Mental Health

Epigenetics and depressive disorders: a review of current progress and future directions

Vania Januar, Richard Saffery and Joanne Ryan

1Cancer & Disease Epigenetics, Murdoch Childrens Research Institute, & Department of Paediatrics, University of Melbourne, Parkville, VIC, Australia and 2Inserm U1061, Hopital La Colombiere & Universite Montpellier, Montpellier, France

*Corresponding author. Cancer & Disease Epigenetics, Murdoch Childrens Research Institute, Royal Children’s Hospital, Flemington Road, Parkville, Victoria 3052, Australia. E-mail: joanne.ryan@mcri.edu.au.

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Abstract

Background: Several broad lines of evidence support the involvement of epigenetic processes in neurodevelopment and psychiatric disorders. Epigenetic disruption also provides a potential mechanism to account for the numerous gene-environment interactions that have been reported in association with neuropsychiatric phenotypes.

Methods: A review of the literature was performed with keywords ‘depression’, ‘depressive disorder’ or ‘antidepressants’ and ‘DNA methylation’, or ‘epigenetics’ in humans. Citations were limited to those written in English and published prior to July 2014.

Results: We present a summary of results to date. Most studies have focused on DNA methylation in various CNS or peripheral tissue, with almost universally small sample sizes. Although seven epigenome-wide association studies have now been reported, the majority of studies have used a candidate-gene approach. Three genes (SLC6A4, BDNF, NR3C1) have been investigated in more than one study, but replication of findings is generally lacking.

Conclusions: Recent evidence provides insights to epigenetic processes in psychiatric disorders; however, replication is lacking and care must be taken in the interpretation of current findings. This applies to epigenetic epidemiology generally, which is subject to various limitations that no single approach can address in isolation. Due to limited focus of most depression studies to date, placing the findings within the broader context of mood disorder pathophysiology may prove challenging. However, identifying peripheral biomarkers for depressive disorder remains a tantalising possibility, especially given the potential for carefully-designed longitudinal studies with multiple biospecimens and ongoing advances in epigenetic technologies.

Key words: Epigenetics; DNA methylation; major depressive disorder; depression; gene-environment; tissue heterogeneity; confounding; causation; CNS tissue; biomarker
Introduction
Depression, mechanisms and risk factors
Depression is one of the most prevalent psychiatric conditions worldwide and is considered a major public health problem, accounting for 40.5% of disability-adjusted life years. Prevalence rates continue to rise, and the World Health Organization estimates that depression will be the leading cause of disease burden by 2030.

Despite much research, diagnosis of depression is still largely based on clinical symptoms, with efforts to find objective and reliable biomarkers of depressive disorders largely unsuccessful. This likely reflects the fact that the pathophysiology and underlying biochemical/molecular events leading to depression remain largely unknown. One major challenge in the field thus lies in identifying systemic and molecular mechanisms of disease pathogenesis.

As with any complex disease, depressive disorders arise from a combination of genetic and environmental risk factors. Evidence of environment-psychopathology association is emerging, and several environmental risk factors have been identified. However, how these factors alter physiological processes, leading to the development of the depressive symptoms and stable changes in behaviour, remain largely unclear.

Genetics of depression
Depression is a familial disorder with several family-based studies suggesting a heritability of around 40%. However despite intense research that includes a number of large genome-wide association studies (GWAS), very few genetic variants have robustly been implicated in depressive disorders. For example, Wray et al. investigating 5763 cases and 6901 controls, found only suggestive evidence of an association between major depressive disorder (MDD) and polymorphisms at or near genes with plausible biological relevance, such as galanin and adenylate cyclase 3, and the largest study to date, a recent mega-analysis involving 9240 MDD cases and 9519 controls with replication in 6783 MDD cases and 50 695 controls, also failed to detect clear genetic variants associated with the phenotype. This contrasts with other psychiatric disorders, such as schizophrenia, where numerous genetic loci implicated in disease pathogenesis have now been identified.

