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Laura M. Hack, *Emory University*
Gabriel R. Fries, *University of Texas Health Science Center*
Harris A. Eyre, *Stanford University*
Chad A. Bousman, *University of Calgary*
Ajeet B. Singh, *Deakin University*
Joa Quevedo, *University of Texas Health Science Center*
Vineeth P. John, *University of Texas Health Science Center*
Bernhard T. Baune, *University of Melbourne*
Boadie Dunlop, *Emory University*

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Laura M. Hack1,2,3, Gabriel R. Fries4, Harris A. Eyre2,5,6,7, Chad A. Bousman8, Ajeet B. Singh6, Joao Quevedo4, Vineeth P. John4, Bernhard T. Baune7, Boadie W. Dunlop1

1Department of Psychiatry and Behavioral Sciences, School of Medicine, Emory University, Atlanta, GA, USA
2Department of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Palo Alto, California, USA
3Sierra Pacific Mental Illness Research Education and Clinical Centers, VA Palo Alto Health Care System, Palo Alto, California, USA
4Department of Psychiatry and Behavioral Sciences, McGovern Medical School, University of Texas Health Science Center at Houston (UTHealth), Houston, TX, USA
5Innovation Institute, Texas Medical Center, Houston, TX, USA
6IMPACT SRC, School of Medicine, Deakin University, Geelong, Victoria, Australia
7Department of Psychiatry, University of Melbourne, Melbourne, Victoria, Australia
8Departments of Medical Genetics, Psychiatry, Physiology & Pharmacology, University of Calgary, Calgary, AB, Canada

Abstract

**Background:** Major depressive disorder (MDD) is a leading cause of disability worldwide, and over half of patients do not achieve symptom remission following an initial antidepressant course. Despite evidence implicating a strong genetic basis for the pathophysiology of MDD, there are no adequately validated biomarkers of treatment response routinely used in clinical practice. Pharmacoepigenetics is an emerging field that has the potential to combine both genetic and environmental information into treatment selection and further the goal of precision psychiatry. However, this field is in its infancy compared to the more established pharmacogenetics approaches.

Corresponding author: Laura M. Hack, Department of Psychiatry and Behavioral Sciences Stanford University School of Medicine 401 Quarry Road, Palo Alto, CA 94305, lhack@stanford.edu.

Contributors: LMH, BWD, and HAE conducted the literature review and LMH wrote the original draft of the manuscript. GRF, HAE, CAB, ABS, JQ, VPJ, BTB, and BWD provided critical feedback and editing of the manuscript. All authors approved of the final submitted version.

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Conflict of Interest: GRF, VPJ, and JQ have no conflicts of interest to declare.
**Methods:** We prepared a narrative review using literature searches of studies in English pertaining to pharmacoepigenetics and treatment of depressive disorders conducted in PubMed, Google Scholar, PsychINFO, and Ovid Medicine from inception through January 2019. We reviewed studies of DNA methylation and histone modifications in both humans and animal models of depression.

**Results:** Emerging evidence from human and animal work suggests a key role for epigenetic marks, including DNA methylation and histone modifications, in the prediction of antidepressant response. The challenges of heterogeneity of patient characteristics and loci studied as well as lack of replication that have impacted the field of pharmacogenetics also pose challenges to the development of pharmacoepigenetic tools. Additionally, given the tissue specific nature of epigenetic marks as well as their susceptibility to change in response to environmental factors and aging, pharmacoepigenetic tools face additional challenges to their development.

**Limitations:** This is a narrative and not systematic review of the literature on the pharmacoepigenetics of antidepressant response. We highlight key studies pertaining to pharmacoepigenetics and treatment of depressive disorders in humans and depressive-like behaviors in animal models, regardless of sample size or methodology. While we discuss DNA methylation and histone modifications, we do not cover microRNAs, which have been reviewed elsewhere recently.

**Conclusions:** Utilization of genome-wide approaches and reproducible epigenetic assays, careful selection of the tissue assessed, and integration of genetic and clinical information into pharmacoepigenetic tools will improve the likelihood of developing clinically useful tests.

**Keywords**
Pharmacoepigenetics; Depression; Antidepressants; Decision Support Tools (DSTs); DNA methylation; Histones

1. **Introduction**

Despite a large body of research examining clinical and biological differences among individuals with major depressive disorder (MDD), findings from this work have not yet produced tools to reliably enhance treatment selection. When opting for a medication approach to treating MDD, clinicians’ choices are largely guided by potential side effects, past treatment responses, and clinical characteristics, such as the presence of comorbid anxiety, psychosis, trauma, or substance misuse. However, there is limited replicated evidence showing these characteristics to be robust moderators of antidepressant efficacy (Dunlop, 2015). Evidence-based moderators that some psychiatrists incorporate into antidepressant selection include the presence of melancholic features, which may preferentially respond to tricyclic antidepressants (TCAs) (Perry, 1996), and atypical features, which may respond better to monoamine oxidase inhibitors (MAOIs) than TCAs (Quitkin et al., 1991; Quitkin et al., 1990). Psychotic depression is the only clinical subtype that has strong evidence for a particular prescriptive pathway; that is, the combination of an antidepressant and an antipsychotic (Malhi et al., 2018).
Unguided selection of antidepressants produces response rates of only 50–60% and even lower remission rates for a single treatment course (Papakostas and Fava, 2009; Rush et al., 2006). Failure to achieve remission results in prolonged suffering, greater impairment in psychosocial functioning, and higher levels of health care use. The trial and error approach to treatment selection also suffers from high rates of side effects, with approximately 55% of patients experiencing at least one bothersome side effect from antidepressant treatment (Papakostas, 2008). While successive trial and error treatment trials appear to boost cumulative remission rates to 67% (Rush et al., 2006), the prolonged process to achieve remission leaves patients suffering from illness morbidity and elevates the risk of disengaging from care.

More recent work focusing on biological predictors, including neuroimaging, neurophysiological, neuroendocrine, and genetic measures has identified potential alternative approaches to treatment selection (Busch and Menke, 2018; Fonseka et al., 2018; Olbrich and Arns, 2013; Williams, 2017). The field of pharmacogenetics (PGx) developed with the idea that specific genetic variants can inform decisions about medication choice by helping to predict response to and tolerability of different medications. The potential utility of analyzing common genetic variants in antidepressant response is supported by the finding that 42% of individual variation in medication outcomes derives from genetic factors (Tansey et al., 2013).

