1 (*DNMT1*), dystrobrevin binding protein 1 (*DTNBP1*), nitric oxide synthase 1 (*NOS1*), 5-hydroxytryptamine receptor 1E (*HTR1E*), glutamate metabotropic receptor 5 (*GRM5*), proline-rich membrane anchor 1 (*PRIMA1*), 5-hydroxytryptamine receptor 2A (*HTR2A*), collagen type I alpha 2 chain (*COL1A2*), LIM domain only 1 (*LMO1*), and immunoglobulin superfamily DCC subclass member 4 (*IGDCC4*). These genes play a role in methylating DNA (*DNMT1*), protein regulation (*PPP3CC*), the serotonin pathway (*HTR1E, HTR2A*), and neurotransmitters activity (*GRM5, PRIMA1*). Additionally, Ho et al. analyzed postmortem cortex gray matter from bipolar disorder patients and healthy controls using the Illumina HumanMethylation 450 K array (Ho et al., 2019). When comparing the methylation profiles between bipolar disorder and healthy control samples, differential methylation was identified in pathways related to glutamatergic dysregulation, synaptogenesis, and synaptic plasticity although none reached the threshold for genome-wide significance. Furthermore, area-specific differences were identified based on diagnosis. Similarly, but in another tissue, Li et al. investigated genome-wide methylation differences by MeDIP in peripheral blood of bipolar disorder patients versus healthy controls (Li et al., 2015). They identified 56 genes including *DNMT1*, calcium voltage-gated channel subunit alpha1 S (*CACNA1S*), PRAME nuclear receptor transcriptional regulator (*PRAME*), myelin transcription factor 1 like (*MYT1L*), and stabilin 1 (*STAB1*)

TABLE 15.2 Whole-genome methylation studies of bipolar diagnosis.

Lead author	Methylome-wide technology	Highlighted findings
Abdolmaleky et al. (2019)	27 K	Increased methylation of *CCND1* gene and lack of brain laterality differences of *TGHFBR2* gene in BD while differences found in HC
Comes et al. (2020)	EPIC	34,776 Nominally significant sites but no single site was significant after correction for multiple testing
Dempster et al. (2011)	27 K	Networks of genes related to neurological disease, psychological disorders, genetic disease, nutritional disease, and gene control. Top 8 genes included: *GPR24, TLE6, STAB1, PPYR1, CTNNA2, ST6GALNAC1, C1orf35*, and *IQCH*
Fries et al. (2017)	450 K	64 Sites were differentially methylated in both high-risk offspring and BD patients vs HCs and were found in *PQLC2L, PCNX, MAGI2, HOOK2, SLC45A4, GLUL, PGCP, LCE2D, NLK*, and *ZNF195*
Gaine et al. (2019)	NGS	Increased methylation in *PDCD1* and *ADAM21P1* and decreased methylation in *MYOM2*, *CES1P1*, and *ARHGEF38* in BD
Ho et al. (2019)	450 K	Broad differential methylation found based on brain region within BD vs HC
Jeremian et al. (2017)	450 K	Decreased methylation in *MPP4, TRE2/BUB2/CDC16* and increased methylation in *NUP133*
Li et al. (2015)	MeDIP	56 Genes including *DNMT1, CACNA1S, PRAME, MYT1L*, and *STAB1*
Mill et al. (2008)	Custom microarray	Increased *RPP21, KEL, WDR18*, and *RPL39* in female BD; *MEK1* associated with lifetime antipsychotic use
Ruzicka, Subburaju, and Benes (2017)	450 K	195 Differentially methylated positions

Lead author	Methylome-wide technology	Highlighted findings
Ruzicka et al. (2018)	450 K	42 Significant sites in B patients distributed across genome
Sabunciyan et al. (2015)	450 K	Increased methylation of *CYP11A1* in BD vs HC validated by pyrosequencing
Walker et al. (2016)	450 K	One CpG site in *NBEAL2* (decreased) and one site in *FANCI* (increased) found to be differentially methylated in affected patients vs HCs
Watkeys et al. (2020)	450 K	Polymethylomic profile scores including 362–1208 CpG sites did not significantly associate with BD diagnosis after correction for multiple testing
Xiao et al. (2014)	MeDIP	Identified several significant sites including: *PPP3CC, DNMT1, DTNBP1, NOS1, HTR1E, GRM5, PRIMA1, HTR2A*, and *HTR2A; COL1A2, LMO1*, and *IGDCC4 l*
Zhao et al. (2015)	MeDIP	Large differential methylation broadly impacting promoter elements, CpG islands, noncoding RNAs, and repetitive elements within introns

