



Drug Response-Related DNA Methylation Changes in Schizophrenia, Bipolar Disorder, and Major Depressive Disorder

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Pharmacotherapy is the most common treatment for schizophrenia (SCZ), bipolar disorder (BD), and major depressive disorder (MDD). Pharmacogenetic studies have achieved results with limited clinical utility. DNA methylation (DNAm), an epigenetic modification, has been proposed to be involved in both the pathology and drug treatment of these disorders. Emerging data indicates that DNAm could be used as a predictor of drug response for psychiatric disorders. In this study, we performed a systematic review to evaluate the reproducibility of published changes of drug response-related DNAm in SCZ, BD and MDD. A total of 37 publications were included. Since the studies involved patients of different treatment stages, we partitioned them into three groups based on their primary focuses: (1) medication-induced DNAm changes ($n = 8$); (2) the relationship between DNAm and clinical improvement ($n = 24$); and (3) comparison of DNAm status across different medications ($n = 14$). We found that only BDNF was consistent with the DNAm changes detected in four independent studies for MDD. It was positively correlated with clinical improvement in MDD. To develop better predictive DNAm factors for drug response, we also discussed future research strategies, including experimental, analytical procedures and statistical criteria. Our review shows promising possibilities for using BDNF DNAm as a predictor of antidepressant treatment response for MDD, while more pharmacoepigenetic studies are needed for treatments of various diseases. Future research should take advantage of a system-wide analysis with a strict and standard analytical procedure.

Keywords: pharmacoepigenetic, DNA methylation, drug response, schizophrenia, bipolar disorder, major depressive disorder

BACKGROUND AND MOTIVATION

Schizophrenia (SCZ), bipolar disorder (BD), and major depressive disorder (MDD) are severe psychiatric disorders, conferring lifelong disability (Hyman, 2012; GBD 2015 Disease and Injury Incidence and Prevalence Collaborators, 2016). A majority of patients with these psychiatric disorders receive medication as the first-line treatment (Moore et al., 2009; Pratt et al., 2017).

Therapeutics for SCZ, BD, and MDD are generally based on similar classes of molecules, targeting similar pathways, with distinct doses and proper combinations. However, drug selection is clinically subjective and treatment typically requires weeks of symptom evaluation to determine treatment efficacy. A large proportion of patients fail to respond to first-line drug treatment. For example, the Sequenced Treatment Alternatives to Relieve Depression trial (STAR*D) study reported that only 35% of patients remit after their primary antidepressant treatment (Rush et al., 2006; Katz et al., 2012). This illustrates the importance of developing biomarkers that can support decisions regarding optimal drug choice, and identify likely poor responders as quickly as possible. This emphasizes the need for a better biomarker-based stratification of patients that could facilitate treatment planning.

Pharmacogenetic approaches for guiding the treatment of psychiatric disorders has been a rapidly expanding area of research in the last two decades (Nelson et al., 2016). The main hypothesis of these studies was that genetic variants could predict the influences of drug treatment. Numerous pharmacogenetic studies have investigated the genetic contribution to the treatment response of these disorders (Baum et al., 2008; Brandl et al., 2016; Shi et al., 2017; Yu et al., 2018). Clinical practice for pharmacogenetic findings is emerging, as a few of the Food and Drug Administration (FDA) approved drug labels changed for SCZ and MDD. Although psychiatric disorders are thought to be highly heritable, gene-environment interactions are relevant, and results of pharmacogenetic studies have been inconsistent, limiting the clinical utilization of this approach.

Emerging evidence suggests that epigenetic marks could be used as a predictor of drug response for psychiatric disorders (Chan and Baylin, 2012; Heerboth et al., 2014). Dysregulation of epigenetic events can be pathological, and associated with SCZ, BD, and MDD (Mill et al., 2008; Sabunciyan et al., 2012; Guintivano et al., 2013; Chen et al., 2014; Liu et al., 2018). This implies that biological pathways and cellular processes are under the impact of epigenome status. Unlike genetics, epigenetic status is dynamic and could better reflect various environmental events during disease progress and drug treatment. Because of the reversibility of epigenetic events, we postulate that modulation of epigenetic regulators could be valuable for therapeutic potential. Pharmacoeigenetics may be more promising for guiding the treatment of psychiatric disorders than pharmacogenetics.

The recent advent of interest in pharmacoeigenetics of psychiatric disorders is an alternative research avenue. The epigenetic modification known as DNA methylation (DNAm), one of major epigenetic modifications, is the product of the interaction between genetic variants and environmental influence, and for this reason might be a better predictor of treatment outcomes (Kubota et al., 2012; Moore et al., 2013). Measurable in peripheral blood, alterations of DNAm have been reported to be involved in both the pathology and drug treatment of SCZ, BD, and MDD (Schroeder et al., 2012; Vialou et al., 2013; Jaffe et al., 2016). Drugs may exert their effects by reversing these DNAm deregulations. Moreover, some FDA-approved drugs, like clozapine and sulpiride, have demonstrated the ability to activate brain DNA demethylation (Dong et al., 2008). Therefore, DNAm

is a promising molecular approach to study mechanisms and prediction of drug response in psychiatric disorders (Goud Alladi et al., 2018; Lisoway et al., 2018). However, the reproducibility of those results, crucial for their clinical application, has not been thoroughly evaluated.

We systematically reviewed the literature on DNAm and drug response in SCZ, MDD, and BD to determine if epigenetic markers have produced any reproducible finding as predictors of drug response. In this review, we focused on studies providing information about the effect of medications on DNAm and the utility of DNAm as a predictor of drug treatment for major psychiatric disorders (Figure 1). First, we evaluated the reproducibility of reported changes of DNAm-related genes associated with drug response in each disorder. We also evaluated whether the results of candidate gene studies could be reproduced in genome-wide studies. Finally, we discuss limitations of existing studies and provide recommendations for future research.

METHODS

We systematically reviewed published studies that examined DNAm in relation to drug response in SCZ, BD, or MDD, using the PubMed, Web of Science, and MEDLINE with the following search builder: ((((((pharmacogenetic) OR pharmacogenomic) OR pharmacoeigenetic) OR drug response) OR treatment) OR drug efficiency) OR #DRUG#) AND (((DNA methylation) OR methylation) OR epigenetic) OR EWAS) AND (#DISORDER#). #DRUG# is the major drug for each disorder: antipsychotic, mood stabilizer, lithium, antidepressant, selective serotonin reuptake inhibitor (SSRI) and selective norepinephrine reuptake inhibitor (SNRI). #DISORDER# is one of the major psychiatric disorders: SCZ, BD and MDD. The inclusion criteria were the following: (1) published in English before April 2020; (2) assessed drug response on DNAm levels; (3) sample size larger than 10. Studies on animal and cell culture models were excluded. We discarded one study testing for drug-related co-methylation modules for BD, since it did not directly compare the efficacy of the individual drugs and was incompatible with the other studies. The literature selection followed the standard by Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (Liberati et al., 2009).