Results from candidate gene studies of antidepressant response have been utilized to develop multiple pharmacogenetic-based decision support tools (DSTs), which vary widely in the number and type of genes assessed, included medications, cost, regulation, and method of results delivery. A recent review identified 76 labs in the US that offer pharmacogenetic testing services (Haga and Kantor, 2018) with 38 DSTs that assess antidepressants (Fabbri et al., 2018). These tools are being marketed directly to patients and clinicians, though current depression treatment guidelines either do not address this type of testing or simply refer to it as an area of future research (Peterson et al., 2017; Zeier et al., 2018). A recent meta-analysis concluded that while there are concerns about the quality of the evidence base, pharmacogenetic testing does hold promise for improving response and remission rates in MDD, particularly among patients with treatment resistance or intolerability to previous psychotropics (Bousman et al., 2019).

More recently, investigators have begun to examine ways in which epigenetic marks predict antidepressant response as part of the field of pharmacoepigenetics. Epigenetic modifications are changes to DNA structure that do not affect the DNA sequence but mediate alterations in gene expression, which, in turn, can influence protein levels. While some epigenetic marks appear inherited, most can be altered throughout a person’s lifetime starting during prenatal development by multiple environmental factors, including exposure to pharmacotherapy (Kanherkar et al., 2014).

Epigenetic factors that have been most studied in the context of antidepressant response include DNA methylation, histone modifications, and the control of gene expression by non-coding RNAs. DNA methylation is the most widely studied and best understood epigenetic modification. It typically involves the addition of a methyl group to a cytosine within a CpG
dinucleotide via DNA methyltransferase (DNMT), generating the modified nucleotide 5-methylcytosine (5mC), but less commonly involves adding a methyl group (or other variants of methyl groups) to cytosines within other types of dinucleotides. Generally, the presence of 5mC at gene promoters is associated with decreased gene expression, whereas intragenic methylation can induce gene transcription or silencing (Bonasio et al., 2010; Maunakea et al., 2010). The enzyme DNMT1 preserves DNA methylation patterns during replication, while DNMT3a and DNMT3b lead to de novo methylation of double-stranded DNA (Menke and Binder, 2014).

Histone modification refers to the enzymatic attachment to or removal of chemical groups from lysine and arginine residues on histones’ N-terminal tails. Histones are found in nucleosomes, which consist of an octamer of histone proteins (two copies of H2A, H2B, H3, and H4 each) around which DNA is coiled (Sun et al., 2013). Acetylation is the most common histone modification and generally produces an increase in gene expression by inducing the formation of a more loosened and accessible chromatin (‘euchromatin’). N-terminal tails of histones can also be methylated with one, two, or three methyl groups. Methylation of histones can lead to transcriptional activation (H3-lysine (K)4, H3K36) or repression (H3K9, H3K27, H4K20) based on which histone and lysine is being methylated (Lachner et al., 2003).

There are multiple mechanisms by which antidepressants and antidepressant-like compounds have been shown to alter the epigenome. Evidence suggests that the TCAs amitriptyline and imipramine, the selective serotonin reuptake inhibitor (SSRI) paroxetine, and the antidepressant-like compound genipin (a molecule extracted from Gardenia jasminoides Ellis, i.e. cape jasmine) decrease DNA methylation by reducing DNMT1 enzymatic activity both in and ex vivo (Perisic et al., 2010; Ye et al., 2018; Zimmermann et al., 2012). Paroxetine has also been found to alter DNMT1 phosphorylation, which affects the enzyme’s activity, in peripheral blood cells obtained from depressed patients (Gassen et al., 2015). Evidence suggests that the SSRI fluoxetine indirectly alters the epigenetic landscape through chronic elevation of serotonin, which in turn increases expression of methyl-CpG-binding protein, a transcription factor involved in DNA methylation, and a specific histone deacetylase (HDAC), an enzyme that removes acetyl groups from histones (Csoka and Szyf, 2009). Furthermore, the serotonin-norepinephrine reuptake inhibitor (SNRI) venlafaxine (Qiao et al., 2019) and imipramine (Tsankova et al., 2006) selectively down-regulate HDAC5 in rodent models of depression. There is also evidence that imipramine decreases activity of HDAC3 and HDAC4 in fetal mouse neocortical neurons (Nghia et al., 2015). In addition to valproic acid, multiple other HDAC inhibitors have antidepressant effects in animal models (Fuchikami et al., 2016).

Although the field of pharmacoepigenetics is quite young compared to the more established pharmacogenetics approach, an increasing body of preclinical and clinical work indicates that epigenetic marks may be useful for the prediction of treatment response in patients with MDD. Here, we review the current state of the field of pharmacoepigenetics of oral antidepressant response in human and animal models of depression with a focus on DNA methylation and histone modifications. We will not discuss non-coding RNAs, as they have recently been reviewed elsewhere in relation to antidepressant response (Belzeaux et al., 2010).
2018; Fiori et al., 2018). Given the issue of high tissue specificity in DNA methylation patterns (Ziller et al., 2013) and the fact that most DNA methylation studies in humans use blood, we used the freely available web application Blood-Brain Epigenetic Concordance, BECon (Edgar et al., 2017), to obtain a sense of the concordance of CpGs between blood and brain in genes for which we found two or more studies, including BDNF, SLC6A4, and HTR1B. We then discuss key issues with the current antidepressant pharmacoepigenetics literature and provide suggestions for future studies to aid in the development of clinically useful pharmacoepigenetic DSTs.

2. Methods

Studies were identified for inclusion in this narrative review by searching PubMed, Google Scholar, PsychINFO, and Ovid Medicine from inception through January 2019 (LMH, GRF, HAE, BTB, and BWD). Articles were limited to those published in English and could include either humans or animals as subjects. The literature search strategy was based on using combinations of the following keywords: epigenetic*, pharmacoepigenetic*, pharmacoepigenomic*, depression, major depressive disorder, DNA methylation, acetylation, histone*, antidepressant response, mice, rats, and animal models.