Table includes author/citation, technology utilized for whole-genome methylation and highlighted findings. Please consult the primary literature for further findings and complete descriptions of findings.*Abbreviations*: *27 K*, Illumina HumanMethylation 27 K Array; *450 K*, Illumina HumanMethylation 450 K Array; *ADAM21P1*, ADAM metallopeptidase domain 21 pseudogene 1; *ARHGEF38*, rho guanine nucleotide exchange factor 38; *BD*, bipolar disorder; *BUB2*, mitotic check point protein; *C1orf35*, chromosome 1 open reading frame 35; *CACNA1S*, calcium voltage-gated channel subunit alpha1 S; *CCND1*, cyclin D1; *CDC16*, cell division cycle 16; *CES1P1*, carboxylesterase 1 pseudogene 1; *COL1A2*, collagen type I alpha 2 chain; *CTNNA2*, catenin alpha 2; *CYP11A1*, cytochrome P450 family 11 subfamily A member 1; *DNMT1*, DNA methyltransferase 1; *DTNBP1*, dystrobrevin binding protein 1; *EPIC*, Illumina HumanMethylation Epic Array; *FANCI*, Fanconi anemia complementation group I; *GLUL*, glutamate-ammonia ligase; *GPR24*, melanin concentrating hormone receptor 1; *GRM5*, glutamate metabotropic receptor 5; *HC*, healthy control; *HOOK2*, hook microtubule tethering protein 2; *HTR1E*, 5-hydroxytryptamine receptor 1E; *HTR2A*, 5-hydroxytryptamine receptor 2A; *IGDCC4*, immunoglobulin superfamily DCC subclass member 4; *IQCH*, IQ motif containing H; *KEL*, Kell blood group, metalloendopeptidase; *LCE2D*, late cornified envelope 2D; *LMO1*, LIM domain only 1; *MAGI2*, membrane-associated guanylate kinase, WW and PDZ domain containing 2; *MeDIP*, methylated DNA immunoprecipitation; *MEK1*, mitogen-activated protein kinase kinase 1; *MPP4*, membrane palmitoylated protein 4; *MYOM2*, myomesin 2; *MYT1L*, myelin transcription factor 1 like; *NBEAL2*, neurobeachin-like 2; *NLK*, nemo-like kinase; *NOS1*, nitric oxide synthase 1; *NUP133*, nucleoporin 133; *PCNX*, pecanex 1; *PDCD1*, programmed cell death 1; *PGCP*, carboxypeptidase Q; *PPP3CC*, protein phosphatase 3 catalytic subunit gamma; *PPYR1*, neuropeptide Y receptor Y4; *PQLC2L*, PQ loop repeat containing 2 like; *PRAME*, preferentially expressed antigen in melanoma; *PRIMA1*, proline-rich membrane anchor 1; *RPL39*, ribosomal protein L39; *RPP21*, ribonuclease P/MRP subunit p21; *SLC45A4*, solute carrier family 45 member 4; *ST6GALNAC1*, ST6 N-acetylgalactosaminide alpha-2,6-sialyltransferase 1; *STAB1*, stabilin 1; *TGHFBR2*, transforming growth factor beta receptor 2; *TLE6*, transducin-like enhancer of split 6; *TRE2*, ubiquitin-specific peptidase 6; *WDR18*, WD repeat domain 18; *ZNF195*, zinc finger protein 195.

that were differentially methylated based on diagnosis. Finally, Comes et al. performed an analysis with the HumanMethylation EPIC array on bipolar disorder patients and healthy controls to evaluate the diagnostic and longitudinal methylation differences (Comes et al., 2020). They found 34,776 sites that were significant at a nominal level ($P < .05$), however, after correction for confounders and multiple testing, none of the sites reached genome-wide statistical significance cutoffs. Altogether the whole-genome analyses of bipolar disorder have identified a diverse set of potential methylation markers that are candidates for further gene-specific replication in longitudinal designs. Such replication is critical to support novel gene findings and to strengthen these findings which have been limited by small sample sizes.

15.2.3 Gene-specific analyses

Exploration into the methylation biomarkers of bipolar disorder disease pathophysiology and risk has also employed targeted and hypothesis-driven analysis of genes (Table 15.1). This has included genes implicated previously in bipolar disorder from genetic variation studies, epigenetic genes such as those that code for the enzymes involved in methylation as well as novel or rarely studied genes identified from genome-wide methylation studies.

A particular gene of interest is *BDNF* which codes for the brain-derived neurotrophic factor which codes for a protein that plays a role in nerve growth factor and has activity in both the central nervous system and periphery. It has been implicated in the development of various psychiatric disease states and their treatment including bipolar disorder and major depressive disorder as well as other disease states (e.g., insulin resistance). *BDNF* methylation has been evaluated in a series of studies comparing methylation in various regions within the gene (promoter methylation, exon methylation, and methylation surrounding the val66met polymorphism) in peripheral blood from bipolar disorder patients compared to healthy controls. Findings have differed across the studies with decreases (Dell'Osso et al., 2014), increases (Nassan et al., 2020; Stenz et al., 2015; Strauss et al., 2013), and no differences (Carlberg et al., 2014) in *BDNF* methylation observed in bipolar disorder patients compared to healthy controls. Importantly, Stentz et al. demonstrated that *BDNF* methylation was highly correlated between brain and peripheral blood suggesting that the measurement of this gene's methylation in the periphery may reflect its methylation status in the brain (Stenz et al., 2015).

Another gene of interest is the HLA complex group 9 (*HCG9*) gene which is a nonprotein coding gene that lies in the major histocompatibility complex I region. This gene was identified as a potential gene of importance in bipolar disorder from previous genome-wide methylation assessments and may play a role in stress response and apoptosis pathways (Seidl et al., 2010). Kaminsky et al., who originally discovered the *HCG9* gene in a full genome scan of methylation markers in psychosis (Mill et al., 2008) validated their findings

by pyrosequencing showing decreased methylation in *HCG9* in postmortem brain of bipolar disorder patients compared to healthy controls (Kaminsky et al., 2012). Another investigation by Pal et al. utilized deep (>30×) bisulfite sequencing of the *HCG9* gene in postmortem brain to evaluate the degree of DNA methylation at both CpG and non-CpG sites (Pal et al., 2016). Although not a readily studied due to the theory that methylation is far less common, non-CpG methylation (also called CpH where the H refers to A, C, or T nucleotides) may have a stronger role in the body of the gene as compared to CpG's role in the promoter regions of the gene (Jang et al., 2017). Pal demonstrated increased CpH methylation on both DNA strands in bipolar disorder patients compared to control that was particularly prevalent on CpA dinucleotides. In contrast to the Kaminsky study described above, they found a decrease in CpG methylation within the *HCG9* gene.

The MINDY lysine 48 deubiquitinase 2 gene (*MINDY2*), more commonly referred to as *FAM63B*, codes for a protein with an unclear function but may be related to circadian rhythm control. This gene was originally discovered as a schizophrenia-risk gene but has been studied in bipolar disorder due to some possible shared comorbidities between the disorders (Aberg et al., 2014). Starnawska et al. demonstrated decreased methylation of *FAM63B* in the peripheral blood of bipolar patients versus healthy controls (Starnawska et al., 2016b). This finding was replicated in another study of peripheral blood of bipolar patients compared to healthy controls (Sugawara et al., 2018). This novel gene may represent a useful future biomarker of bipolar disorder risk with future work validating these findings and connecting them to mechanisms, disease progression, or treatment response.