We included 37 publications about drug response-related DNAm in SCZ, BD, and MDD (Figure 2). Studies included SCZ (number of studies, $n = 7$), BD ($n = 6$), MDD ($n = 18$), and cross-disorder studies (BD and MDD, $n = 2$; SCZ and BD, $n = 4$). Since the studies involved patients of different treatment stages, we partitioned them into three groups based on their primary focuses (Figure 3): (1) medication-induced DNAm changes; (2) the relationship between DNAm and clinical improvement; and (3) comparison of DNAm status across different medications. To evaluate the reproducibility of published changes of DNAm-related genes associated with treatment response in psychiatric disorders, we examined the stability of the reported changes in each disorder. Here, we defined stability as the presence of significant results ($p < 0.05$) that were consistent in more than one independent study. We also evaluated whether the

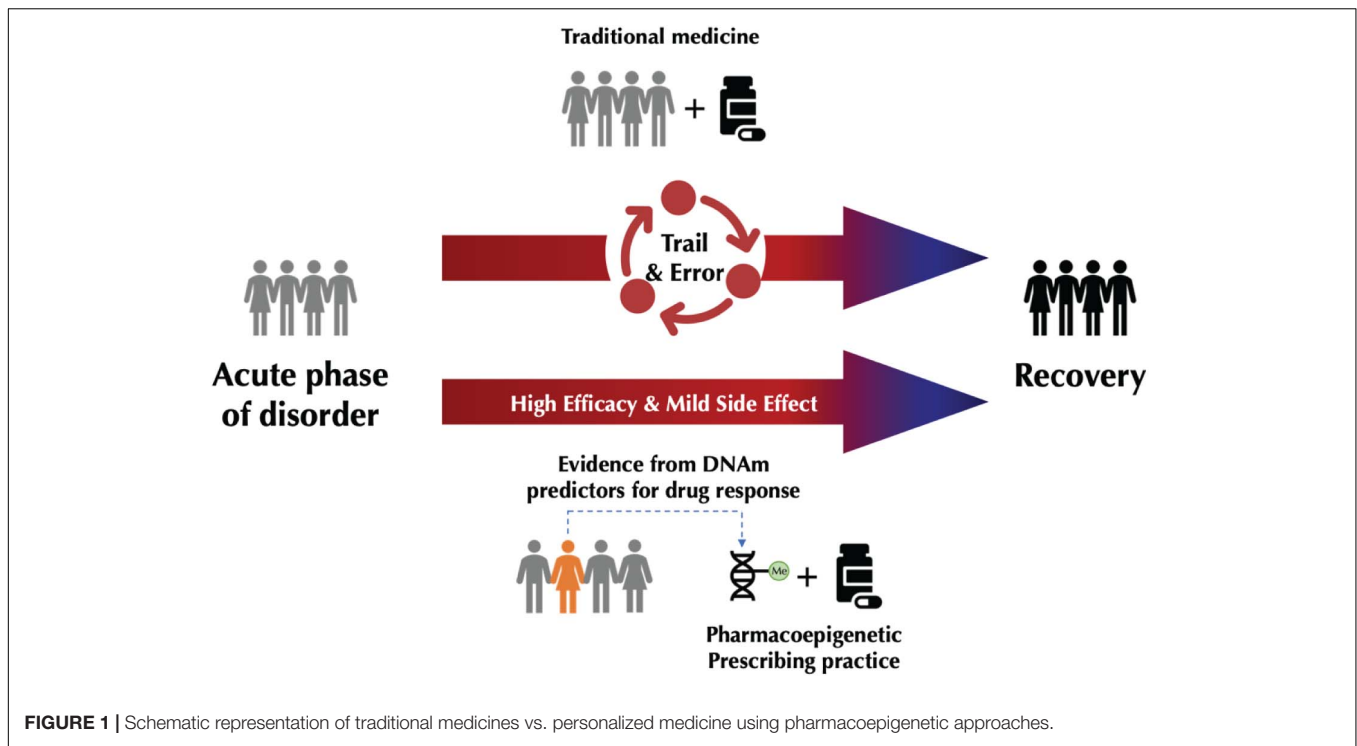


FIGURE 1 | Schematic representation of traditional medicines vs. personalized medicine using pharmacoeigenetic approaches.

results of candidate gene studies could be reproduced in genome-wide studies.

KEY CONCEPTS, DEFINITIONS, AND PRINCIPLES IN PHARMACOEPIGENETICS

Pharmacoeigenetics research in psychiatry focuses on the effects of treatments on DNAm and their potential influences on treatment response. The main aim of pharmacoeigenetics is the identification of DNAm of specific genes that are associated with treatment outcomes of psychiatric disorders, with the ultimate goal of translating this information into strategies that can support personalized medicine.

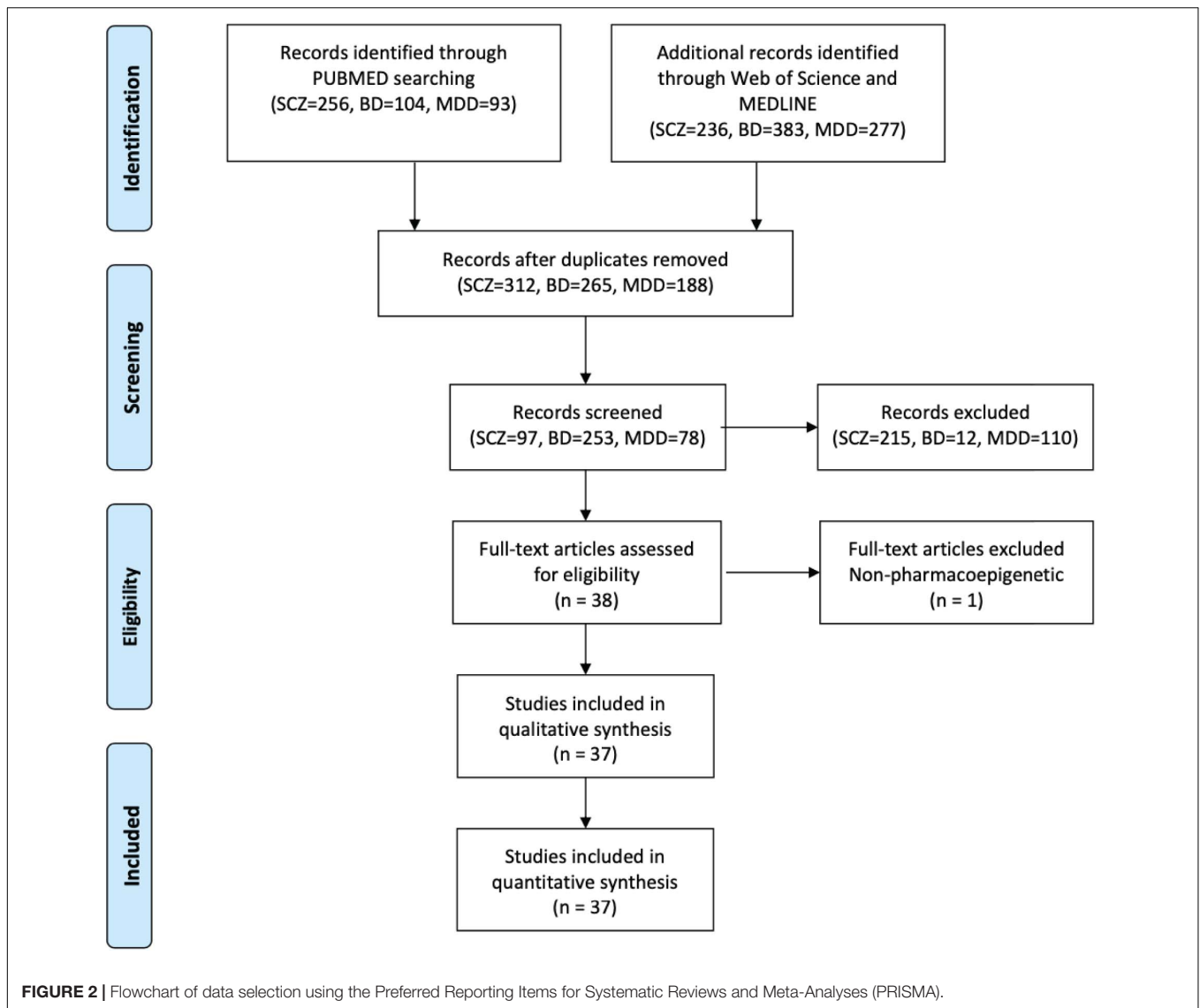
DNA Methylation

DNA methylation is the epigenetic modification by which methyl groups are added to DNA nucleotides, primarily cytosine, and adenine. DNAm is thought to have a prominent influence on the structure and functions of DNA, particularly in the regulation of gene expression. However, DNAm regulates gene expression differently according to its genomic context. Previous studies revealed that DNAm levels of CpG sites near the transcription start sites could repress gene expression levels, while DNAm levels of gene body CpGs could activate the expression of their target genes (Holliday and Pugh, 1975; Hellman and Chess, 2007; Jones, 2012; Moore et al., 2013). Conflicting results were reported regarding intergenic CpGs: some claimed that they might regulate gene expression less frequently (Chen et al., 2014; Wagner et al., 2014; Liu et al., 2018), while others suggested