3. Results

3.1. Pharmacoepigenetic studies

The majority of studies of antidepressant pharmacoepigenetics have examined SSRIs in relation to epigenetic marks, although SNRIs, TCAs, MAOIs, and mirtazapine have also been assessed. Furthermore, compounds not traditionally considered to be antidepressants, including HDAC inhibitors, DNMT inhibitors, and genipin have also been studied. Below, we discuss the animal and human literature findings, structured by the gene studied and the observed baseline predictors or post-treatment changes reported. Table 1 lists pharmacoepigenetic studies of antidepressant response in humans and Table 2 shows the same in rodents.

3.1.1. Locus-specific epigenetic marks

3.1.1.1. Baseline: Brain-Derived Neurotrophic Factor: Brain-derived neurotrophic factor (BDNF) is a neurotrophin belonging to a family of proteins involved in neuronal proliferation, migration, differentiation, and survival (Huang and Reichardt, 2001). For review of epigenetic marks affecting BDNF and their role in depression in both humans and animals, see Duclot and Kabbaj (2015). Multiple human and animal studies have found evidence that effective therapy with antidepressants increases peripheral levels of BDNF (Molendijk et al., 2011) and that an early lack of increase in plasma levels of BDNF predicts non-response to antidepressants (Tadić et al., 2011). Given this background, investigators examined the baseline methylation status of the promoter of BDNF exon IV in the leukocytes of MDD patients (n=39) in a naturalistic setting and found lower levels at this locus to be predictive of non-response to antidepressants belonging to multiple classes, including SSRIs, SNRIs, atypical antidepressants, TCAs, and MAOIs (Tadic et al., 2014). Another study of Korean patients with acute coronary syndrome and depressive disorder
found that pre-treatment BDNF hypermethylation in the exon VI promoter assessed in whole blood predicted improvement in depressive symptoms with escitalopram treatment (Kim et al., 2015). Using blood, Wang and colleagues found that baseline DNA hypermethylation in 4 CpGs across the promoters of multiple BDNF exons predicted better response to 8 weeks of escitalopram in a cohort of Han Chinese participants with MDD (n=85) (Wang et al., 2018b). Furthermore, they showed that average BDNF methylation across multiple exons was significantly increased after escitalopram treatment in remitters in a subset of participants (n=44). The investigators found that the interaction between lower life stress scores and higher DNA methylation predicted better treatment response, providing an example of how epigenetic marks and environmental exposures may be combined in future pharmacoepigenetic tools.

Figure 1 depicts a truncated version of the human BDNF gene and sites of baseline and post-treatment epigenetic changes related to antidepressant response. The study by Wang and colleagues (2018b) is not included because CpGs across multiple exons in BDNF were evaluated. The BECon application compares DNA methylation levels of 75 CpGs within the BDNF gene or promoters and three Brodmann areas (7, 10, and 20) of 16 individuals (Edgar et al., 2017) between blood and brain. Given the relevance of Brodmann area 10 to depression (Bludau et al., 2016), we focused on the correlation between methylation sites in blood and this area of the brain. The analysis revealed that only 2 CpGs (2.7%) had strong positive or negative correlations (r >= 0.5), while 13 CpGs (17.3%) had moderate positive or negative correlations (r >= 0.3).

3.1.1.2. Post-treatment: Brain-Derived Neurotrophic Factor: Multiple antidepressants induce epigenetic effects in the Bdnf gene in animal models of depression. Using hippocampal tissue, fluoxetine has been shown to increase methylation in the Bdnf promoter IV associated with a decrease in depressive-like behaviors in post-stroke mice (Jin et al., 2017) and enrich acetylated histone 4 lysine 12 (H4K12) at the Bdnf promoter III in the forebrain neocortex of maternally separated mice when administered with an HDAC inhibitor (Schmauss, 2015). Bdnf expression was upregulated in both of these studies. Imipramine administration increased histone 3 acetylation at the Bdnf gene promoter III and IV in the hippocampus of mice exposed to social stress (Tsankova et al., 2006). In another study of mice using medial prefrontal cortex (PFC), Xu and colleagues found that the MAOI tranylcypromine reversed stress-induced increases in dimethylation of histone H3 at lysine 9 immediately downstream of the Bdnf exon IV promoter, suppression of gene transcription, and cognitive inflexibility (Xu et al., 2018). Furthermore, evidence suggests that genipin reverses depressive-like behaviors in prenatally stressed mice while inducing DNA demethylation of multiple Bdnf exon promoters in the hippocampus through inhibition of the enzyme DNMT1 (Ye et al., 2018). The authors suggest that genipin may inhibit DNMT1 activity indirectly by interfering with its transcription and/or more directly by disrupting the DNMT1 DNA-binding domain. DNA demethylation was accompanied by the normalization of previously downregulated Bdnf expression in the hippocampus of these mice. Furthermore, chronic systematic administration of the HDAC inhibitor suberoylanilide hydroxamic acid (SAHA) was found to improve depressive-like behavior in a mouse model of treatment-resistant depression and increase the expression of Bdnf in the prefrontal cortex.
(Meylan et al., 2016). The results of this animal work align well with evidence from humans mentioned previously that increased expression of BDNF correlates with successful antidepressant treatment (Molendijk et al., 2011).

In a small cross-sectional study, Chen and colleagues showed that the use of antidepressant medications from multiple classes was associated with decreased repressive histone methylation at the BDNF IV promoter in post-mortem PFC tissue from medicated MDD subjects (n=7) as compared to unmedicated subjects (n=11) (Chen et al., 2011). Additionally, using whole blood, Lopez and colleagues found a decrease in histone 3 lysine 27 trimethylation (H3K27me3) at the BDNF exon IV promoter correlated negatively with BDNF IV expression and depression severity after treatment with citalopram for 8 weeks as compared to baseline (Lopez et al., 2013).