There are several other genes that have been investigated in a gene-specific manner within bipolar disorder including *CACNA1C*, FKBP prolyl isomerase 5 (*FKBP5*) (Saito et al., 2020), catechol-O-methyltransferase (*COMT*) (Abdolmaleky et al., 2006; Nohesara et al., 2011), and more. Future work replicating the findings with these studied genes will be the next step in understanding their utility as a future biomarker in bipolar disorder.

15.3 DNA methylation and treatment response in bipolar disorder

DNA methylation regarding treatment of bipolar disorder has been evaluated in terms of its role as an explanatory mechanism and as a biomarker that can be used to predict response (efficacy or side effects) to treatment. Like the investigations of DNA methylation with bipolar disorder disease development and risk, the investigations of treatment response have utilized a wide range of DNA methylation technologies and assessed several different medication types including mood stabilizers and antipsychotics (Table 15.3).

TABLE 15.3 **DNA methylation studies of bipolar disorder pharmacotherapy.**

Lead author	Medications studied	Type of methylation study	Genes, if applicable	Technology	Summarized results
Abdolmaleky et al. (2011)	AP and VPA	Gene specific	HTR2A	qPCR	Methylation at *HTR2A* surrounding -1438 G/A variant was higher in AP-free vs HCs. Methylation at *HTR2A* surrounding T102C variant was decreased in patients on APs and VPA vs HCs
Abdolmaleky et al. (2014)	AP	Full genome	NA	27 K; 450 K	No differences based on diagnosis but higher methylation in AP-free SCZ patients vs BD patients or HCs
Backlund et al. (2015)	AAP, Ms, and Li	Global	NA	ELISA	No difference between BD patients and HC. Decreased methylation in Li patients, increased in Li + VPA and Li + AAP
Bengesser et al. (2018)	Li and MS	Gene specific	ARNTL	PSQ	Increased methylation vs HC with significant association with Li and Ms treatment
Bromberg et al. (2009)	MS	Global	NA	Radio-labeled extension assay	No difference in global methylation
Burghardt et al. (2015)	AAP and MS	Global	NA	Restriction Enzyme based	Decreased global methylation in AAP group vs Ms group
Burghardt et al. (2016)	AAP and MS	Full genome	NA	450 K	Identified decreased *FAR2* gene methylation (validated by PSQ) associated with increased insulin resistance in AAP-treated patients but not Ms-treated patients
Burghardt et al. (2018)	AAP and MS	Gene specific	AKT1, AKT2, AKT3	qPCR	Increased *AKT1* and *AKT2* in AAP-treated group compared to Ms group

Lead author	Medications studied	Type of methylation study	Genes, if applicable	Technology	Summarized results
Burghardt et al. (2019)	AAP and MS	Global	NA	ELISA	Increased 5-mC and 5-fC (not 5-hmC) in bipolar patients vs healthy controls. Hypermethylation (5-mC and 5-fC) in AAP-treated patients while no differences observed for those on Ms
D'Addario et al. (2012)	Ms, Li, and AD	Gene specific	BDNF	qPCR	AD increased methylation vs Li and VPA. Increased methylation in BDII vs control but no difference for BDI
Dell'Osso et al. (2014)	AAP, Ms, Li, and AD	Gene specific	BDNF	qPCR	Increased methylation (not significant) with AAP, pregabalin, and AD therapy vs Li and VPA. Increased methylation in MDD and BDII patients vs BDI
Houtepen et al. (2016)	AAP, MS	Gene specific		27 K; 450 K	Quetiapine and VPA have significantly altered methylation profiles. Only one site in *GABRA1* was significant with carbamazepine treatment after correction for multiple tests.
Kaminsky et al. (2015)	Li and VPA	Gene specific	KCNQ2 and KCNQ3	PSQ	Decreased *KCNQ3* methylation in BD patients vs HCs after correcting for Ms use suggesting Ms treatment may increase methylation to "control" levels
Schröter et al. (2020)	AAP, Ms, Li, and AD	Gene specific	BDNF	Pyrosequencing	Decreased methylation in BD vs MDD patients at one site with average methylation across all sites being higher in AD-treated patients.

Table provides study/citation, treatment evaluated, type of methylation approach, genes studied (if applicable), methylation technology, and summarized findings.*Abbreviations*: *5-fc*, 5-formylcytosine; *5-hmC*, 5-hydroxymethylcytosine; *5-mC*, 5-methylcytosine; *AAP*, atypical antipsychotics; *AD*, antidepressants; *AKT1*, AKT serine/threonine kinase 1; *AKT2*, AKT serine/threonine kinase 2; *AKT3*, AKT serine/threonine kinase 3; *AP*, antipsychotics; *ARNTL*, aryl hydrocarbon receptor nuclear translocator like; *BD*, bipolar disorder; *BDNF*, brain-derived neurotrophic factor; *GABRA1*, gamma-aminobutyric acid type A receptor subunit alpha1; *HC*, healthy control; *HTR2A*, 5-hydroxytryptamine receptor 2A; *KCNQ2*, potassium voltage-gated channel subfamily Q member 2; *KCNQ3*, potassium voltage-gated channel subfamily Q member 3; *Li*, lithium; *MDD*, major depressive disorder; *Ms*, mood stabilizer; *SCZ*, schizophrenia; *VPA*, valproic acid.

15.3.1 Global methylation

Studies have looked at the associations between global methylation and treatment in bipolar disorder and found contrasting findings depending on the study. Work by Backlund et al. investigated global methylation by ELISA and did not find differences when comparing bipolar patients on any treatment compared to matched healthy controls without regard to treatment (Backlund et al., 2015). However, they found decreased global methylation in patients on lithium monotherapy while increased global methylation was detected in patients on lithium plus valproic acid or lithium plus an antipsychotic. The latter hypermethylation in patients on lithium plus an antipsychotic did not remain when controlling for age and sex. Increased global methylation (including changes in alternative methylation such as 5-hydroxymethylation) with antipsychotic monotherapy has also identified in other studies including a study of methylation in skeletal muscle (Burghardt et al., 2019) yet, decreased methylation has been identified in other studies (Burghardt et al., 2015). Bromberg et al. utilized a restriction enzyme-based approach coupled with radio-labeled extension and found that there were no differences in peripheral blood global DNA methylation between bipolar patients on valproic acid and lithium or a combination of the two as compared to age and gender-matched controls (Bromberg et al., 2009). Although this work suggests possible effects by several different bipolar disorder pharmacotherapies, total sample sizes are relatively small using cross-sectional or case-control designs so further confirmation is needed utilizing similar approaches to measure global methylation and in prospective treatment designs.