that intergenic CpGs could repress the gene expression (Gaudet et al., 2004; Hutnick et al., 2010; Moore et al., 2013). Though CpGs are the primary sites for DNAm, it has also been observed at non-CpG sites (CpA, CpT, and CpC) in mammals (Pinney, 2014). Non-CpG methylation sites are common in several tissue types, including skeletal muscle (Yan et al., 2011) and brain (Lister et al., 2013; Guo et al., 2014). However, the functions of non-CpG methylation are still largely unknown and information on their function is just beginning to emerge. Our review includes only studies on the DNAm at CpG sites.

Tissues from postmortem samples, preserved clinical specimens from humans, can be used to study DNAm. For study pharmacoeigenetics for psychiatric disorders, peripheral tissues, such as blood, saliva, and buccal cells, are useful for developing clinically informative biomarkers. Psychiatric disorders are considered primarily a brain dysregulation. Therefore, we prefer to investigate genomic or epigenomic features in peripheral tissues that are similar to brain. Given that DNAm signals can be tissue-specific, it is important to establish the pros and cons of different types of tissue for investigation (Mill and Petronis, 2007).

Several studies investigated correlations of DNAm in blood and multiple regions of the brain, with inconsistent results (Davies et al., 2012; Horvath et al., 2012; Kaminsky et al., 2012; Masliah et al., 2013; Walton et al., 2016). Studies also indicated that DNAm in saliva and buccal cells were more informative surrogate tissues than blood (Lowe et al., 2013; Smith et al., 2015). To address this issue, Braun et al. (2019) performed genome-wide DNAm comparison across the human brain, blood, saliva and buccal cells. They proposed that to determine the optimal surrogate tissue for representing brain



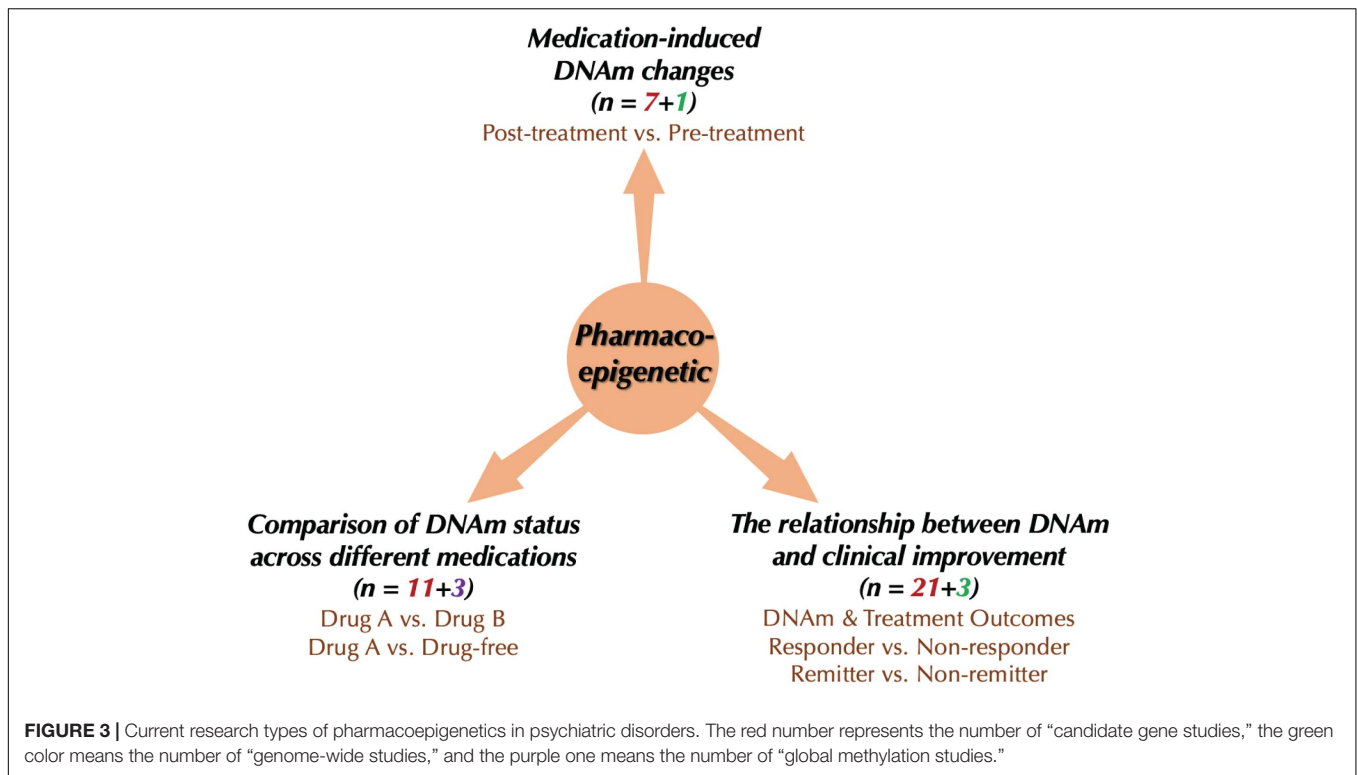
DNAm, the DNAm patterns specific to the genomic region of interest between these two tissues must be considered (Braun et al., 2019). More importantly, the proxy tissue should have similar responsive changes to environmental influences such as drugs. Building a reliable peripheral-brain relationship will be critical in proving the biological relevance and illustrating the underlying biological mechanisms of available *in vivo* human tissue for clinical application.

Laboratory techniques have been developed for measuring DNAm not only at global methylation levels but also individual nucleotide levels. In this review, the research strategies used to assess drug response-related DNAm included target analyses for candidate genes, the discovery of genome-wide DNAm with microarrays, and assessment for global methylation. Microarray-based technologies such as Illumina Infinium HumanMethylation27 BeadChip® Array (HM27K) (Bibikova et al., 2009), Infinium® HumanMethylation450 BeadChips (HM450K) (Dedeurwaerder et al., 2011, 2014) and Infinium®

MethylationEPIC BeadChip (EPIC) (Moran et al., 2016), have been widely used for methylation profiling. Methylated DNA immunoprecipitation sequencing (MeDIP), Reduced Representation Bisulfite Sequencing (RRBS) and others are also used for DNAm measurement (Song et al., 2005; Couldrey and Cave, 2014). Whole-genome bisulfite sequencing is the gold standard for measuring CpG and non-CpG methylation, but due to its cost, few published pharmacoeigenetic studies have used it.