Despite this, numerous case-control studies have implicated specific genes involved in depression pathophysiology. Some of the most common genes investigated include brain-derived neurotrophic factor (BDNF), serotonin receptor (5-HT), serotonin transporter (SLC6A4, or 5-HTT) and catechol-o-methyltransferase (COMT). These genes regulate pathways that are implicated in the development of mood disorders; BDNF regulates neural plasticity and connectivity, and 5-HT, SLC6A4 and COMT are involved in the regulation of neurotransmitter signalling. Furthermore, polymorphisms in these genes have been linked to increased risk for depression, anxiety, stress and various cognitive functions. However meta-analyses have yielded mixed results, again highlighting the lack of clear replication in this field. Interestingly, none of these genes have been consistently found in any GWAS. This could relate in part to heterogeneity in the phenotype being measured, for example the American Psychiatric Association’s Diagnostic and Statistical Manual (DSM) diagnosed MDD vs depressive symptoms, assessed using a variety of different instruments, as well as depression comorbid with other psychiatric conditions. Another possible reason for the absence of direct links between genes and depressive disorders is the multifactorial nature of the disorder, including the likelihood that numerous genes, each with a relatively small effect, play a contributory role.

Adding another layer of complexity to mood disorder pathophysiology is the potential for interactions between genes and environment (GxE). Several models of psychiatric illness incorporate GxE effects; for example, the diathesis-stress model of depression purports that individual resilience, or genes, modify an individual’s susceptibility to environmental stressors. Yet despite intense research efforts, there remains a lack of direct evidence for such interactions. A seminal study by Caspi et al. found that polymorphisms of the SLC6A4 gene moderated the effect of childhood adversity.
of stress on susceptibility to depression. A plethora of subsequent studies have attempted to replicate these initial findings with mixed results, and recent meta-analyses have thus not been conclusive. Preliminary evidence of GxE interaction has been reported for other candidate genes implicated in depression, including dopamine receptor D2 (DRD2), monoamine oxidase A (MAOA) and BDNF, though replication is again lacking and most studies are likely to be underpowered to examine such associations.

More recently, a number of studies have attempted to elucidate the complex process by which environmental factors could combine with genetic susceptibility to influence an individual’s risk of disease, potentially providing a functional explanation for apparent GxE interactions. A number of putative mechanisms have been proposed, particularly in relation to the disruption of gene expression levels seen in post-mortem brain tissue in both animal models and humans in some psychiatric illnesses. However, the mechanisms by which gene expression changes lead to prolonged depression are incompletely understood, particularly as changes in nuclear regulatory proteins or transcription factors associated with disruption in gene activity often do not persist as long as behavioural changes do. Biological mechanisms that can integrate the various hypotheses for the pathogenesis of depressive disorders must fit certain paradigms, including: (i) ability to induce stable changes in physiology; (ii) plasticity and responsiveness to both environment and genetic factors; and (iii) ability to reconcile genetic and environmental contributions to disease. Persistent epigenetic changes present a possible candidate for such a mechanism.

Epigenetic processes, which regulate the activity of DNA (including gene expression) in the absence of induced DNA sequence change, have been implicated in several human diseases, including cancer and neuropsychiatric disorders. Proper cellular function depends on the homeostatic regulation of the epigenome and, as this is sensitive to environmental cues, small alterations in environment may theoretically lead to long-term changes in gene expression, contributing to disease. Some of the most widely studied mechanisms of epigenetic regulation include DNA methylation, histone modifications and non-coding RNAs. Due to its stability and ease of measurement, DNA methylation has been the most often studied.

The aim of this review is to provide an overview of the current research investigating the role of epigenetics in depression. This review mainly focuses on studies involving humans, although we also describe some of the key findings from animal models. A critical appraisal of current limitations in this field is then presented.

Methodology

A literature search was performed using MEDLINE, Web of Knowledge and PsycINFO databases with keywords ‘depression’, ‘depressive disorder’ or ‘antidepressants’ and ‘DNA methylation’ or ‘epigenetics’ in humans. Studies were also identified by manually searching the reference list of relevant articles. Citations were limited to those written in English and published up to July 2014. Research regarding the effect of maternal depression on neonates was excluded from the search, as were studies investigating hormone-associated depression (i.e. premenstrual dysphoric disorder, postpartum depression, postmenopausal mood disorders).