15.3.2 Whole-genome analyses

In addition to analyses of bipolar disorder treatment on global methylation, two studies have analyzed whole-genome or epigenome-wide DNA methylation associations with treatment in bipolar disorder. Houtepen et al. utilized the Illumina HumanMethylation 27 K and 450 K arrays to analyze whole blood DNA methylation in subjects on either mood stabilizer or atypical antipsychotic therapy (Houtepen et al., 2016). Overall, they found that treatment with valproic acid and quetiapine was associated with significantly altered DNA methylation signatures when adjusting for cell composition differences inherent in whole blood. Despite promising models, none of the associations between any treatment and a single-array CpG site remained significant after correcting for multiple testing possibly due to limited sample sizes. The only significant site that remained after multiple testing correction was in the gamma-aminobutyric acid type A receptor subunit alpha1 (*GABRA1*) gene with carbamazepine treatment. This gene codes for the receptor of the associated inhibitory neurotransmitter and has been linked to epilepsy in genetic

variation studies. Finally, the authors also performed a "targeted" analysis of set of candidate genes pulled from the array, however, no associations were statistically significant. These candidate genes covered transporters, brain-associated proteins, neurotrophins, and factors potentially associated with medication side effects. The second study by Burghardt et al. utilized the 450 K array on peripheral blood samples from bipolar patients on atypical antipsychotics or lithium to detect associations with antipsychotics-induced insulin resistance (Burghardt et al., 2016). They found two genome-wide significant hits in a discovery sample (bipolar patients on antipsychotics) within the *FAR2* gene and an intergenic probe that was significantly associated with insulin resistance in atypical antipsychotic-treated bipolar patients. They then replicated their findings in an additional sample of bipolar patients on either atypical antipsychotics or lithium monotherapy and found decreased *FAR2* methylation with increased insulin resistance in only patients on atypical antipsychotics.

15.3.3 Gene-specific studies

A series of studies have also investigated DNA methylation of candidate or target genes in bipolar disorder patients treatment. Three studies have looked at *BDNF* methylation in peripheral blood of bipolar patients on combination mood stabilizer with antipsychotic treatment compared to healthy controls (D'Addario et al., 2012; Dell'Osso et al., 2014; Schröter et al., 2020). In studies by Dell'Osso and D'Addario, regional *BDNF* methylation was studied by qPCR methods in a cross-sectional sample of bipolar disorder patients and controls (D'Addario) or depressed patients (Dell'Osso). These studies identified increased *BDNF* methylation in patients on antidepressant therapy while lithium and valproic acid therapy had decreased methylation, albeit not in a statistically significant manner. D'Addario also identified increased *BDNF* methylation in patients with bipolar II diagnoses which appeared to be driven by antidepressant treatment (no different in bipolar I vs controls). Similarly, Dell'Osso, found increased *BDNF* methylation in patients with major depressive disorder and bipolar II when comparing to bipolar I, again suggesting a possible role for antidepressant treatment. Schroter et al. investigated *BDNF* methylation in bipolar disorder and depressed patients before and after treatment response by pyrosequencing. They observed a significant decrease in *BDNF* methylation in bipolar patients compared to depressed patients at one CpG site (of 24 sites studied) and identified, with all patients combined, a significant increase in average methylation across CpG sites with antidepressant treatment.

In addition to possible pharmacodynamic target genes like *BDNF*, two studies by Abdolmakey et al. investigated the pharmacokinetic candidates of serotonin transporter and receptor gene methylation by MSqPCR and array (27 K combined with 450 K array) respectively, in saliva and brain samples of bipolar patients versus healthy controls (Abdolmaleky et al., 2011; Abdol-

maleky et al., 2014). In their former study, they found serotonin 2A receptor (*5HTR2A*) methylation was significantly increased in bipolar disorder patients versus healthy controls in methylation sites around a nearby, common genetic variant within the same gene called –1438A/G. This methylation was also elevated in antipsychotic-free patients while valproic acid did not have an effect. In contrast, methylation around another genetic variant called T102C was decreased in bipolar patients versus healthy controls with no detectable effect of treatment. In the latter study using the array technology to evaluate serotonin transporter (*SLC6A4*) methylation, they found no differences based on diagnosis but identified an increase in methylation for antipsychotic-free schizophrenia patients when compared to bipolar disorder patients.

Finally, a group of studies have evaluated several different genes including aryl hydrocarbon receptor nuclear translocator like (*ARNTL*), AKT serine/threonine kinases (*AKT1/2/3*), potassium voltage-gated cChannel genes (*KCNQ1/2*), and FA complementation group A (*FANC*) in bipolar disorder patients. Bengesser et al. analyzed *ARNTL* gene methylation, a gene that codes for a transcription activator, by pyrosequencing and identified increased methylation in bipolar patients versus healthy controls with effects on methylation detectable by both lithium and mood stabilizer (Bengesser et al., 2018). Burghardt et al. evaluated *AKT* gene methylation (three isoforms involved in protein activation and numerous pathways) in skeletal muscle biopsies of patients on atypical antipsychotic and mood stabilizer therapy (Burghardt et al., 2018). They found increased *AKT1* and *AKT2* gene methylation in patients taking atypical antipsychotics but not mood stabilizers which are the two isoforms thought to be involved in the process of glucose uptake in the muscle. This may demonstrate a possible connection between atypical antipsychotic-induced metabolic side effect and gene methylation. Notably, *AKT3*, the isoform predominately involved in central nervous system activity, was not differentially methylation in the skeletal muscle between bipolar patients on antipsychotic compared to mood stabilizers. Kaminsky et al. analyzed *KCNQ2* and *KCNQ3* methylation in postmortem brain samples of bipolar patients and healthy controls (Kaminsky et al., 2015). This family of genes codes for the proteins that make up voltage-gated potassium channels and has been subject to investigation as a possible cause of bipolar disorder illness (Judy & Zandi, 2013). They identified significantly decreased methylation in *KCNQ3* (not *KCNQ2*) in bipolar disorder patients. Furthermore, their analyses identified that mood stabilizers including valproic acid, "restored" *KCNQ3* methylation back to healthy control levels (i.e., increased *KCNQ3* methylation) in human postmortem brain samples but not in rodent samples treated with the same drugs. Lithium did not have an effect on *KCNQ3* methylation and these patients still had significantly lower methylation compared to healthy controls.