### 3.1.1.3. Baseline: Monoaminergic transporter genes

The serotonin transporter, encoded by the solute carrier family 6, member 4 (SLC6A4) gene, is a key target of antidepressant drug action. In one study, higher average baseline methylation of the promoter of the SLC6A4 gene across 9 CpG sites assessed in whole blood was associated with better escitalopram response after 6 weeks of treatment in Caucasian MDD patients (n=94) (Domschke et al., 2014). However, another study found no association between methylation in the SLC6A4 promoter from leukocytes and response in MDD patients (n=108) treated with antidepressants for 12 weeks after correction for multiple testing but nominal associations were found between higher average methylation as well as at a particular CpG and worse response (Kang et al., 2013). Despite examining the same CpG sites, Domschke and colleagues suggest the differing results between these studies may be due to variability in treatment duration, sample ethnicity, or pharmacogenetic heterogeneity in the study by Kang et al. (Domschke et al., 2014). When we assessed the correlation between blood and brain of the 15 CpGs for SLC6A4 listed in BECon in Brodmann area 10 (Edgar et al., 2017), we found that 2 CpGs (13.3%) had strong positive or negative correlations ($r \geq 0.5$), while 11 CpGs (73.3%) had moderate positive or negative correlations ($r \geq 0.3$). To our knowledge, no other monoaminergic transporter genes have been tested with respect to baseline epigenetic status.

### 3.1.1.4. Post-treatment: Monoaminergic transporter genes

One small cross-sectional study found that two CpG sites assessed in whole blood in the SLC6A4 promoter were more highly methylated in MDD patients (n=33) treated with SSRIIs compared to those who were on dual acting antidepressants (Booij et al., 2015). Additionally, post-treatment methylation of a particular CpG in SLC6A4 in whole blood was found to be significantly higher than baseline in a sample of Japanese MDD subjects (n=50) who showed better therapeutic responses after 6 weeks of antidepressant treatment with paroxetine, fluvoxamine, or milnacipran compared to those with poor responses (Okada et al., 2014). Solute carrier family 6, member 2 (SLC6A2) encodes the norepinephrine transporter, another key target of antidepressant drug action. Investigators measured methylation of the promoter of SLC6A2 in whole blood before and after SSRI treatment in a sample of patients with MDD (n=5) and panic disorder (n=4) and found no significant change (Bayles et al., 2013). However, the
sample is too small to draw any conclusions about the role of SLC6A2 methylation in response to SSRI treatment.

3.1.1.5. **Baseline: Serotonin receptor genes:** Baseline hypomethylation of two CpG sites within serotonin receptor type 1A (HTR1A) and 1B (HTR1B) genes derived from whole blood was predictive of poor escitalopram response in a sample of Han Chinese patients with MDD (n=85) after 8 weeks of treatment (Wang et al., 2018a). This was the same group of subjects tested in the Wang et al. (2018b) BDNF article previously discussed. The authors further showed that the interaction of high recent life stress with low methylation in 3 CpG sites in the HTR1A gene and 1 CpG site in the HTR1B gene predicted poor escitalopram response. Another group showed that improvement in depressive symptoms after treatment with fluoxetine was correlated with lower baseline average methylation of the HTR1B gene promoter assessed in whole blood in 83 children and adolescents with MDD, obsessive compulsive disorder, or generalized anxiety disorder (Gasso et al., 2017). Wang and colleagues provide several possible reasons for the discrepancy in findings between their study and that of Gasso et al., including differences in ethnicity, diagnosis, antidepressant studied, and duration of treatment (Wang et al., 2018a). Other possible reasons for the contrasting results include differences in the 5mC analysis method and ages of the participants. Using the BECon application (Edgar et al., 2017) to evaluate the correlation between blood and brain of the 12 CpGs for HTR1B in Brodmann area 10, there were 0 CpGs (0%) with a strong positive or negative correlations (r >= 0.5) and 5 CpGs (41.7%) with a moderate positive or negative correlations (r >= 0.3).

3.1.1.6. **Post-treatment: Serotonin receptor genes:** One preclinical study assessed the effect of an antidepressant on methylation status of the Htr1a gene. Chronic imipramine administration reversed chronic stress-induced increases in the methylation of a CpG site within the promoter of the Htr1a gene in the prefrontal cortex and midbrain of mice, along with an associated increase in Htr1a expression (Le Francois et al., 2015).

3.1.1.7. **Baseline: Other genes:** A potential epigenetic moderator for choosing between nortriptyline and escitalopram was identified from the Genome-based Therapeutic Drugs for Depression study. MDD patients with baseline hypermethylation of a CpG site in the interleukin-11 (IL11) gene assessed in whole blood achieved better antidepressant response to escitalopram (n=80) but worse response to nortriptyline (n=33) (Powell et al., 2013). If replicated, this finding is an example of the clinical utility pharmacoepigenetic approaches could bring to clinical care in predicting differential response to treatment. The authors further demonstrated that a variant in the IL11 gene interacted with a CpG site to predict antidepressant response, providing an example of how both genetic and epigenetic information could be incorporated into future tools. Takeuchi and colleagues conducted a genome-wide DNA methylation analysis using peripheral leukocytes in a small sample of Japanese MDD patients (n=20) treated with paroxetine for 6 weeks (Takeuchi et al., 2017). The investigators showed that 218 CpGs were nominally significantly different between the 10 best responders and 10 worst responders, while 2 CpGs passed multiple test correction. These 2 sites were located in the PPFIA4 gene, which encodes the protein liprin-alpha-4 shown to be important in neural transmission and HS3ST1, which encodes the enzyme...
heparin sulfate glucosamine 3-O sulfotransferase 1 and has no known relationship with MDD or antidepressant response.

### 3.1.1.8. Post-treatment: Other genes

Epigenetic changes induced by antidepressants have been studied in multiple other genes in animal models of depression. Chronic escitalopram treatment reversed hypermethylation of the promoter of the S100 Calcium Binding Protein A10 (S100a10) gene in the PFC of the Flinders Sensitive Line (FSL) genetic rat model of depression (Melas et al., 2012), suggesting a potentially useful novel target worthy of examination in clinical MDD samples treated with escitalopram. Furthermore, investigators reported that imipramine reversed stress-induced methylation and expression changes of the corticotropin-releasing factor (Crf) gene in the hypothalami of mice exposed to social defeat and attenuated their social avoidance behavior (Elliott et al., 2010). In FSL rats, another HDAC inhibitor, L-acetylcarnitine (LAC), acted as a rapid and long-lasting antidepressant while inducing increased levels of acetylated H3K27 bound to the promoter of the glutamate metabotropic receptor type (Grm2) gene, thus enhancing transcription of this gene in the hippocampus and prefrontal cortex (Nasca et al., 2013).