Epigenetic Modifications In Depression

Several broad lines of evidence support the involvement of epigenetic processes in psychiatric disorders. Firstly, the complexity of the neural connectome (the complex neural pathways or wiring of the brain) is several orders of magnitude greater than the human genome, such that the genome per se carries insufficient information to code for the connectome. Furthermore, epigenetic change is critical for neurogenesis and is implicated in behavioural abnormalities. Epigenetic disruption therefore provides a plausible mechanism to account for the numerous gene-environment interactions that have been reported in association with neuropsychiatric phenotypes.

Animal models

The interest in behavioural epigenetics ‘exploded’ in recent years, following a seminal study in rats, in which offspring with a nurturing mother were found to be less anxious and have decreased methylation in the promoter region of the glucocorticoid receptor gene (NR3C1) from hippocampal tissue, compared with those with neglectful mothers (effect size = 20–60% across 15 CpG sites, p < 0.01 at seven sites). Changes in methylation persisted until adulthood.

Despite provocative findings such as this, the use of animal models to mimic any type of human behaviour is always very difficult and fraught with limitations. Depression represents a particularly challenging disorder to model in animal studies due to: (i) its complexity and unknown aetiology; (ii) vast and varied symptomatologies across individuals; and (iii) the difficulty of objectively measuring these phenotypes in animals (i.e. self-esteem, feelings of guilt, suicidal thoughts). Most animal models therefore focus on specific depression endophenotypes, such as behavioural despair, with rodents exposed to chronic stress or social defeat. Whereas some consider
them appropriate models of depression, others argue that they provide a quite poor reflection of the complex phenotype in humans. A summary of some of these animal models investigating epigenetic changes is given in Table 1. The majority of such studies use a candidate gene approach, examining histone modifications and/or DNA methylation in brain tissue, although the exact brain region varies. Chronic stress has been associated with increased methylation of GDNF and CRF, although in the latter study the effects were gender- and brain region-dependent. Increased dimethylation of histone H3 at lysine-27 (H3K27me2) was also observed in response to chronic social defeat, at a global and a gene-specific level at BDNF. Antidepressant use has been associated with different epigenetic modifications, as well as reversing effects observed with chronic defeat stress. Replication is lacking, as most studies used different animal strains and/or models, and care must be taken when extrapolating the results to the human context.

Human studies of DNA methylation in depression

The vast majority of studies that have explored the link between depression and/or antidepressant use and epigenetic change in humans have focused on DNA methylation. These studies are described in Table 2. At the time of writing, a total of 29 studies were identified and over 70% were published in the past 2 years. This highlights the rapid growth in the field. Methylation has been measured in various tissues, including peripheral tissues of blood, buccal tissue and saliva, plus several brain regions using post-mortem samples. Most studies have used an informed candidate gene approach. However seven epigenome-wide association studies (EWAS), using a variety of different platforms and tissues and examining many thousands of genes, have also been reported. Interestingly, samples sizes have been almost universally small, with only two EWAS studies having a sample of 100 or more. A discussion of some of the main findings is given below, and limitations to current research are described in the next section.

EWAS studies have the advantage that they are hypothesis-free, examining thousands of genomic locations in an unbiased manner. As such, these studies are generally considered superior to candidate-gene approaches that assess a small proportion of the genome, and are listed first in Table 2. The majority of EWAS studies on depression used array-based technologies; three used the Illumina Infinium Human Methylation 450 Bead Chip Array which interrogates 485,000 CpG sites, but all used different tissues (blood, buccal tissue, saliva); another used the older 27K array (27,578 probes) with blood; and a fifth used a comprehensive high-throughput relative methylation (CHARM) approach covering 3.5 million CpGs on brain tissue from the cerebral cortex. A couple of EWAS studies in blood or prefrontal cortex tissue have used affinity enrichment with methyl binding proteins to ‘pull down’ regions of methylation in the genome, identified using DNA sequencing of enriched fractions. In this instance information on specific CpG sites is not obtained, though regions of altered DNA methylation in association with depression, spanning the entire genome, are identifiable. Overall the findings have not been replicated across studies, as discussed in further detail below, with differences in the platforms and tissues examined being at least two of the factors contributing to the variable findings.