The work to discover DNA methylation biomarkers that assist with the prediction of medication response in bipolar disorder is still an ongoing endeavor. Like the investigation into disease biomarkers in the previous section,

BDNF appears to be a promising gene that may prove clinically useful with additional replication and analysis in prospective studies before and after treatment.

15.4 DNA methylation and animal and preclinical model studies of bipolar disorder

Although the analysis of DNA methylation biomarkers in bipolar disorder is the most useful when pursued in human populations for a variety of reasons including specific genetic and environmental influences, the use of preclinical and animal models is an important step to establishing the molecular mechanisms underlying any identified biomarker and supporting their theoretical basis as a biomarker. Here we will highlight a few studies (Table 15.4) to demonstrate the work in this area but direct the reader elsewhere for further, in-depth reviews (Fries et al., 2016; Gardea-Resendez et al., 2020; Grayson & Guidotti, 2018).

Preclinical in vitro and in vivo models have been particularly useful in evaluating epigenetic and DNA methylation changes secondary to lithium treatment. For example, in support of the work described above on *BDNF* methylation, Dwivedi et al. investigated the effects of lithium on rat hippocampal neuronal *BDNF* methylation (Dwivedi & Zhang, 2014). They identified decreased *BDNF* methylation in exon IV following lithium treatment with concomitant increases in *BDNF* gene expression. Lee et al. also analyzed the rat hippocampus for changes in leptin receptor (*LEPR)* gene methylation secondary to treatment with lithium or valproic acid (Lee et al., 2015). They did not find significant evidence of either drug on DNA methylation of the *LEPR* gene, a candidate gene chosen based on evidence suggesting a role in both bipolar disorder and response to the drugs commonly used in the treatment of bipolar disorder. Finally, Asai et al. treated human neuroblastoma cell cultures with lithium, valproic acid, and carbamazepine and analyzed genome-wide profiles with the Illumina HumanMethylation 27 K array (Asai et al., 2013). Overall, their analyses showed that the DNA methylation profiles significantly differed between the treatment and control experiments. Specifically, they identified over 100 genes that had increased DNA methylation and over 100 genes that had decreased methylation with lithium compared to controls. They found approximately 60 genes changed in either direction (increased or decreased methylation) for valproic acid and carbamazepine as well.

In summary, the use of preclinical and animal models may prove useful for both discovery and validation of human methylation biomarkers. Both lines of investigation should seek to inform and support each other as candidate biomarkers move from the discovery to application phase.

TABLE 15.4 Summary of studies of DNA methylation in preclinical and animal models of bipolar disorder.

Lead author	Type of methylation study	Summarized results
Asai et al. (2013)	Full genome and gene specific	Epigenetic changes identified with VPA, Li, and carbamazepine and included 41 hypermethylated and 11 hypomethylated genes. For *SLC4A*, the third and fourth CpG sites were hypomethylated by all MSs, except for VPA.
Dong et al. (2019)	Gene specific	Clozapine corrected (decreased) methylation of *BDNF*, *Reln*, and *Gad1* genes in mouse hippocampus. Haloperidol and risperidone had no effect.
Luoni et al. (2016)	Full genome	*Ank3*, a candidate gene for BD, showed increased methylation in prefrontal cortex of rats exposed to prenatal stress with concomitant changes in RNA and protein expression. Additional genes from genome-wide analysis included *Cnga4*, *Dars2*, *Gabrg2*, *Htr4*, *Lphn2*, *Slc22a2*, and *Tiam1*.
Dwivedi and Zhang (2014)	Gene specific	At the dose of 1mM, Li decreased *BDNF* methylation of exon IV by 38%, whereas at the dose of 2mM, this decrease was 50%.
Scola et al. (2014)	Full genome	Rotenone treatment, used to induce mitochondrial dysfunction, increased DNA methylation levels. Li prevents this increased DNA methylation and hydroxymethylation in rat cortical primary neurons.
Dong et al. (2016)	Gene specific	Clozapine and VPA, but not haloperidol, correct promoter hypermethylation of *BDNF*, *Reln*, *Gad1*, and *Gad2* in the frontal cortex of prenatally stressed mice. In nonstressed mice, clozapine treatment failed to alter the level of promoter methylation in these genes.
Lee et al. (2015)	Gene specific	Despite upregulation of gene expression of *LEPR* with Li and VPA treatment in hippocampal tissues, DNA methylation of *LEPR* was not significantly altered with treatment.

Lead author	Type of methylation study	Summarized results
Aizawa and Yamamuro (2015)	Gene specific	VPA alters the methylation pattern of the distal CpG island of the *p21* gene. TaqI and BstUI sites at the distal CpG island were decreased from 85% to 32% and from 48% to 13%, respectively.
Dong et al. (2008)	Gene specific	Clozapine and sulpiride, but not haloperidol or olanzapine exhibited cortical and striatal demethylation of *Reln* and *Gad1* promoters. These effects were further enhanced by administration of VPA.