### 3.1.2. Global epigenetic marks

Multiple preclinical studies have examined global epigenetic changes in response to antidepressant administration in animal models of depression. Chronic but not acute imipramine administration decreased stress-induced increases in global DNA methylation as well as DNMT3a and DNMT3b expression in the PFC of rats (Sales and Joca, 2018). The DNMT inhibitors 5-aza-2′-deoxycytidine (5-AzaD) and 5-azacytidine (5-AzaC) were found to improve depressive-like behaviors in mice, both when administered directly into the hippocampus and systemically (Sales et al., 2011). Moreover, the same authors demonstrated that DNMT inhibitors potentiate the behavioral effects of known antidepressant drugs, such as desipramine and fluoxetine, in a preclinical model of stress (Sales and Joca, 2016). Similar evidence exists with drugs that target histone modifications, including HDAC inhibitors. Investigators showed that chronic administration of the HDAC inhibitor sodium butyrate induced antidepressant-like effects in FSL rats with accompanying upregulation of the enzyme ten-eleven translocation methylcytosine dioxygenase 1 (TET1) in PFC (Wei et al., 2014). This enzyme converts 5mC into 5-hydroxymethylcytosine (5hmC), an important step in the DNA demethylation process. Qiao and colleagues treated rats exposed to unpredictable stress with venlafaxine for 4 weeks and found the medication rescued depressive-like behaviors, significantly inhibited the global decrease of H3K9ac in the hippocampus, and downregulated the elevated expression of HDAC5 induced by stress (Qiao et al., 2019). In a human study, researchers demonstrated a significant association between clinical response to paroxetine after 6 weeks of treatment in MDD patients and phosphorylation of DNMT1 in isolated peripheral blood mononuclear cells from the same patients (Gassen et al., 2015).

### 3.2. Summary of current pharmacoepigenetic studies

Overall results of published antidepressant pharmacoepigenetic studies in humans and animal models of depression are of limited power and have inconsistent findings. This may partially be because the studies are heterogeneous in terms of genetic loci, tissue source, sample clinical characteristics, and type of antidepressant assessed. Given the limited
number of studies and relatively small sample sizes to date, it is premature to make firm conclusions about the potential clinical utility of such as treatment biomarkers. However, preliminary data suggests that antidepressant medications from multiple classes modulate epigenetic mechanisms. Some of these changes have been correlated with antidepressant response in samples of depressed patients, providing a valuable opportunity for potential clinical application. Additionally, baseline epigenetic differences have been found between responders and non-responders to antidepressants. Thus, the emerging field of psychiatric pharmacoepigenetics may complement that of pharmacogenetics and offer ways to integrate genetic and environmental information. This may be particularly relevant in MDD, as there is evidence that environmental exposures, such as early-life adversity, affect treatment outcomes and progression of illness in some patients (Klein et al., 2009), probably partially through epigenetic modulation (Jawahar et al., 2015; Turecki and Meaney, 2016).

4. Discussion

How can pharmacoepigenetic tools be prepared for clinical use?

Unlike genetic assays, which measure static allele states that are immutable and constant across all cell types, epigenetic measures are tissue specific and susceptible to change over time with aging and environmental exposures (both physical and psychological). These tissue specific and dynamic aspects of epigenetic profiles increase both the challenge and the opportunities for developing pharmacoepigenetics tools to aid in antidepressant treatment selection. Much work needs to be done before pharmacoepigenetic measures could be incorporated into DSTs to guide treatment selection for patients with MDD. Key issues with the current evidence base and suggestions for future research are outlined in Table 3.

One concern is that most studies thus far of antidepressant pharmacoepigenetics have been conducted using a candidate approach. Just as genome-wide association studies (GWAS) of antidepressant response have suggested regions and associated molecular pathways not considered using hypothesis-driven approaches (Gadad et al., 2018), there are likely candidates in the epigenetics of antidepressant response that have not been evaluated based on our understanding of the biology of depression. Data from individual studies will likely need to be pooled into consortia to have adequate power to detect effects significant at the epigenome-wide level. The aggregation of epigenetic variants significant at sub-threshold levels (i.e. polyepigenetic risk scores) may prove useful in predicting antidepressant response even if epigenome-wide significant marks are not initially identified, as has been the case with genetic variants of antidepressant response (GENDEP Investigators et al., 2013). As the field begins to accumulate evidence from EWAS of antidepressant response, it will benefit from curation of associated epigenetic marks into a knowledgebase like the EWAS Atlas (Li et al., 2018). Incorporating findings from EWAS along with well replicated loci from candidate studies with convergent evidence (e.g. from gene expression studies) into future DSTs will increase the likelihood of clinical utility.

Significant, clinically-useful marks must be validated by large controlled clinical trials with replication of findings in independent populations. In doing so, we will gain a better sense of whether epigenetic marks that predict treatment response are the same as those that change in response to treatment. As more evidence accumulates for particular epigenetic marks,
inclusion in the pharmacogenetic knowledgebase (PharmGKB) (Whirl-Carrillo et al., 2012) would facilitate the integration and implementation of this information in the future. It would be ideal if the validity of replicated marks were determined by an international, expert group, like the Clinical Pharmacogenetics Implementation Consortium (CPIC) has done for the pharmacogenetics of depression (Hicks et al., 2015; Hicks et al., 2017), prior to incorporation into future tools.

It will be important for investigators who conduct future pharmacoepigenetic studies to select reproducible assays to ensure that pharmacoepigenetic tools have high analytic validity. For DNA methylation, extant pharmacoepigenetic studies of antidepressant response have also used a variety of assays. A study comparing 21 locus-specific DNA and 6 global methylation methods for clinical applications found good agreement across all approaches, although the amplicon bisulfite sequencing and bisulfite pyrosequencing showed the best all-round performance (Bock et al., 2016). Forthcoming studies should avoid methylation assays found to have poorer performance, such as enrichment bisulfite sequencing, which produced a high number of outliers compared to other assays (Bock et al., 2016). Furthermore, investigators who engage in future epigenome-wide studies of antidepressant response should be aware of the low concordance found for some CpGs between two widely used Illumina BeadChips and the potential need to filter out discordant probes (Logue et al., 2017).