Research Strategies

The most widely used approach for studying drug response-related DNAm is still a “candidate gene” method. We found 31 studies of 26 candidate genes completed in the three disorders of interest to us, and findings from 19 of them (73%) reached nominal significance ($p < 0.05$). These candidate gene studies only focus on a limited number of genes, based on their established association with a disorders’ pathogenesis or hypothesized relation to response to drug treatment. Although



previous candidate gene studies have identified target genes that may predict or reflect the clinical effect of drug response, most studies had limited statistical power, a concern especially for dense array data. Because of this limitation, the candidate gene method can provide little comprehensive understanding regarding the mechanism of action of drugs, but can only confirm previous knowledge or test relevance of a particular gene methylation (Harrison, 2015).

Along with next-generation sequencing, array-based analyses are commonly used in DNAm measurement to assess site-specific methylation in the genome. These genome-wide analyses offer a non-biased experimental approach to identify novel candidates (Kurdyukov and Bullock, 2016), but we found only 3 studies that that implemented genome-wide analysis, using microarrays to evaluate the drug response. These studies identified several loci associated with clinical improvement, almost all of which were not previously predicted to be relevant. Unfortunately, genome-wide DNAm measurement faces two challenges, first its cost (added to costs of a clinical trial) and second, the challenge of having large samples in a study of patients receiving controlled treatment regimens. These problems limit the size of data sets, and therefore statistical power, for array analysis of drug-related treatment effects. However, genome-wide approaches using a microarray do provide more comprehensive data and could identify novel and unexpected DNAm effects (Barros-Silva et al., 2018).

Additionally, three studies evaluated global DNAm in a specific tissue for a cross-medications comparison. Global DNAm reflects the DNAm status of total genomic content within a sample. Several methods exist to assess global DNAm status,

including enzyme-linked immunosorbent assays (ELISA), the use of a previously validated protocol of Linear Interspersed Nuclear Element 1 (LINE-1) and the use of restriction enzymes (Burghardt et al., 2020). However, this approach only assesses DNAm changes at the genome level, and thus does not further provide data on region-specific DNAm changes to characterize critical regulatory regions (e.g., CpG islands and promoter regions).

PHARMACOEPIGENETIC FINDINGS IN SCZ, BD, AND MDD

The included pharmacoeugenetic studies were divided into three types: (1) medication-induced DNAm changes; (2) relationships between DNAm and clinical improvement, and (3) comparison of DNAm status across different medications (Figure 3). In this section, we summarize recent findings from pharmacoeugenetic studies in SCZ, BD and MDD to evaluate the reproducibility of drug response-related DNAm changes in these disorders.

Medication-Induced DNAm Changes

The changes of DNAm after a period of drug treatment were examined in studies comparing DNAm changes between pre- and post-treatment, which could reflect the effects of drug treatment directly. There were eight studies (Table 1, SCZ = 2, BD = 1, and MDD = 5) that examined medication-induced DNAm changes, including seven that were candidate gene studies and one genome-wide study.

For candidate gene studies, there were 9 candidate genes included and only 5 reached nominal significance ($p < 0.05$).

TABLE 1 | summary of studies exploring medication-induced DNAm changes.

Studies	Genome Region analyzed	Sample size	Drugs (duration)	Tissue	Platform	Main results
Schizophrenia						
Venugopal et al., 2018	IL6 Promoter regions	40 SCZ	Co-medications (3 months)	Blood	BIMSA	<ul style="list-style-type: none"> • Increase of DNAm at IL6 promoter region after drug treatment ($p < 0.001$); • No significant differences in DNAm at IL6 promoter region between endpoint and controls ($p = 0.17$)
Kinoshita et al., 2017	Whole genome	21 SCZ	Clozapine (1 year)	Blood	HM450K	<ul style="list-style-type: none"> • 29134 CpG sites were differentially methylated after treatment without correction for multiple testing ($p < 0.05$)
Bipolar disorder						
Zhang et al., 2018	COMT&PPIEL Promoter regions	150 BD; 50 HC	Co-medications (18 months)	Blood	MS-PCR	<ul style="list-style-type: none"> • No significant differences in DNAm status at COMT and PPIEL promoter region after 12 months treatment compared with controls ($p < 0.05$)
Major depressive disorder						
Tadić et al., 2014	BDNF Exon IV promoter region	39 MDD	Monoaminergic drugs (6 weeks)	Leus	NA	<ul style="list-style-type: none"> • No significant differences in DNAm status at BDNF promoter region between pre- and post-treatment
Wang et al., 2018b	BDNF Promoter regions	44 MDD	Escitalopram* (8 weeks)	Blood	Illumina Hiseq	<ul style="list-style-type: none"> • Increase of DNAm at BDNF after escitalopram treatment ($p < 0.01$) • REMs: increase of DNAm after escitalopram treatment • NREMs: no significant differences in DNAm after escitalopram treatment
Okada et al., 2014	5-HTT CpG island at the 5' region	40 MDD	ADs (6 weeks)	Blood	MCS	<ul style="list-style-type: none"> • Increase of DNAm at CpG 3 of 5-HTT ($p = 0.0004$, Bonferroni set at $p < 0.0017$)
Kahl et al., 2016	GLUT1&GLUT4 Promoter regions	37 MDD	ADs (6 weeks)	Blood	Bisulfite sequencing	<ul style="list-style-type: none"> • REMs: decrease of DNAm at GLUT1 gene after treatment ($p < 0.05$) • NREMs: increase of DNAm at GLUT1 gene after treatment ($p < 0.05$) • No significant differences in GLUT4 between pre- and post-treatment
Wang et al., 2018a	HTR1A&HTR1B Promoter regions	44 MDD	Escitalopram* (8 weeks)	Blood	Illumina Hiseq	<ul style="list-style-type: none"> • No significant differences in average DNAm of HTR1A/1B after treatment • Increase of DNAm at 4 CpG sites in HTR1B after treatment ($p < 0.05$)

Bisulfite Sequencing DNA Methylation Analysis, BIMSA; Leukocytes, Leus; MassARRAY Compact System, MCS; Methylation-specific Polymerase Chain Reaction, MS-PCR; Healthy Control, HC; Remitters, REMs; Non-remitters, NREMs; Antidepressants, ADs.

*Monotherapy was permitted for co-medications with other psycho-pharmacological agents or medications for insomnia.

Of those five, only brain-derived neurotrophic factor (BDNF) was analyzed for MDD patients in 2 independent studies (Tadić et al., 2014; Wang et al., 2018b). A significant increase of DNAm in BDNF after 8 weeks of escitalopram treatment for 44 MDD patients based on blood samples was reported by Wang and collaborators (Wang et al., 2018b). The other study did not find significant DNAm

changes in leukocytes after 6 weeks of monoaminergic drug treatment for 39 MDD patients (Tadić et al., 2014). One explanation for this discrepancy is that escitalopram belongs to the SSRI class which has distinctly different mechanisms of action from monoaminergic drugs. The treatment duration and tissue types were also different between these two studies.

The genome-wide study identified 29,134 sites ($p < 0.05$) that were significantly differentially methylated after 1 year of treatment with clozapine for 21 SCZ patients based on blood samples, using HM450K assays (Kinoshita et al., 2017). This relatively small study has not been replicated to date. We further evaluated whether the results of candidate gene studies for antipsychotics treatment could be reproduced in genome-wide studies. We found none of those significant candidate genes tested reached a nominal significance in genome-wide analysis.