Table provides study/citation, type of methylation approach, and a summary of results.*Abbreviations*: *Ank3*, ankyrin 3; *BD*, bipolar disorder; *BDNF*, brain-derived neurotrophic factor; *Cnga4*, cyclic nucleotide-gated channel alpha 4; *Dars2*, aspartyl-tRNA synthetase 2, mitochondrial; *Gabrg2*, gamma-aminobutyric acid type A receptor gamma2 subunit; *Gad1*, glutamate decarboxylase 1; *Gad2*, glutamate decarboxylase 2; *Htr45*, hydroxytryptamine receptor 4; *LEPR*, leptin receptor; *Li*, lithium; *Lphn2*, adhesion G protein-coupled receptor L2; *Ms*, mood stabilizer; *p21*, cyclin-dependent kinase inhibitor 1A; *Reln*, reelin; *Slc22a2*, solute carrier family 22 member 2; *SLC4A*, solute carrier family 4 member 1; *Tiam1*, T-cell lymphoma invasion and metastasis 1; *VPA*, valproic acid.

15.5 Summary

There has been considerable work into the discovery of DNA methylation biomarkers that potentially associate with bipolar disorder disease and treatment. This work is still at its infancy and, as such, is not ready for utilization at the clinical level. Future work concentrating on replication, establishing strong correlation or preferably causation in larger populations will be essential to bringing DNA methylation to the biomarker "toolbox."

References

Abdolmaleky, H.M., et al. (2006). Hypomethylation of MB-COMT promoter is a major risk factor for schizophrenia and bipolar disorder. Human Molecular Genetics, 15(21), 3132–3145.

Abdolmaleky, H.M., et al. (2011). Epigenetic dysregulation of HTR2A in the brain of patients with schizophrenia and bipolar disorder. Schizophrenia Research, 129(2–3), 183–190.

Abdolmaleky, H.M., et al. (2014). DNA hypermethylation of serotonin transporter gene promoter in drug naive patients with schizophrenia. Schizophrenia Research, 152(2–3), 373–380.

Abdolmaleky, H.M., et al. (2015). Antipsychotic drugs attenuate aberrant DNA methylation of DTNBP1 (dysbindin) promoter in saliva and post-mortem brain of patients with schizophrenia and psychotic bipolar disorder. American Journal of Medical Genetics Part B: Neuropsychiatric Genetics, 168(8), 687–696.

Abdolmaleky, H.M., et al. (2019). Aberrant transcriptomes and DNA methylomes define pathways that drive pathogenesis and loss of brain laterality/asymmetry in

schizophrenia and bipolar disorder. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics, 180(2), 138–149.

Aberg, K.A., et al. (2014). Methylome-wide association study of schizophrenia: Identifying blood biomarker signatures of environmental insults. JAMA Psychiatry, 71(3), 255–264.

Aizawa, S., & Yamamuro, Y. (2015). Valproate administration to mice increases hippocampal p21 expression by altering genomic DNA methylation. Neuroreport, 26(15), 915–920.

Asai, T., et al. (2013). Effect of mood stabilizers on DNA methylation in human neuroblastoma cells. The International Journal of Neuropsychopharmacology/ Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP), 16(10), 2285–2294.

Backlund, L., et al. (2015). Mood stabilizers and the influence on global leukocyte DNA methylation in bipolar disorder. Molecular Neuropsychiatry, 1(2), 76–81.

Bakulski, K.M., et al. (2016). Epigenetic research in neuropsychiatric disorders: The "tissue issue.". Current Behavioral Neuroscience Reports, 3(3), 264–274.

Bengesser, S.A., et al. (2018). Is the molecular clock ticking differently in bipolar disorder? Methylation analysis of the clock gene ARNTL. The World Journal of Biological Psychiatry: The Official Journal of the World Federation of Societies of Biological Psychiatry, 19(sup2), S21–S29.

Bengesser, S.A., et al. (2019). Epigenetics of the molecular clock and bacterial diversity in bipolar disorder. Psychoneuroendocrinology, 101, 160–166.

Bromberg, A., et al. (2009). Global leukocyte DNA methylation is not altered in euthymic bipolar patients. Journal of Affective Disorders, 118(1), 234–239.

Burghardt, K.J., et al. (2015). DNA methylation, insulin resistance and second-generation antipsychotics in bipolar disorder. Epigenomics, 7(3), 343–352.

Burghardt, K.J., et al. (2016). Gene-specific DNA methylation may mediate atypical antipsychotic-induced insulin resistance. Bipolar Disorders, 18(5), 423–432.

Burghardt, K.J., et al. (2018). Association of protein kinase B (AKT) DNA hypermethylation with maintenance atypical antipsychotic treatment in patients with bipolar disorder. Pharmacotherapy, 38(4), 428–435.

Burghardt, K.J., et al. (2019). Skeletal muscle DNA methylation modifications and psychopharmacologic treatment in bipolar disorder. European Neuropsychopharmacology: the Journal of the European College of Neuropsychopharmacology, 29(12), 1365–1373.

Carlberg, L., et al. (2014). Brain-derived neurotrophic factor (BDNF)-epigenetic regulation in unipolar and bipolar affective disorder. Journal of Affective Disorders, 168, 399–406.

Ceylan, D., et al. (2018). DNA redox modulations and global DNA methylation in bipolar disorder: Effects of sex, smoking and illness state. Psychiatry Research, 261, 589–596.

Chagnon, Y.C., et al. (2020). A multimodal attempt to follow-up linkage regions using RNA expression, SNPs and CpG methylation in schizophrenia and bipolar disorder kindreds. European Journal of Human Genetics, 28(4), 499–507.

Comes, A.L., et al. (2020). The role of environmental stress and DNA methylation in the longitudinal course of bipolar disorder. International Journal of Bipolar Disorders, 8(1), 9.

Cruceanu, C., et al. (2016). DNA hypomethylation of Synapsin II CpG islands associates with increased gene expression in bipolar disorder and major depression. BMC Psychiatry, 16(1), 286.

D'Addario, C., et al. (2012). Selective DNA methylation of BDNF promoter in bipolar

disorder: differences among patients with BDI and BDII. Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology, 37(7), 1647–1655.