An additional concern for pharmacoepigenetic studies is tissue source. Most human studies of epigenetic variation in relation to antidepressant treatment response have been conducted using blood samples, as neural tissue is unlikely to be obtained from live subjects. This is a concern given that, while some epigenetic marks have been shown to be highly correlated between brain and blood in the context of MDD (Oh et al., 2015), it is known that DNA methylation generally has strong tissue specificity (Farré et al., 2015). Because only easily accessible tissues could be collected in the clinical setting for use in a pharmacoepigenetics panel, we suggest the use of buccal cells (Lowe et al., 2013) or saliva samples (Smith et al., 2015), given that they show more similar methylation patterns than blood tissue. If blood is used in future studies for identifying additional loci, one possibility is to limit the selected CpGs to those that show high correlation between blood and brain using the freely available web application BECon (Edgar et al., 2017). The NIH’s Epigenomics Roadmap is another resource for identifying epigenetic marks in blood that may serve as proxies for those in brain (Kundaje et al., 2015). An additional possibility for future studies to identify additional loci of interest is to generate induced pluripotent stem cell (iPSC)-derived neurons from skin fibroblasts or from peripheral blood cells and expose these to antidepressants in vitro (Wang et al., 2015). Research has demonstrated that iPSC-derived neurons show similar methylation patterns as compared to human embryonic stem cells (de Boni et al., 2018); however, it is still unknown how similar their epigenetic profiles are compared to native neurons. Another limitation of this approach is the time required to generate iPSCs. Nevertheless, treating cells derived from depressed patients ex vivo and tracking epigenetic changes has already been shown to be a viable approach (Gassen et al., 2015). Researchers demonstrated that epigenetic changes observed in peripheral blood mononuclear cells derived from depressed patients and treated ex vivo with paroxetine mirrored changes seen
in the same patients treated with various antidepressants. Furthermore, these changes correlated with clinical response.

While extant antidepressant pharmacoepigenetic studies assessing DNA methylation and histone modifications have only examined pharmacodynamic genes, we recommend future work also assess pharmacokinetic genes, given evidence that epigenetic marks affect the expression of cytochrome P450 genes (Ingelman-Sundberg et al., 2007). Blood would be a good tissue for this purpose, as there is evidence that blood methylation patterns are similar to those in the liver (Bysani et al., 2017). A concern related to tissue specificity is that, to our knowledge, no published studies of antidepressant pharmacoepigenetics have corrected for cell type, although DNA methylation is known to vary by cell type within the blood (Jaffe and Irizarry, 2014), saliva (Langie et al., 2017), and brain (Iwamoto et al., 2011). Thus, it will be important for future studies to correct for cellular proportions within the studied tissue.

Another limitation of the current pharmacoepigenetics literature is the focus on methylation of CpG dinucleotides given that approximately 25% of methylation is of cytosines within CpH (H = A/C/T) dinucleotides in the adult brain (Guo et al., 2014). Non-CpG dinucleotides display varying levels of methylation in different parts of the brain and there is evidence that they play a role in multiple neurological disorders, including Alzheimer’s Disease and Parkinson’s Disease (Jang et al., 2017; Oh et al., 2015), so it is reasonable to assume that they may be important in psychiatric disorders as well. Furthermore, common methods for assessing DNA methylation (i.e. bisulfite-based technologies) do not differentiate between 5mC and 5hmC, an intermediate in the active demethylation pathway. 5hmC is most abundant in the brain of mammals (Yardimci and Zhang, 2015), enriched in genes involved in neurodevelopment (Gross et al., 2015), and is modulated by stress (Hack et al., 2016), supporting an independent (and possibly stable) role for this epigenetic mark, particularly in psychiatric disorders.

Furthermore, most extant pharmacoepigenetics studies lack a comparator arm. Head-to-head trials of different antidepressants are needed to determine if epigenetic marks predict differential response to medications. Moreover, there needs to be greater consistency among studies in terms of patient characteristics, treatment duration, use of covariates, and multiple testing correction. This standardization will help decrease the possibility that lack of replication among studies assessing the same variants is due to heterogeneity in these variables. Future studies must also consider the impact of age, gender, other medications, and environmental factors on epigenetic marks.

Given evidence that antidepressant response likely arises from a complex interplay of genetic, epigenetic, and environmental factors, it would be advantageous to the field of pharmacoepigenetics to integrate genetic background, high-quality environmental information, and epigenetic marks in the same analysis and assess interactions between these variables. An interesting approach to address some limitations of currently available commercial pharmacogenetic panels would be to incorporate epigenetic marks in them along with clinical and environmental exposures, as opposed to creating new independent pharmacoepigenetic tools. This will be highly dependent upon clinical trials integrating...
multiple genetic and epigenetic marks at once. To take this one step further, given evidence from animal and human work that other therapies for depression, including TMS (Etievant et al., 2015), ECT (de Jong et al., 2014), and cognitive behavioral therapy (Roberts et al., 2014) induce epigenetic changes, it is reasonable to think that there is a potential to incorporate marks related to these treatments in future tools.

A unique opportunity of pharmacoepigenetic approaches is the potential to use early changes (1–2 weeks) after initiating as a means of predicting eventual likely response. Most predictor studies of outcomes in MDD examine pre-treatment (baseline) variables. However, because unguided assessment of efficacy of antidepressants in individuals usually requires a six-week trial, tools that identify changes in a biomarker early in treatment could prove very valuable for indicating whether a medication should be continued or whether a switch should be made to an alternative treatment, thereby aborting the unnecessary time and potential side effects associated with a full six-week trial. Unreplicated studies using electroencephalogram measures (Hunter et al., 2010; Leuchter et al., 2009) or metabolomic (Kaddurah-Daouk et al., 2013; Zhu et al., 2013) changes have demonstrated the possibility of applying early change biomarkers for personalizing treatment. However, developing this application of pharmacoepigenetics will require improved understanding of the time course of change in epigenetic marks after initiating treatment. This is an area where human iPSCs may be particular valuable. Epigenetic assessments may also provide insights into the tachyphylaxis observed in some patients where a previously effective antidepressant loses benefit over time (Lisoway et al., 2018). This is a serious and understudied problem that affects a significant percentage of antidepressant-treated patients (Rothschild et al., 2009).