The Relationship Between DNAm and Clinical Improvement

More studies explored the relationship between DNAm levels at baseline and clinical improvement after a period of drug treatment. The most frequently used method to assess the clinical improvement is still the scale score (e.g., PANSS, HAM-D). Patients are separated into remission, non-remission, response or non-response based on changes of scale scores. Remission, the symptom of virtual absence of disease, is the goal of treatment (Paykel, 1998). Response is defined as a clinically meaningful reduction in symptoms (e.g., a reduction of at least 50% in baseline symptom levels) (Aaronson et al., 2017). In this section, there were 24 studies included (Table 2). Examining the correlation between baseline DNAm and treatment outcomes for patients, these studies address the question of whether pre-treatment DNAm features could predict clinical improvement.

Twenty-one candidate genes were evaluated and DNAm of 12 genes showed a significant correlation with clinical improvement ($p < 0.05$). Only BDNF (Carlberg et al., 2014; Tadić et al., 2014; Lieb et al., 2018; Wang et al., 2018b; Hsieh et al., 2019; Wagner et al., 2019), 5-HTT (Kang et al., 2013; Domschke et al., 2014; Okada et al., 2014; Iga et al., 2016), and HTR1B (Gassó et al., 2017; Wang et al., 2018a) were assessed in multiple MDD studies. No replication efforts were found in studies of other major psychiatric disorders. Baseline DNAm levels of BDNF in MDD patients in both blood and leukocytes was positively correlated with remission of depressive symptomatology after antidepressant treatment in 4 of 6 independent studies (Tadić et al., 2014; Lieb et al., 2018; Wang et al., 2018b; Hsieh et al., 2019).

Three studies used a genome-wide approach to evaluate the correlation between baseline DNAm and clinical improvement. Takeuchi et al. (2017) analyzed the changes of baseline DNAm between responders and non-responders of 20 paroxetine-treated MDD patients based on blood samples, using HM450K assays. Responders and non-responders had two CpG sites on genes PPFIA4 and HS3ST1 that were differently methylated ($q < 0.05$). Another study compared the DNAm changes in blood between responders and non-responders with 8 weeks of escitalopram treatment for 177 MDD patients, using the EPIC assays (Ju et al., 2019). They identified 303 ($p < 0.05$) sites with nominally significant differences between responders and non-responders, but none were significantly different after correction for multiple comparisons. Since these two drugs, paroxetine and escitalopram, are both SSRIs, we compared the reproducibility of the nominally significant results between these two studies. We found that none

of the significant loci found to be associated with paroxetine could be replicated in the escitalopram treatment analysis. This failure at replication has to be considered in the context of differences in the platforms used, limitations in statistical power, and the 303 escitalopram-related sites detected by EPIC analysis were not included in HM450K assays. In evaluating whether results of candidate gene studies for MDD could be reproduced in genome-wide studies, we did not detect any overlap.

We did find one study involving schizophrenia, which evaluated the correlation between DNAm changes in blood after 1-year of clozapine treatment and changes of PANSS scores for 21 SCZ patients (Kinoshita et al., 2017). They found that DNAm in a site located at the CREBBP gene was negatively correlated with clinical improvement after multiple testing correction. However, this finding remains to be replicated. Further analysis showed that none of these significant candidate genes tested for antipsychotics (e.g., HTR1A, CYP3A4, COMT), especially clozapine, reached a nominal significance in genome-wide analysis.

Comparison of DNAm Status Across Different Medications

Major medications for SCZ, BD, and MDD included antipsychotics, mood stabilizers and antidepressants. Antipsychotic medications are one of the primary treatments for the acute phases and the long-term prevention of recurrence for SCZ patients (Leucht et al., 2009; Hasan et al., 2017). Mood stabilizers, especially lithium, are a cornerstone in the long-term treatment of BD (Rybakowski, 2011; Geddes and Miklowitz, 2013). MDD patients are often treated with antidepressants (Drozda et al., 2014). However, the drug medications for each disorder are not mutually exclusive, as antipsychotic drugs may also be used in BD and MDD patients (Cruz et al., 2010; Patkar and Pae, 2013; Poo and Agius, 2014). The therapeutic efficacy of individual drugs is quite variable, and treatment trials to establish efficacy are laborious, so establishing biomarkers to improve guidance for treatment planning of individual patients is an important aim of psychiatric research. Predicting response to specific therapies could promote personalized medicine.

In this review, we evaluated 14 studies that compared DNAm after treatment with different medications, including 11 studies that used a candidate gene method and 3 that analyzed global methylation (Table 3). We found that antidepressant medication increased the DNAm status of promoter regions of the BDNF gene in both BD and MDD patients beyond the level seen in antidepressant-free patients. With advantages and disadvantages, it is worth noting that drug selection and treatment duration varied across studies.

CHALLENGES AND FUTURE DIRECTIONS

Underpowered Studies — Large Cohort Collaborations Are Needed

Insufficient statistical power is a major challenge for pharmacoeigenetic studies in psychiatric disorders. The

TABLE 2 | Summary of studies exploring the relationship between DNAm and clinical improvement.

Studies	Genome Region analyzed	Sample size	Drugs (duration)	Tissue	Platform	Main results
Schizophrenia						
Tang et al., 2014	HTR1A Promoter region	82 SCZ	Co-medications (10 weeks)	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> • DNAm at CpG 13 showed a positive correlation with changes in total PANSS scores ($p = 0.006$) and changes in negative factor ($p < 0.001$) • DNAm at CpG 13 was positively correlated with baseline negative factor ($p = 0.019$)
Shi et al., 2017	CYP3A4, CYP2D6, ABCB1, HTR2A, DRD2 Upstream of promoter region	288 SCZ	Risperidone* (4 weeks)	Blood	MassARRAY Analyzer 4	<p>RES vs. NRES:</p> <ul style="list-style-type: none"> • Decrease of DNAm at CpG_193 ($p = 0.0008$, $q = 0.012$), CpG_242:244:250 ($p = 0.000051$, $q = 0.00076$) and CpG_284 ($p = 0.0023$, $q = 0.034$) in CYP2D6 • Increase of DNAm at CpG_-367:-372:-374 ($p = 0.0018$, $q = 0.028$), CpG_-222 ($p = 0.012$, $q > 0.05$) and CpG_-243 ($p = 0.02$, $q > 0.05$) in CYP3A4 • Decrease of DNAm at CpG_-36 ($p = 0.000091$, $q = 0.0014$), CpG_-258 ($p = 0.000082$, $q = 0.0013$), CpG_-296 ($p = 0.000091$, $q = 0.0014$) in CYP3A4
Kinoshita et al., 2017	Whole genome	21 SCZ	Clozapine (1 year)	Blood	HM450K	<ul style="list-style-type: none"> • Increases in DNAm of the CREBBP (cg05151055, $p = 2.7 \times 10^{-7}$, $q < 0.05$) gene was significantly correlated with clinical improvements in treatment-resistant SCZ.
Nour El Huda et al., 2018	COMT CpG island of the 5' upstream	138 SCZ 132 HC	Co-medications (NA)	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> • DNAm of COMT was negatively correlated with excitement ($p < 0.001$) and depressed ($p = 0.001$) scores
Miura et al., 2018	ANKK1 -162C to +260C of the 5' region	34 SCZ	Aripiprazole*(6 weeks)	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> • RES vs. NRES: no differences in DNAm levels at overall CpG sites; hypermethylation at CpG site 387 ($p = 0.017$) • DNAm at CpG sites 387 was negatively correlated with changes in total PANSS ($p = 0.031$), positive ($p = 0.037$) and negative scores ($p = 0.039$)
Bipolar disorder						
Burghardt et al., 2018	AKT1, AKT2, AKT3 Promoter region	30 BD	AAPs or MSs at least 3 months	FSM	PCR + MS-HRM	<ul style="list-style-type: none"> • AAPs patients: AKT2 DNAm and HOMA-IR were positively correlated ($p = 0.3$) • MSs patients: AKT2 DNAm and HOMA-IR was negatively correlated ($p = 0.1$)
Major depressive disorder						
Kang et al., 2013	5-HTT Promoter region	108 MDD	Ads (12 weeks)	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> • CpG site 2 and the average DNAm of 5-HTT were negatively correlated with changes of HAM-D scores ($p < 0.05$) • CpG sites 1 was negatively correlated with changes of HAMA scores ($p < 0.05$); • average DNAm was negatively correlated with changes of SOFAS scores ($p < 0.05$)
Powell et al., 2013	IL11 CpG island	113 MDD	Escitalopram or Nortriptyline (12 weeks)	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> • DNAm of CpG unit 5 was negatively correlated with changes of MADRS scores ($p = 0.005$, $q = 0.055$); • Escitalopram group: CpG unit 4 was positively correlated with changes of MADRS scores ($p = 0.005$, $q = 0.055$); • Nortriptyline group: CpG unit 4 was negatively correlated with changes of MADRS scores ($p = 0.005$, $q = 0.055$)