D'Addario, C., et al. (2018). Regulation of gene transcription in bipolar disorders: Role of DNA methylation in the relationship between prodynorphin and brain derived neurotrophic factor. Progress in Neuro-psychopharmacology & Biological Psychiatry, 82, 314–321.

Dell'Osso, B., et al. (2014). Epigenetic modulation of BDNF gene: Differences in DNA methylation between unipolar and bipolar patients. Journal of Affective Disorders, 166, 330–333.

Dempster, E.L., et al. (2011). Disease-associated epigenetic changes in monozygotic twins discordant for schizophrenia and bipolar disorder. Human Molecular Genetics, 20(24), 4786–4796.

Dong, E., et al. (2008). Clozapine and sulpiride but not haloperidol or olanzapine activate brain DNA demethylation. Proceedings of the National Academy of Sciences of the United States of America, 105(36), 13614–13619.

Dong, E., et al. (2016). Behavioral and molecular neuroepigenetic alterations in prenatally stressed mice: Relevance for the study of chromatin remodeling properties of antipsychotic drugs. Translational Psychiatry, 6(1), e711.

Dong, E., et al. (2019). N-phthalyl-l-tryptophan (RG108), like clozapine (CLO), induces chromatin remodeling in brains of prenatally stressed mice. Molecular Pharmacology, 95(1), 62–69.

Duffy, A., et al. (2019). Epigenetic markers in inflammation-related genes associated with mood disorder: A cross-sectional and longitudinal study in high-risk offspring of bipolar parents. International Journal of Bipolar Disorders, 7(1), 17.

Dwivedi, T., & Zhang, H. (2014). Lithium-induced neuroprotection is associated with epigenetic modification of specific BDNF gene promoter and altered expression of apoptotic-regulatory proteins. Frontiers in Neuroscience, 8, 457.

Fries, G.R., et al. (2014). Hypothalamic-pituitary-adrenal axis dysfunction and illness progression in bipolar disorder. The International Journal of Neuropsychopharmacology/Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP), 18(1), pyu043.

Fries, G.R., et al. (2016). The role of DNA methylation in the pathophysiology and treatment of bipolar disorder. Neuroscience and Biobehavioral Reviews, 68, 474–488.

Fries, G.R., et al. (2017). Integrated transcriptome and methylome analysis in youth at high risk for bipolar disorder: A preliminary analysis. Translational Psychiatry, 7(3), e1059.

Gaine, M.E., et al. (2019). Differentially methylated regions in bipolar disorder and suicide. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics, 180(7), 496–507.

Gardea-Resendez, M., et al. (2020). Dissecting the epigenetic changes induced by non-antipsychotic mood stabilizers on schizophrenia and affective disorders: A systematic review. Frontiers in Pharmacology, 11, 467.

Ghadirivasfi, M., et al. (2011). Hypomethylation of the serotonin receptor type-2A gene (HTR2A) at T102C polymorphic site in DNA derived from the saliva of patients with schizophrenia and bipolar disorder. American Journal of Medical Genetics. Part B, Neuropsychiatric Genetics: The Official Publication of the International Society of Psychiatric Genetics, 156b(5), 536–545.

Gouil, Q., & Keniry, A. (2019). Latest techniques to study DNA methylation. Essays in Biochemistry, 63(6), 639–648.

Grayson, D.R., & Guidotti, A. (2018). DNA methylation in animal models of psychosis. Progress in Molecular Biology and Translational Science, 157, 105–132.
Guo, J.U., et al. (2014). Distribution, recognition and regulation of non-CpG methylation in the adult mammalian brain. Nature Neuroscience, 17(2), 215–222.
He, Y., et al. (2018). Childhood adversity is associated with increased KITLG methylation in healthy individuals but not in bipolar disorder patients. Frontiers in Psychiatry, 9, 743.
Ho, A.M., et al. (2019). Genome-wide DNA methylomic differences between dorsolateral prefrontal and temporal pole cortices of bipolar disorder. Journal of Psychiatric Research, 117, 45–54.
Houtepen, L.C., et al. (2016). DNA methylation signatures of mood stabilizers and antipsychotics in bipolar disorder. Epigenomics, 8(2), 197–208.
Huzayyin, A.A., et al. (2014). Decreased global methylation in patients with bipolar disorder who respond to lithium. International Journal of Neuropsychopharmacology, 17(4), 561–569.
Jang, H.S., et al. (2017). CpG and non-CpG methylation in epigenetic gene regulation and brain function. Genes (Basel), 8(6), 148.
Jeremian, R., et al. (2017). Investigation of correlations between DNA methylation, suicidal behavior and aging. Bipolar Disorders, 19(1), 32–40.
Jia, Y.F., et al. (2017). Differential SLC1A2 promoter methylation in bipolar disorder with or without addiction. Frontiers in Cellular Neuroscience, 11, 217.
Judy, J.T., & Zandi, P.P. (2013). A review of potassium channels in bipolar disorder. Frontiers in Genetics, 4, 105.
Kaminsky, Z., et al. (2012). A multi-tissue analysis identifies HLA complex group 9 gene methylation differences in bipolar disorder. Molecular Psychiatry, 17(7), 728–740.
Kaminsky, Z., et al. (2015). DNA methylation and expression of KCNQ3 in bipolar disorder. Bipolar Disorders, 17(2), 150–159.
Kaushal, A., et al. (2017). Comparison of different cell type correction methods for genome-scale epigenetics studies. BMC Bioinformatics, 18(1), 216.
Kuratomi, G., et al. (2008). Aberrant DNA methylation associated with bipolar disorder identified from discordant monozygotic twins. Molecular Psychiatry, 13(4), 429–441.
Lee, R.S., et al. (2015). Search for common targets of lithium and valproic acid identifies novel epigenetic effects of lithium on the rat leptin receptor gene. Translational Psychiatry, 5(7), e600.
Li, S., et al. (2018). Hypomethylation of LINE-1 elements in schizophrenia and bipolar disorder. Journal of Psychiatric Research, 107, 68–72.
Li, S., et al. (2019a). Altered DNA methylation of the AluY subfamily in schizophrenia and bipolar disorder. Epigenomics, 11(6), 581–586.
Li, W., et al. (2019b). Association of SYNE1 locus with bipolar disorder in Chinese population. Hereditas, 156, 19.
Li, Y., et al. (2015). Genome-wide methylome analyses reveal novel epigenetic regulation patterns in schizophrenia and bipolar disorder. Biomed Research International, 2015, 201587.
Luoni, A., et al. (2016). Ankyrin-3 as a molecular marker of early-life stress and vulnerability to psychiatric disorders. Translational Psychiatry, 6(11), e943.
Mill, J., et al. (2008). Epigenomic profiling reveals DNA-methylation changes associated with major psychosis. The American Journal of Human Genetics, 82(3), 696–711.
Murata, Y., et al. (2020). Global DNA hypomethylation and its correlation to the betaine level in peripheral blood of patients with schizophrenia. Progress in