An Australian study of 24 adult monozygotic twin pairs (50% female) discordant for depression, assessed methylation levels throughout the genome with the Infinium 450K array in white blood cells. No differences in global mean methylation across all probes between discordant twin pairs were found, although among female discordant monozygotic twins, depression was associated with lower mean methylation levels (no effect size provided; p = 0.005). Furthermore, the variance in methylation in depressed twins compared with their unaffected co-twin was higher (52.4% of probes having higher variance in cases; binomial p < 2.2 x 10^{-16}). Uddin et al. interrogated methylation levels using the Infinium 27K platform in blood DNA, and used functional annotation clustering to investigate uniquely methylated/unmethylated genes in each group. They found different methylation profiles between individuals (mean age 45.3 years, 60% female) with a lifetime history of depression (n = 33) and controls (n = 67). Unaffected individuals had more genes with a defined methylation state (genes which are either unmethylated or methylated only in cases or controls, with methylated genes being defined as those with > 80% methylation, and unmethylated genes < 20%) relative to depressed individuals (unmethylated: controls 205 vs cases 107; methylated: controls 329 vs cases 21; p < 0.0001). Functional annotation and pathway analysis suggested that these uniquely methylated or unmethylated genes cluster into distinct biological pathways, involved in brain development, tryptophan (a serotonin precursor) production and psychopathology-associated genes. The authors also performed gene expression analyses of commonly methylated and unmethylated genes, and found that higher methylation generally corresponded to lower gene expression. This study provides some interesting insights into how changes in epigenomic landscape in peripheral blood may alter cellular function and physiology in association with depression.

An EWAS using 66 post-mortem frontal cortex samples found methylation differences in depressed individuals
<table>
<thead>
<tr>
<th>Reference</th>
<th>N</th>
<th>Strain</th>
<th>Exposure</th>
<th>Tissue</th>
<th>Gene</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fuchikami et al. 2009</td>
<td>6</td>
<td>Sprague-Dawley rats</td>
<td>Single stress immobilization</td>
<td>Hippocampus</td>
<td>BDNF</td>
<td>At t = 0 h: ↑ histone 3 ac; ↓ histone 4 ac; At t = 24 h: no effect</td>
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<tr>
<td>Melas et al. 2012</td>
<td>7-10</td>
<td>FSL/FRL mice</td>
<td>AD</td>
<td>Prefrontal cortex</td>
<td>P11</td>
<td>In FSL: ↓ DNA methylation &amp; ↓ DNMT1, DNMT3a</td>
</tr>
<tr>
<td>Onishchenko et al. 2008</td>
<td>6</td>
<td>Male C57BL/6/Bk1 mice</td>
<td>Methyl mercury AD</td>
<td>Hippocampus</td>
<td>BDNF</td>
<td>Methyl mercury-induced depression-like symptoms &amp; ↑ DNA methylation;</td>
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<td></td>
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<td></td>
<td>↑ histone 3k27me3; ↓ histone 3ac; AD: restores BDNF mRNA; o histone 3k27me3; ↑ histone 3ac</td>
</tr>
<tr>
<td>Sales et al. 2011</td>
<td>5-7</td>
<td>Wistar rats, Swiss mice</td>
<td>DNMT inhibitor</td>
<td>Hippocampus</td>
<td>BDNF</td>
<td>Dose-dependent AD-like effect ↓ DNA methylation; ↓ BDNF expression</td>
</tr>
<tr>
<td>Sterrenburg et al. 2011</td>
<td>12</td>
<td>Wistar rat</td>
<td>Chronic stress</td>
<td>Paraventricular nucleus of</td>
<td>CRF</td>
<td>↑ DNA methylation in females only</td>
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<td></td>
<td></td>
<td></td>
<td>hypothalamus</td>
<td>Bed nucleus of the stria</td>
<td></td>
<td>↓ DNA methylation in males only</td>
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<td></td>
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<td>terminalis</td>
<td>Central nucleus of amygdala</td>
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<td></td>
<td>50% female</td>
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<td>Tsankova et al. 2006</td>
<td>6</td>
<td>B16/C37 mice</td>
<td>Chronic social defeat, AD</td>
<td>Hippocampus</td>
<td>BDNF</td>
<td>↓ DNA methylation in females only</td>
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<tr>
<td>Uchida et al. 2011</td>
<td>8</td>
<td>B6 (resilient) &amp; BALB</td>
<td>Chronic stress, AD</td>
<td>Nucleus accumbens</td>
<td>GDNF</td>
<td>Both strains: ↑ DNA methylation (AD-reversible) BALB: o histone 4ac, histone 3k27me3 (AD-ineffective) &amp; ↓ histone 3ac, histone 3me, histone 4me (AD-reversible) B6: ↑ histone 3ac; o histone 4ac &amp; ↓ histone 3k27me, histone 3k4me (AD-reversible)</td>
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<td></td>
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<td>(susceptible) mice</td>
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<tr>
<td>Wan et al. 2014</td>
<td>30</td>
<td>Sprague-Dawley rats</td>
<td>Chronic stress</td>
<td>Nucleus accumbens</td>
<td>CRHR1</td>
<td>↓ histone 3k9me3; o histone 3k4me3</td>
</tr>
<tr>
<td>Wilkinson et al. 2009</td>
<td></td>
<td>Not indicated</td>
<td>Chronic social defeat, isolation</td>
<td>Nucleus accumbens</td>
<td>Global</td>
<td>↑ histone 3k27me2; ↑ histone 3k9me2</td>
</tr>
</tbody>
</table>