Because the field of antidepressant pharmacoepigenetics is only in its infancy and there are many pending milestones as reviewed in this manuscript, it will likely require several more years for pharmacoepigenetic tools to become clinically available for patients with MDD. Nevertheless, initial results are promising and warrant more substantial translational research. The development of DSTs that incorporate epigenetic marks is poised to be part of the exciting and rapidly growing area known as precision or personalized psychiatry, which holds the promise of providing personalized, patient-centered treatment based on an individual’s unique biological and environmental characteristics (Fernandes et al., 2017).

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• Antidepressant response is associated with multiple epigenetic marks.
• Many limitations exist in the study base of antidepressant pharmacoepigenetics.
• Future studies should focus on overcoming these limitations.
• Integrating multiple predictors into new tools will likely have the greatest utility.
Figure 1.
Sites of findings in pharmacoepigenetic studies of antidepressant response across the human BDNF gene. Gene truncated to focus on exons that have been studied in relation to antidepressant response. Arrows point to promoters associated with individual exons.
Abbreviations: 5mC – 5-methylcytosine; ACS – acute coronary syndrome; H3K27me3 – histone 3 lysine 27 trimethylation; MDD – major depressive disorder.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Epigenetic mark</th>
<th>Baseline or Post-treatment</th>
<th>Antidepressant</th>
<th>Sample</th>
<th>Time</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BDNF</td>
<td>5mC of 12 CpGs in exon IV promoter</td>
<td>Baseline</td>
<td>SSRIs, SNRIs, atypicals, TCAs, and MAOIs</td>
<td>MDD patients (n=59)</td>
<td>Leukocytes</td>
<td>↓ 5mC in 1 CpG</td>
<td>2-6 weeks</td>
</tr>
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<td>5mC of 9 CpGs in exon VI promoter</td>
<td>Baseline</td>
<td>Escitalopram</td>
<td>ACS patients with depressive disorder (n=25)</td>
<td>Whole blood</td>
<td>↑ average 5mC</td>
<td>24 weeks</td>
</tr>
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<td></td>
<td>5mC of 90 CpGs in exon I-IV</td>
<td>Baseline and Post-treatment</td>
<td>Escitalopram</td>
<td>MDD (n=85)</td>
<td>Whole blood</td>
<td>↑ baseline 5mC in 4 CpGs</td>
<td>↑ response</td>
</tr>
<tr>
<td></td>
<td>H3K27me3 at exon IV promoter</td>
<td>Post-treatment</td>
<td>Multiple classes</td>
<td>Medicated MDD patients (n=7) and unmedicated MDD patients (n=1)</td>
<td>Post-mortem PFC</td>
<td>↓ H3K27me3</td>
<td>↑ BDNF expression in medicated group</td>
</tr>
<tr>
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<td>H3K27me3 at exon IV promoter</td>
<td>Post-treatment</td>
<td>Citalopram</td>
<td>MDD patients (n=65)</td>
<td>Whole blood</td>
<td>↓ H3K27me3 and ↑ BDNF</td>
<td>↑ response</td>
</tr>
<tr>
<td>SLC6A4</td>
<td>5mC of 9 CpGs within exon 1A</td>
<td>Baseline</td>
<td>Escitalopram</td>
<td>MDD patients (n=94)</td>
<td>Whole blood</td>
<td>↑ average 5mC</td>
<td>6 weeks</td>
</tr>
<tr>
<td></td>
<td>5mC of 7 CpGs within promoter</td>
<td>Baseline</td>
<td>Multiple antidepressants</td>
<td>MDD patients (n=86)</td>
<td>Leukocytes</td>
<td>↑ 5mC in 2 CpGs associated with SSRI use but not dual acting antidepressants</td>
<td>↑ response</td>
</tr>
<tr>
<td></td>
<td>5mC of 10 CpGs within promoter</td>
<td>Post-treatment</td>
<td>SSRIs, Dual acting antidepressants</td>
<td>MDD patients (n=65)</td>
<td>Whole blood</td>
<td>↑ 5mC in 2 CpGs associated with SSRI use but not dual acting antidepressants</td>
<td>↑ response</td>
</tr>
<tr>
<td></td>
<td>SLC6A2</td>
<td>5mC of CpGs in promoter</td>
<td>Citalopram, fluoxetine, or sertraline</td>
<td>MDD patients (n=5), panic disorder patients (n=6)</td>
<td>Whole blood</td>
<td>↑ 5mC in 1 CpG</td>
<td>↑ response</td>
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<td>HTR1A</td>
<td>5mC of CpGs in promoter</td>
<td>Baseline</td>
<td>Escitalopram</td>
<td>MDD (n=85)</td>
<td>Whole blood</td>
<td>↑ 5mC in 1 CpG</td>
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<td>HTR1B</td>
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<td>Baseline</td>
<td>Escitalopram</td>
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<td>Whole blood</td>
<td>↑ 5mC in 1 CpG</td>
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<td></td>
<td>5mC of 7 CpGs within promoter</td>
<td>Baseline</td>
<td>Fluoxetine, Children and adults with MDD, OCD, or GAD (n=85)</td>
<td>Whole blood</td>
<td>No association between 5mC in 2 CpGs associated with SSRI use but not dual acting antidepressants</td>
<td>↑ response</td>
<td>12 weeks</td>
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<td>IL-11</td>
<td>5mC of 11 CpGs in intron</td>
<td>Baseline</td>
<td>Escitalopram and nortriptyline</td>
<td>MDD patients (n=133)</td>
<td>Whole blood</td>
<td>↑ 5mC of CpG site 5</td>
<td>↑ response to escitalopram</td>
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<td>MAOA</td>
<td>5mC of 45 CpGs in exon 1, intron 1</td>
<td>Baseline</td>
<td>Paroxetine</td>
<td>MDD patients (n=94)</td>
<td>Whole blood</td>
<td>No association among 5mC in 2 CpGs associated with SSRI use but not dual acting antidepressants</td>
<td>↑ response</td>
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<tr>
<td>PFHE1 and KDRST1</td>
<td>Genome-wide 5mC study</td>
<td>Baseline</td>
<td>Paroxetine</td>
<td>MDD patients (n=20)</td>
<td>Leukocytes</td>
<td>↑ 5mC in 2 CpGs after multiple test correction</td>
<td>↑ response</td>
</tr>
</tbody>
</table>