Neuropsychopharmacology & Biological Psychiatry, 99, 109855.
Nassan, M., et al. (2020). Methylation of brain derived neurotrophic factor (BDNF) Val66Met CpG site is associated with early onset bipolar disorder. Journal of Affective Disorders, 267, 96–102.
Nohesara, S., et al. (2011). DNA hypomethylation of MB-COMT promoter in the DNA derived from saliva in schizophrenia and bipolar disorder. Journal of Psychiatric Research, 45(11), 1432–1438.
Pal, M., et al. (2016). High precision DNA modification analysis of HCG9 in major psychosis. Schizophrenia Bulletin, 42(1), 170–177.
Perroud, N., et al. (2016). Methylation of serotonin receptor 3A in ADHD, borderline personality, and bipolar disorders: Link with severity of the disorders and childhood maltreatment. Depression and Anxiety, 33(1), 45–55.
Rao, J.S., et al. (2012). Epigenetic modifications in frontal cortex from Alzheimer's disease and bipolar disorder patients. Translational Psychiatry, 2(7), e132.
Rubin, L.H., et al. (2016). Sex and diagnosis specific associations between DNA methylation of the oxytocin receptor gene with emotion processing and temporal-limbic and prefrontal brain volumes in psychotic disorders. Biological Psychiatry: Cognitive Neuroscience and Neuroimaging, 1(2), 141–151.
Ruzicka, W.B., et al. (2018). Location matters: Distinct DNA methylation patterns in GABAergic interneuronal populations from separate microcircuits within the human hippocampus. Human Molecular Genetics, 27(2), 254–265.
Ruzicka, W.B., Subburaju, S., & Benes, F.M. (2015). Circuit- and diagnosis-specific DNA methylation changes at γ-aminobutyric acid-related genes in postmortem human hippocampus in schizophrenia and bipolar disorder. JAMA Psychiatry, 72(6), 541–551.
Ruzicka, W.B., Subburaju, S., & Benes, F.M. (2017). Variability of DNA methylation within schizophrenia risk loci across subregions of human hippocampus. Genes (Basel), 8(5), 143.
Sabunciyan, S., et al. (2015). Association of DNA methylation with acute mania and inflammatory markers. PLOS ONE, 10(7), e0132001.
Saito, T., et al. (2020). Effect of interaction between a specific subtype of child abuse and the FKBP5 rs1360780 SNP on DNA methylation among patients with bipolar disorder. Journal of Affective Disorders, 272, 417–422.
Schröter, K., et al. (2020). Longitudinal multi-level biomarker analysis of BDNF in major depression and bipolar disorder. European Archives of Psychiatry and Clinical Neuroscience, 270(2), 169–181.
Schür, R.R., et al. (2018). Glucocorticoid receptor exon 1(F) methylation and the cortisol stress response in health and disease. Psychoneuroendocrinology, 97, 182–189.
Scola, G., et al. (2014). Lithium reduces the effects of rotenone-induced complex I dysfunction on DNA methylation and hydroxymethylation in rat cortical primary neurons. Psychopharmacology (Berl), 231(21), 4189–4198.
Seidl, C., et al. (2010). Differential gene expression triggered by highly cytotoxic alpha-emitter-immunoconjugates in gastric cancer cells. Investigational New Drugs, 28(1), 49–60.
Soeiro-de-Souza, M.G., et al. (2013). Number of manic episodes is associated with elevated DNA oxidation in bipolar I disorder. The International Journal of Neuropsychopharmacology/Official Scientific Journal of the Collegium Internationale Neuropsychopharmacologicum (CINP), 16(7), 1505–1512.
Starnawska, A., et al. (2016a). CACNA1C hypermethylation is associated with bipolar disorder. Translational Psychiatry, 6(6), e831.
Starnawska, A., et al. (2016b). Hypomethylation of FAM63B in bipolar disorder

patients. Clinical Epigenetics, 8, 52.
Stenz, L., et al. (2015). BDNF promoter I methylation correlates between post-mortem human peripheral and brain tissues. Neuroscience Research, 91, 1–7.
Strauss, J.S., et al. (2013). Quantitative leukocyte BDNF promoter methylation analysis in bipolar disorder. International Journal of Bipolar Disorders, 1, 28.
Sugawara, H., et al. (2018). DNA methylation analyses of the candidate genes identified by a methylome-wide association study revealed common epigenetic alterations in schizophrenia and bipolar disorder. Psychiatry and Clinical Neurosciences, 72(4), 245–254.
Walker, R.M., et al. (2016). DNA methylation in a Scottish family multiply affected by bipolar disorder and major depressive disorder. Clinical Epigenetics, 8, 5.
Watkeys, O.J., et al. (2020). Derivation of poly-methylomic profile scores for schizophrenia. Progress in Neuropsychopharmacology & Biological Psychiatry, 101, 109925.
Xiao, Y., et al. (2014). The DNA methylome and transcriptome of different brain regions in schizophrenia and bipolar disorder. PLOS ONE, 9(4), e95875.
Zhao, H., et al. (2015). Genome-wide DNA methylome reveals the dysfunction of intronic microRNAs in major psychosis. BMC Medical Genomics, 8, 62.