Methylation association: ↑ = increased levels (hypermethylation); ↓ = decreased levels (hypomethylation); o = no association significant.

FSL, Flinders Sensitive Line; FRL, Flinders Resistant Line; AD, antidepressants; DNMT, DNA methyltransferase; H3K27me3, tri-methylated histone H3 on lysine 27; H3K27me2, di-methylated histone H3 on lysine 27; H3K9me2, di-methylated histone H3 on lysine 9; H3ac, acetylated histone H3; H4ac, acetylated histone H4.
Table 2. Studies investigating the association between depression and/or antidepressant use and DNA methylation

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study characteristics</th>
<th>Methylation analysis, N</th>
<th>Depression diagnosisa and AD treatment</th>
<th>DNA Source</th>
<th>Gene, region</th>
<th>Methylation association</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Byrne et al. 2013</td>
<td>MZ twins from Australia; case-control, 30–70 yrs, 50% female</td>
<td>12 MDD discordant; 12 controls</td>
<td>MDD/depression: SSAGA/CIIDI. 0% discordant pairs used AD</td>
<td>White blood cells</td>
<td>EWAS (450K) global &amp; individual genes</td>
<td>↓ global, female discordant pairs. o global &amp; genome-wide all probes</td>
<td>Matched for age, alcohol, smoking</td>
</tr>
<tr>
<td>Davies et al. 2014</td>
<td>MZ twins from registries in UK and Australia; case-control, 23–73 yrs, 86% female; replication cohort 100% female</td>
<td>27 and 23 depression discordant; replication cohort: 118 MDD, 236 controls</td>
<td>Depression/MDD: CIIDI/SSAGA. AD information available for UK twins only</td>
<td>Blood</td>
<td>EWAS (MeDIP-seq). AGTPBP1, CLSTN1, TBC1D8, ZBTB20 replicated</td>
<td>↑ ZBTB20, o others</td>
<td>Adjusted for age, BMI, smoking, AD use for UK twins only</td>
</tr>
<tr>
<td>Dempster et al. 2014</td>
<td>Caucasian MZ twins in Genesis Study, 12–19 yrs, 72% female; Replication: post-mortem SMRI samples most Caucasian, 29–68 yrs, 41% female</td>
<td>18 depression discordant; replication cohort: 14 MDD, 15 controls</td>
<td>Depression: SMFQ. Discordance identified as SMFQ score difference ≥ 6. AD use: not indicated</td>
<td>Buccal, cerebellum</td>
<td>EWAS (450K). Ten topmost genes replicated</td>
<td>↑ MDD in STK32C. ↓ MDD in DEPDC7</td>
<td>None, but no association between gender and methylation levels</td>
</tr>
<tr>
<td>Nagy et al. 2014</td>
<td>Post-mortem suicide samples from Douglas-Bell Brain Bank, Canada, 10–25 yrs, 28% females in initial screen, 0% in replication. Cases with astrocytic dysfunction</td>
<td>76 depression, 45 controls; replication cohort: 22 depression, 17 controls</td>
<td>Depression: clinical histories from proxy-based interviews. AD use: not indicated</td>
<td>Prefrontal cortex</td>
<td>EWAS (MBD2-seq). 115 DMRs mainly related to astrocytic function. BEGAIN &amp; GRIK2 selected for replication</td>
<td>↑BEGAIN. ↓ GRIK2. ↓ expression in both genes</td>
<td>Matched for age, RNA integrity number, post-mortem interval, gender</td>
</tr>
<tr>
<td>Sabuncian et al. 2012</td>
<td>Post-mortem SMRI samples 20–70 yrs, 23% female; additional brain samples for replication cohort</td>