Abbreviations: 5mC – 5-methylcytosine; ACS – acute coronary syndrome; BDNF – brain-derived neurotrophic factor; DNMT1 – DNA methyltransferase 1; GAD – generalized anxiety disorder; HTR1A – 5-Hydroxytryptamine receptor 1A; HTR1B – 5-Hydroxytryptamine receptor 1B; HS3ST1 – heparin sulfate-glucosamine 3-sulfotransferase 1; IL11 – interleukin 11; MAOA – monoamine oxidase A; MAO-B – monoamine oxidase B; MDD – major depressive disorder; OCD – obsessive compulsive disorder; PFC – prefrontal cortex; PPFIA4 – PTPRF Interacting Protein Alpha 4; SLC6A2 – solute carrier family 6 member 2; SLC6A4 – solute carrier family 6 member 4; SNRI – serotonin-norepinephrine reuptake inhibitor; SSRI – selective serotonin reuptake inhibitor; TCA – tricyclic antidepressants.
<table>
<thead>
<tr>
<th>Gene</th>
<th>Epigenetic Mark</th>
<th>Baseline or Post-treatment</th>
<th>Antidepressant</th>
<th>Species/strain, model</th>
<th>Tissue</th>
<th>Outcome</th>
<th>Reference</th>
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<td>Bdnf</td>
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<td>Fluoxetine</td>
<td>C57BL/6 J mice, post-stroke depression model</td>
<td>Hippocampus</td>
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<td>↑ Bdnf expression</td>
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<td></td>
<td>↓ depression-like behaviors</td>
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<td>Post-treatment</td>
<td>Imipramine</td>
<td>Adult male BALB/c Mice exposed to unpredictable chronic mild stress</td>
<td>PFC, midbrain</td>
<td>↓ 5mC of 1 CpG</td>
<td>Le Francois et al. (2015)</td>
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<td>reversed depressive-like phenotype</td>
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<td>Post-treatment</td>
<td>Escitalopram</td>
<td>Female FSL rodent model of depression</td>
<td>PFC</td>
<td>↓ 5mC</td>
<td>Melas et al. (2012)</td>
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<td>↑ S100a10 expression</td>
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<td>Crf</td>
<td>5mC within promoter</td>
<td>Post-treatment</td>
<td>Imipramine</td>
<td>Adult mice exposed to social defeat</td>
<td>Hypothalamus</td>
<td>↑ 5mC at 5 CpGs</td>
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<td>↓ Crf</td>
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<td>↓ social avoidance behavior</td>
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<td>Nr2b</td>
<td>H3K9ac and H3K27ac within promoter</td>
<td>Post-treatment</td>
<td>Imipramine</td>
<td>Mouse fetuses</td>
<td>Neocortical neurons</td>
<td>↑ H3ac</td>
<td>Nghia et al. (2015)</td>
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<td>↑ Nr2b expression</td>
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<td>↓ HDAC3 and HDAC4 activity</td>
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<td>Grm2</td>
<td>H3K27ac within promoter</td>
<td>Post-treatment</td>
<td>LAC</td>
<td>FSL rodent model of depression</td>
<td>Hippocampus, PFC</td>
<td>↑ H3K27ac</td>
<td>Nasca et al. (2013)</td>
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<td>↑ Grm2 expression</td>
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</tbody>
</table>

Abbreviations: 5mC – 5-methylcytosine; Bdnf – brain-derived neurotrophic factor; Crf – corticotrophin-releasing factor; Htr1a – serotonin 1A; LAC – L-acetylcarnitine; Nr2b – N-methyl D-aspartate receptor subtype 2B; PFC – prefrontal cortex; S100a10 – S100 Calcium Binding Protein A10; SAHA – suberoylanilide hydroxamic acid.
Table 3.

Key issues and suggestions for future research in pharmacoepigenetic studies of antidepressant response

<table>
<thead>
<tr>
<th>Key Issues</th>
<th>Suggestions for future research</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. May be missing relevant genes/loci due to lack of epigenome-wide association studies of antidepressant response</td>
<td>● Conduct whole genome epigenetic studies in humans by leveraging consortia data in order to have the sample sizes needed for appropriate power.</td>
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<tr>
<td>2. Lack of replication of particular epigenetic marks</td>
<td>● Test for replication of epigenetic marks in large controlled clinical trials and determine validity of these marks by expert group.</td>
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<tr>
<td>3. Inconsistent methods for measuring epigenetic marks</td>
<td>● Avoid methods known to have poor performance compared to other assays, such as enrichment bisulfite sequencing.</td>
</tr>
</tbody>
</table>
| 4. Use of blood given low concordance between brain and blood methylation patterns | ● Use saliva or buccal samples given higher concordance with brain methylation patterns as compared to blood.  
  ● If blood is used, limit to markers showing high concordance between brain and blood or generate iPSC-derived neurons.  
  ● Blood may be appropriate when assessing pharmacokinetic genes. |
| 5. Lack of studies assessing non-CpG dinucleotides and 5hmC                | ● Incorporate testing of non-CpG dinucleotides and 5hmC into future studies.                   |
| 6. Lack of studies with a comparator arm                                 | ● Design studies to determine if differential response to antidepressants can be predicted by epigenetic marks. |
| 7. Variability in treatment duration, covariates included in the models, and use of correction for multiple testing | ● Standardize treatment duration and covariates in order to increase comparability between studies and correct for multiple testing. |

Abbreviations: 5hmC – 5-hydroxymethylcytosine; iPSC – induced pluripotent stem cell