(Continued)

TABLE 2 | Continued

Studies	Genome Region analyzed	Sample size	Drugs (duration)	Tissue	Platform	Main results
Tadić et al., 2014	BDNF Promoter region	39 MDD	Monoaminergic drugs (6 weeks)	Leus	NA	<ul style="list-style-type: none"> RES vs. NRES: higher DNAm at CpG-87 of BDNF ($p = 0.003$, $q = 0.03$)
Okada et al., 2014	5-HTT CpG island	50 MDD 50 HC	ADs (6 weeks)	Blood	MCS	<ul style="list-style-type: none"> DNAm of CpG 76 was positively correlated with total HAM-D scores ($p = 0.03$); DNAm of CpG 3 was positively correlated with improvement ratio (HAM-D, $p = 0.02$)
Domschke et al., 2014	5-HTT Upstream of exon 1A	94 MDD	Escitalopram*(6 weeks)	Blood	Bisulfite sequencing	<ul style="list-style-type: none"> CpG 1 ($p = 0.048$), CpG 2 ($p = 0.002$, $q < = 0.05$), CpG 4 ($p = 0.029$) and average DNAm ($p = 0.005$, $q < = 0.05$) of 5-HTT were positively correlated with changes of HAMD-21 scores
Domschke et al., 2015	MAO-A Promoter/exon1/intron1 regions	94 MDD	Escitalopram* (6 weeks)	Blood	Bisulfite sequencing	<ul style="list-style-type: none"> Females: DNAm of CpG 1 ($p = 0.04$) and CpG 5 ($p = 0.009$) were positively correlated with changes of HAMD-21 scores; while average DNAm across all CpGs showed no association with treatment response Males: no significant associations detected
Kahl et al., 2016	GLUT1/4 Promoter region	37 MDD	ADs (6 weeks)	Blood	Bisulfite sequencing	<ul style="list-style-type: none"> REMs vs. NREMs: hypomethylation of the average DNAm of GLUT1 ($p < 0.001$); No significant differences were observed for GLUT4 DNAm.
Iga et al., 2016	5-HTT Promoter region relate to TSS	28 MDD; 29 HC	ADs (8 weeks)	Leus	Bisulfite conversion + PCR	<ul style="list-style-type: none"> DNAm of CpG 3 ($p = 0.003$) and CpG 5 ($p = 0.004$) were negatively correlated with baseline HAMD-17 scores DNAm of CpG 2 ($p = 0.04$) was negatively correlated with clinical improvement as assessed with HAMD-17
Gassó et al., 2017	HTR1B Promoter region	57 MDD	Fluoxetine*(12 weeks)	Blood	Pyrosequencing	<ul style="list-style-type: none"> A negative correlation was found between the average DNAm level and GAF/CGAS changes ($p = 0.004$)
Lieb et al., 2018	BDNF Exon IV or P11 promoter	561 MDD	Escitalopram*(8 weeks)	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> DNAm status at CpG-87 of promoter exon IV ($p = 0.029$) was positively correlated with remission of the depressive symptomatology, especially in severe MDD patients ($n = 199$, $p = 0.031$)
Wang et al., 2018a	HTR1A/B promoter region	85 MDD	Escitalopram* (8 weeks)	Blood	Illumina Hiseq	<p>REM VS. NREM:</p> <ul style="list-style-type: none"> Hypermethylation of CpG 668 (HTR1A, $p = 0.025$) and CpG 1401 (HTR1B, $p = 0.033$); Higher DNAm changes after treatment in CpG 2793 ($p = 0.015$), CpG 2834 ($p = 0.002$), CpG 2927 ($p = 0.023$) and CpG 2937 ($p = 0.003$) of HTR1A and CpG-100 ($p = 0.021$) and CpG 1401 ($p = 0.029$) of HTR1B, compared with NREMs
Wang et al., 2018b	BDNF Promoter region	85 MDD	Escitalopram* (8 weeks)	Blood	Illumina Hiseq	<ul style="list-style-type: none"> REM vs. NREM: hypermethylation of amplicon BDNF_1 ($p = 0.006$), BDNF_3 ($p = 0.016$), BDNF_4 ($p = 0.029$) and BDNF_5 ($p = 0.036$); BDNF_1 ($p = 0.004$), BDNF_3 ($p = 0.013$), BDNF_4 ($p = 0.034$), BDNF_5 ($p = 0.027$) and average ($p = 0.038$) DNAm of BDNF was positively correlated with HAMD-17 changes

(Continued)

TABLE 2 | Continued

Studies	Genome Region analyzed	Sample size	Drugs (duration)	Tissue	Platform	Main results
Wagner et al., 2019	BDNF Exon IV and P11 promoter	110 MDD	Escitalopram* (8 weeks)	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> The BDNF exon IV promoter and P11 gene methylation did not predict a normalization of executive dysfunctions
Hsieh et al., 2019	BDNF Exon IX promoter	36 MDD	SSRIs (4 weeks)	Blood	Pyrosequencing	<ul style="list-style-type: none"> RES vs. NRES: hypermethylation of CpG 24 ($p = 0.029$) and CpG 324 ($p = 0.031$) of BDNF promoter region
Draganov et al., 2019	IL6, IL6R, IL1- β CpG island relative to 5' regulatory region	153 MDD	SSRIs (NA)	Blood	Bisulfite-pyrosequencing	<ul style="list-style-type: none"> RES vs. NRES: hypermethylation of CpG IL6R_4 ($p = 0.05$)
Takeuchi et al., 2017	Whole genome	20 MDD	Paroxetine* (6 weeks)	Blood	HM450K	<ul style="list-style-type: none"> BR vs. WR: 218 sites were nominally significant ($p < 0.05$), and 2 sites (PP1A4 and HS3ST1) were significantly hypomethylated after FDR correction ($q < 0.05$).
Ju et al., 2019	Whole genome	177 MDD	Escitalopram (8 weeks)	Blood	EPIC	<ul style="list-style-type: none"> RES vs. NRES: 303 sites were nominally significant ($p < 0.05$, $AP \geq \pm 2\%$)
Bipolar disorder & Major depressive disorder						
Carlberg et al., 2014	BDNF Exon I promoter	207 MDD; 59 BD; 278 HC	ADs (NA)	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> No significant correlations were found for DNAm levels and the total BDI sum score (available from $n = 81$ MDD)

Maudsley Staging Method, MSM; Responder, RES; Non-responder, NRES; Antipsychotics, APs; Typical Antipsychotics, TAPs; Atypical Antipsychotics, AAPs; Mood Stabilizers, MS; Valproate, VPA; Lithium, Li; Fasting skeletal muscle, FSM.