<td>39 MDD (12 psychotic), 27 controls; replication cohort: 16 MDD, 13 controls</td>
<td>MDD: medical records and family interviews. AD: 32 of 39 MDD cases</td>
<td>Frontal cortex</td>
<td>EWAS (CHARM)</td>
<td>CPSF3, LASS2, ZNF263 &amp; PRIMA1 strongest, only PRIMA1 after genome-wide correction. None replicated in other cohort</td>
<td>Matched for age, sex checked adjustment for other factors including AD</td>
</tr>
<tr>
<td>Uddin et al. 2011</td>
<td>Participants from longitudinal Detroit Neighbourhood Health Study, 20–60 yrs, 60% females</td>
<td>33 lifetime depression history, 67 healthy controls</td>
<td>Depressive symptoms: PHQ-9. Cases = depressed mood/anhedonia, ≥ 1 other symptom for ≥ 2 weeks, and/or suicidal thoughts. 5 of 33 cases and 5 of 67 controls using AD/anti-psychotics/anti-anxiolytic</td>
<td>Blood</td>
<td>EWAS (27K)</td>
<td>Different psychiatric methylation profiles identified by Clustering. ↓ IL6 methylation, serum IL6 &amp; CRP in cases</td>
<td>No difference in demographic factors between cases and controls</td>
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</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study characteristics</th>
<th>Methylation analysis, N</th>
<th>Depression diagnosis\ and AD treatment</th>
<th>DNA Source</th>
<th>Gene, region</th>
<th>Methylation association</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weder et al. 2014</td>
<td>Maltreated children removed from parents in USA, 5–14 yrs, 58% female</td>
<td>96 controls without abuse history; 94 abused (35% with a depressive disorder)</td>
<td>Dimensional depression ratings: K-SADS-PL, CBCL, &amp; MFQ. AD use: no information</td>
<td>Saliva</td>
<td>EWAS (450K) &amp; candidate-genes NR3C1, BDNF, FKBP5, SLC6A4</td>
<td>↓ 3 genes from EWAS (GRIN1, ID3, TPPP) with depression, o candidate-genes after correction</td>
<td>Adjusted for age, sex, ethnicity</td>
</tr>
<tr>
<td>Oh et al. 2014</td>
<td>Caucasian case-control studies, 18–75 yrs: MZ twin pairs from Australia, UK and Netherlands, 80% female; Post-mortem QSBB &amp; SMRI samples, 22.5% female; bipolar cohort sperm samples, 0% female.</td>
<td>100 MDD discordant; 41 depression (30 with psychosis), 30 control brain samples; 17 bipolar, 17 control sperm samples</td>
<td>MDD/depression: SSAGA/ CIDI (Twin Study). Brain samples: not indicated. AD use: not indicated</td>
<td>White blood cells. Prefrontal cortex brain tissue. Sperm</td>
<td>8.1K CpG island micro-arrays, validation of top hits using pyrosequencing</td>
<td>A number of differentially methylated loci in cohorts, but no overlap: 44 in MZ twins, 40 in QSBB, 35 in SMRI, 34 in sperm samples</td>
<td>Matched for age, other details not indicated</td>
</tr>
<tr>
<td>Domschke et al. 2014</td>
<td>German hospital patient cohort, 45–50 yrs, 65% female</td>
<td>94 MDD</td>
<td>MDD: SCID-I. AD: patients given 6-week treatment &amp; response assessed with HAM-D</td>
<td>Blood</td>
<td>SLC6A4, promoter</td>
<td>↑ AD response. o 5-HTTLPR &amp; rs25531</td>
<td>Adjusted for age, smoking, chronicity of depression</td>
</tr>
<tr>
<td>Kang et al. 2015</td>