*Monotherapy was permitted for co-medications with other psycho-pharmacological agents or medications for insomnia.

cost of using methylation panels, the high cost of clinical trials where drug therapy is controlled along with diverse drugs used across studies, and the large number of sites for methylation investigation, together conspire to limit sample sizes and statistical power to detect effects of interest. These factors limit our ability to evaluate reproducibility of current pharmacoeigenetic findings. Although several interesting associations between DNAm and drug treatment outcomes occurred in relatively small sample sizes, findings from these studies should be considered preliminary until replication in a larger sample size is pursued. So, given that collecting clinical data with a large-sample is challenging and costly, small-sample studies remain the focus of most investigators in this area via hypothesis-generating studies.

To fill this gap, multi-site collaborations with ethnically diverse groups are needed in studies of the pharmacoeigenetics of psychiatric disorders. Prominent consortia have been established which looked at pharmacogenomics in psychiatric disorders, like the STAR*D project (Rush et al., 2004), the Genome-based Therapeutic Drugs for Depression (GENDEP) project (Uher et al., 2010), the Chinese Antipsychotics Pharmacogenomics Consortium (CAPC) (Yu et al., 2018), and others. These consortia benefit ongoing and future pharmacoeigenetic studies by providing preliminary findings that can be examined in new samples. For example, Powell et al. (2013) found that DNAm in IL11 could predict the clinical response to antidepressants using samples from the GENDEP project. Large international consortia, as have been developed for genetic association studies, are needed to study adequate numbers of patients with samples collected using a uniform procedure and with standardized drug treatment.

Recommendation for Clinical Design: Drug Selection, Treatment Duration, Evaluation, and Tissue Selection for Pharmacoeigenetic Studies

Neuropsychiatric drugs are grouped into various classes with slightly different mechanisms of action. It is clear that from an efficacy perspective, first line treatments for all these disorders leaves considerable room for improvement, potentially in new drug development and in strategies for personalized medicine (Leucht et al., 2012; Cipriani et al., 2018; Huhn et al., 2019; Pillinger et al., 2020). Identification of new biomarkers for these disorders is difficult, primarily because of the lack of knowledge about disease pathophysiology and mechanisms of drug action.

A major aim of psychopharmacology research is to develop valid strategies for selecting the right drug with the right dose for the “right” patients to optimize outcomes with low risks of side effects and relapse. Prior work is limited in supporting progress in this area. Across the published studies in this area, the types of medications were not uniform for each disorder, making comparison of studies difficult. Only 8% of studies used monotherapy for patients. While 27% of studies claimed to use monotherapy for patients, but we noticed that besides the same

TABLE 3 | Summary of studies exploring comparison of DNAm status across different medications.

Studies	Genome Region analyzed	Sample size	treatment duration	Tissue	Platform	Main results
Schizophrenia						
Melas et al., 2012	Global methylation	129 SCZ; 171 HC	NA	Blood	LUMA	<ul style="list-style-type: none"> Haloperidol vs. other APs: higher (control-like) global DNAm ($p < 0.001$)
Nour El Huda et al., 2018	COMT CpG islands	138 SCZ; 132 HC	NA	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> AAP vs. TAP: lower DNAm status ($p = 0.004$); AAP vs. Sulpiride: lower DNAm status ($p = 0.004$) Risperidone vs. TAP: lower DNAm status ($p = 0.049$)
Bipolar disorder						
D'Addario et al., 2012	BDNF Promoter region	49 BD I; 45 BD II; 52 HC	at least 1 months	PBMCs	Bisulfite conversion + RT-qPCR	<ul style="list-style-type: none"> AD vs. AD-free: higher DNAm levels ($p < 0.01$, AD-free had a control-like DNAm levels); Li vs. other drugs: lower (control-like) DNAm levels ($p < 0.05$) VPA vs. other drugs: lower (control-like) DNAm levels ($p < 0.05$)
Backlund et al., 2015	Global methylation	61 BD; 26 HC	at least 3 months	Blood	ELISA	<ul style="list-style-type: none"> Li vs. HC: lower DNAm levels ($p = 0.036$) Li + VPA vs. Li: higher DNAm levels ($p = 0.011$); Li + AP vs. Li: higher DNAm levels ($p = 0.071$)
Burghardt et al., 2018	AKT1&AKT2 &AKT3 Promoter region	30 BD	at least 3 months	FSM	PCR + MS-HRM	<ul style="list-style-type: none"> AAP vs. MSs: increase of AKT1 and AKT2 DNAm ($p = 0.03$; $p = 0.02$)
D'Addario et al., 2018	PDYN Promoter region	54 BD I; 45 BD II; 41 HC	at least 1 months	PBMCs	Bisulfite conversion + RT-qPCR	<ul style="list-style-type: none"> Li or VPA vs. other drugs: lower (control-like) DNAm levels ($p < 0.05$)
Burghardt et al., 2019	Global methylation	28 BD; 13 HC	at least 3 months	FSM	LINE1	<ul style="list-style-type: none"> AAP vs. HC: higher DNAm levels ($p = 0.0004$, $q = 0.0048$) MSs vs. HC: higher DNAm levels ($p = 0.12$) AAP vs. HC: higher DNAm levels ($p = 0.0156$, $q = 0.037$)
Major depressive disorder						
Ryan et al., 2017	IL6 Promoter region	92 MDD; 288 HC	4 waves of follow-up	Buccal swabs	SEQUENOM MassARRAY	<ul style="list-style-type: none"> AD use was associated with a mean 4.6% increase in CpG2 DNAm of IL6 ($p = 0.017$)
Schizophrenia & Bipolar disorder						
Abdolmaleky et al., 2006	MB-COMT promoter region	40 SCZ; 35 BD; 40 HC	life times	Post-mortem brain	qMSP	<ul style="list-style-type: none"> AP vs. drug-free: no differences VPA did not specifically alter DNAm status
Abdolmaleky et al., 2011	HTR2A promoter region	35 SCZ; 35 BD; 35 HC	life times	Post-mortem brain	qMSP	<ul style="list-style-type: none"> AP vs. drug-free: lower DNAm levels ($p = 0.012$) drug-free vs. HC: higher DNAm levels in BD and SCZ and BD combined ($p = 0.002$ and $p = 0.0027$, respectively) AAP vs. TAP: no differences
Abdolmaleky et al., 2014	5-HTT promoter region	35 SCZ; 35 BD; 35 HC	life times	Saliva; Post-mortem brain	qMSP	<ul style="list-style-type: none"> AP vs. HC: no differences in both saliva and brain drug-free vs. AP: higher DNAm levels in brain ($p = 0.038$) drug-free vs. HC: higher DNAm levels in brain ($p = 0.04$)

(Continued)

TABLE 3 | Continued

Studies	Genome Region analyzed	Sample size	treatment duration	Tissue	Platform	Main results
Abdolmaleky et al., 2015	DTNBP1 Promoter flanking SP1 binding site	35 SCZ; 35 BD; 35 HC	life times	Saliva; Post-mortem brain	qMSP	<ul style="list-style-type: none"> • Drug-free vs. AP: higher DNAm status in BD ($p = 0.045$) • CAP vs. AAP + CAP: lower DNAm status in SCZ ($p = 0.037$) • CAP vs. AAP + CAP and AAP combined: decrease of DNAm status in SCZ ($p = 0.017$)
Bipolar disorder & Major depressive disorder						
Dell'Osso et al., 2014	BDNF Exon I promoter	43 MDD; 111 BD; 44 HC	NA	Blood	Bisulfite conversion + PCR	<ul style="list-style-type: none"> • Li or VPA vs. other drugs: tended to decrease (control-like) DNAm levels (no.sig)
Carlberg et al., 2014	BDNF Exon I promoter	207 MDD; 59 BD; 278 HC	NA	Blood	MS-qPCR	<ul style="list-style-type: none"> • For 165 MDD: • AD vs. AD-free: higher DNAm levels ($p = 0.0019$) • AD vs. HC: higher DNAm levels ($p < 0.0001$) • AD-free vs. HC: no differences