<td>Hospital patients Korea, 35–90 yrs, 41% female</td>
<td>108 MDD</td>
<td>MDD: SCID, depression severity: HAM-D. AD: 12-week treatment</td>
<td>Blood</td>
<td>SLC6A4, promoter</td>
<td>↑ family history. o depression age onset, severity, duration, ↓ trend AD response</td>
<td>None, but no associations between age, gender, education, prior depression and methylation</td>
</tr>
<tr>
<td>Okada et al. 2014</td>
<td>Case-control study. Cases are Japanese hospital patients, 21–62 yrs, 46% female</td>
<td>50 MDD, 50 healthy controls</td>
<td>MDD: unstructured interviews, medical records, MINI. Depression severity: HAM-D. AD: 6-week treatment &amp; follow-up for 40 patients</td>
<td>Blood</td>
<td>SLC6A4, promoter</td>
<td>o MDD, o baseline AD, o 5HTTLPR, ↑ CpG3 with AD response, ↑ CpG76 with severity</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Olsson et al. 2010</td>
<td>Australian participants from longitudinal Victorian Adolescent Health Cohort study, adolescents, 55% female</td>
<td>25 depression, 125 healthy controls</td>
<td>Assessed at 8 waves over 10 yrs. Depression: CIS-R ≥ 11. Persistence of depression: no. of waves CIS-R ≥ 11 (excluding wave 8), cases: no. of waves ≥ 5. AD use: not indicated</td>
<td>Buccal</td>
<td>SLC6A4, promoter</td>
<td>o depression, overall or within gene subregions o 5HTTLPR, ↑ both depression and s-allele carriers</td>
<td>Examined effect of alcohol, tobacco, cannabis</td>
</tr>
<tr>
<td>Reference</td>
<td>Study characteristics</td>
<td>Methylation analysis, N</td>
<td>Depression diagnosis(a) and AD treatment</td>
<td>DNA Source</td>
<td>Gene, region</td>
<td>Methylation association</td>
<td>Adjustment</td>
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<tr>
<td>Philibert et al. 2008(^{67})</td>
<td>Iowa Adoption Studies participants, USA, 93% White, 20–60 yrs, 50% female</td>
<td>68 MDD lifetime history, 124 healthy controls</td>
<td>Depressive symptom: counts derived from SSAGA-II. MDD ≥ 5 symptoms. AD use: not indicated</td>
<td>EBV-transformed lymphoblast</td>
<td>SLC6A4, promoter</td>
<td>o MDD. Trend for negative expression correlation. ↑ female sex</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Zhao et al. 2013(^{68})</td>
<td>MZ twins from Vietnam Era Twin Registry, Caucasian, 48–61 yrs, 0% female</td>
<td>43 depression discordant, 41 concordant pairs</td>
<td>Current depressive symptoms/severity: BDI-II. MDD: SCID (DSM-III). AD use: not indicated</td>
<td>Leukocytes</td>
<td>SLC6A4, promoter</td>
<td>↑ intra-pair differences in depression &amp; methylation, ↑ depression o 5-HTTLPR</td>
<td>Adjusted for BMI, smoking, physical activity, alcohol; no effect from AD use</td>
</tr>
<tr>
<td>Kim et al. 2013(^{69,70})</td>
<td>Stroke hospital patients, Korea, 35–87 yrs, 41% female</td>
<td>80 prevalent depression (25 persistent), 28 incident, 136 controls</td>
<td>Depression: MINI. Depression severity: HADS-D &amp; HAM-D at baseline &amp; 1 year. AD use: considered</td>
<td>Blood</td>
<td>SLC6A4, promoter, BDNF, I promoter</td>
<td>↑ for 5HTTLPR/s only. ↑ prevalence &amp;incident. o rs6265</td>