Luminometric Methylation Assay, LUMA; Methylation-sensitive High-resolution Melting, MS-HRM; Linear Interspersed Nuclear Element 1, LINE1; Enzyme-linked Immunosorbant Assay, ELISA; Antipsychotics, APs; Atypical Antipsychotics, AAPs; Typical Antipsychotics, TAPs; Classic Antipsychotics, CAPs; Mood Stabilizers, MSs; Valproate, VPA; Lithium, Li; Fasting skeletal muscle, FSM; Peripheral blood mononuclear cells, PBMCs; Quantitative methylation specific PCR, qMSP.

primary medication, they temporarily permitted secondary medications (e.g., benzodiazepines, flunitrazepam) for insomnia or some side effects, which themselves can also exert epigenetic effects.

Choice of duration for drug treatment trials is also important, yet variable across prior work. Treatment-resistance, non-adherence, late-emerging side effects and relapse are frequent events in clinical practice, some of which are seen only in longer term protocol participation. Aside from these factors, DNAm status is dynamic and can be affected by various environmental events during treatment that are unrelated to drug effects (Bjornsson et al., 2008).

Definition of treatment outcomes for psychiatric disorders is also a challenge, as different approaches may be used across studies. A recent study suggested that integrating genomics and phenotypic measures data could increase the accuracy of prediction of drug response (Kauppi et al., 2018). Thus, future research should consider an integrated examination of genetic, DNAm and dense phenotype assessment of drug effects not only with behavioral ratings but with direct *in vivo* assessment of brain physiology and treatment (Huang et al., 2019). Notably, all of these prediction models need to be covered across the relevant age span, ethnic groups, and sexes.

Predictors of drug response for psychiatric disorders ideally need to have biological relevance based on a solid mechanistic understanding of the pathophysiology of brain dysfunction. Most of the pharmacoeigenetic studies utilized clinically accessible tissues such as blood, saliva, and others. However, whether biomarkers identified in these tissues could reflect similar DNAm changes in the brain needs further study. Several brain banks with post-mortem tissue from patients that had neuropsychiatric disorders have been established,

which generated big data sets at multiple regulatory levels (e.g., DNAm, gene expression, and other omics) (Wang et al., 2019). These multidimensional data sets could facilitate the exploration of the peripheral-brain relationship. Since epigenetic modifications are known to fluctuate over the lifespan, resolving the causal relationships in the epigenetic study of drug response using postmortem brains will be challenging. Cellular models will likely fill the gaps where both peripheral tissue and postmortem brain will not be able to deliver the answers.

System-Wide Analysis Could Benefit Pharmacoeigenetic Studies

Candidate gene studies contributed to answering specific questions in biology, but they do not provide system-wide information. In this review, we noticed that the assessed candidate genes were generally not the top signals in genome-wide studies, and could not even reach a genome-wide significance. The genes that change the most between pre- and post-treatment, or are most strongly associated with clinical improvement, remain to be discovered. Also, pathway-based research rather than gene-based approaches may be more efficient. Different genes that converge on the same pathway may each contribute modestly, but together may robustly co-influence the functional abnormality. Therefore, system-wide research could provide better biomarkers than individual candidate genes.

Regulation of drug responses occurs at various levels including genetics, epigenetics, transcriptional, and protein modification, and also involves many functional pathways (Amare et al., 2017). To date, the underlying system of drug response-related

DNAm remains poorly understood. Some have proposed that one or more components involved in a pathway, even if not risk genes, may be a better drug target than risk genes themselves (Wang et al., 2010; Harrison, 2015). One study assessed the interaction between genetic variates with DNAm for the IL-11 gene, which identified a possible regulatory relationship that could be used to predict antidepressant response for MDD patients (Powell et al., 2013). However, studies to date only scratched the surface of the complex regulatory system of drug response. Systematic integration of genomic data and genome-wide DNAm data will be more effective and unbiased for learning about these regulatory processes, hopefully leading to the discovery of underlying drug response networks.

Data Analysis for Pharmacoeigenetic Studies

Strict statistical criteria and sufficient attention to covariances are critical for reducing false-positive rates. DNAm status is not a constant like DNA sequence are therefore is subject to influences from more confounders. Further, approaches for DNAm assessment can be easily confounded by technical artifacts. DNAm levels are also affected by factors including age (Horvath, 2013; Jaffe et al., 2016; Merid et al., 2020), sex (Maschietto et al., 2017; Xia et al., 2019), circadian rhythms (Lim et al., 2014), smoking (Shenker et al., 2013), drinking (Philibert et al., 2012), and others. All of these considerations for study design and data analysis need to meet standard requirements for drug response-related DNAm studies as in most genomic studies.

Methylation profiling could be easily confounded by batch effects. Systematic error can be introduced when samples are processed in multiple batches (e.g., the same sample measured at different times), which cannot be eliminated unless all samples are run in a single batch (Chen et al., 2011). It is especially important that cases and controls not be put in separate batches. Except for batch effects, positional effects also exist in the microarray and bias analysis (Jiao et al., 2018). Positional effects are emerging when the same sample in different physical positions on the array and could bias methylation levels and lead to false findings. From our review, we observed that only one study did the batch effects correction (Ju et al., 2019), and none of these studies corrected for positional effects according to the method description in those papers. We cannot rule out the possibility that the data were properly processed but failed to be reported in the papers, but not reporting such details at least indicated the lack of attention to the serious issues.

Considering the influence of demographic information (e.g., age, sex, BMI) is also important. In this review, nearly 49% of studies did not control for covariates in their analyses. When there was a correction for covariates, typically only sex and age was controlled. Since DNAm is highly cell-type-specific, cellular heterogeneity of blood may skew DNAm patterns, influencing findings in drug response for psychiatric disorders (You et al., 2020). All studies did not control cell-type

compositions. Collecting data about potentially useful covariates, and controlling for them in analyses, should be standard practice in future research for drug response studies. However, we note that large samples will be needed to develop appropriate modeling for covariate effects, as a few outliers or complex covariate interactions can exert effects that are challenging to deal with in smaller sample studies.

Additionally, strict and consistent statistical criteria for defining significance need to be applied that correct for multiple comparisons. Most published studies considered a nominal $p < 0.05$ to be the threshold for statistical significance, even when multiple genes or CpG sites were tested. We noticed that only 59% of the previous studies did the correction for multiple testing. False-positive results likely occurred in published work (Joerber et al., 2012).

Summary

In this review, we found that only DNAm of BDNF was consistently related to clinical outcomes, an effect observed in four independent studies of MDD patients. Although pharmacoeigenetic studies to date are still limited in number and sample size, preliminary studies to date that evaluated changes in drug response-related DNAm across major psychiatric disorders provide evidence for an intriguing possibility of predictive DNAm biomarkers for drug treatment. A system-wide study using strict analytical and statistical procedures in large multisite studies may best advance identification of useful DNAm predictors for drug response in psychiatric disorders.

AUTHOR CONTRIBUTIONS

JZ, ML, XW, and YH did the manuscript collection. JZ did the manuscript writing. JZ and ML designed and generated the manuscript figures and tables. YX, JS, and RK helped with manuscript writing. CC and CL helped with study design and manuscript writing. All authors contributed to the article and approved the submitted version.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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