<td>Considered a number of potential covariates</td>
</tr>
<tr>
<td>D’Addario et al. 2012(^{71})</td>
<td>MDD patients and controls in Italy (further details not provided)</td>
<td>41 MDD, 44 controls</td>
<td>MDD: SCID. AD: all patients stable treatment (with/without mood stabilizers)</td>
<td>PBMCs</td>
<td>BDNF, promoter</td>
<td>↑ MDD, ↑ AD without stabilizers</td>
<td>Matched for age</td>
</tr>
<tr>
<td>Dell’Osso et al. 2014(^{72})</td>
<td>Comparison of unipolar and bipolar depression (further details not provided)</td>
<td>61 unipolar, 50 bipolar, 43 MDD, 44 controls</td>
<td>Unipolar/bipolar/MDD: SCID. AD: all patients stable treatment</td>
<td>PBMCs</td>
<td>BDNF, I promoter</td>
<td>↑ MDD &amp; depressed vs bipolar</td>
<td>Matched for age; no association between gender, AD and methylation</td>
</tr>
<tr>
<td>Fuchikami et al. 2011(^{73})</td>
<td>Case-control study. Cases are Japanese hospital patients, 20–70 yrs, 53% female</td>
<td>20 MDD, 18 controls</td>
<td>MDD: MINI. Depression severity: HAM-D. AD use: not indicated</td>
<td>Blood</td>
<td>BDNF, I &amp; IV promoter</td>
<td>↑ I promoter, o IV promoter</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Song et al. 2014(^{74})</td>
<td>Japanese employees, 20–60 yrs, 9% female</td>
<td>Quartiles with lowest &amp; highest depression, 90 each (from 360)</td>
<td>Non-specific depressive symptoms: Kessler’s K6. AD use: not indicated</td>
<td>Saliva</td>
<td>BDNF overall &amp; exon 1 promoter using 450K</td>
<td>↓ average (but both ↓ &amp; ↑ at individual sites). ↓ promoter</td>
<td>None, but only alcohol use differed between groups</td>
</tr>
<tr>
<td>Tadic et al., 2013(^{75})</td>
<td>German hospital patients, mean 44.9 yrs, 49% female</td>
<td>39 MDD</td>
<td>Depression: HAM-D. AD: 2–4 week response monitored</td>
<td>Leukocytes</td>
<td>BDNF, exon IV promoter</td>
<td>↑ AD response</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Alt et al. 2010(^{76})</td>
<td>Post-mortem samples, Dutch brain bank, 45–90 yrs, 42% female</td>
<td>6 MDD, 6 controls with no psychiatric history</td>
<td>Clinical patient history for lifetime MDD. AD/antipsychotics: all cases, 1 control using anxiolytic at death</td>
<td>Several brain regions</td>
<td>NR3C1, promoter regions 1J, 1E, 1B, 1F</td>
<td>o MDD</td>
<td>Matched for gender, age, brain weight, post-mortem delay, CSF fluid pH</td>
</tr>
</tbody>
</table>

(Continued)
<table>
<thead>
<tr>
<th>Reference</th>
<th>Study characteristics</th>
<th>Methylation analysis, N</th>
<th>Depression diagnosisa and AD treatment</th>
<th>DNA Source</th>
<th>Gene, region</th>
<th>Methylation association</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na et al. 201477</td>
<td>Korea University Hospital, including MDD outpatients, 18–65 yrs, 72% female</td>
<td>45 MDD, 72 healthy controls</td>
<td>MDD: SCID. Depression: HAM-D (excluding psychotic features). AD: excluded users</td>
<td>Blood</td>
<td>NR3C1, 1F</td>
<td>↓ 2 CpGs, mixed associations with hippocampal volumes</td>
<td>Matched for age, gender; excluded low IQ, neurological diseases, brain lesions</td>
</tr>
<tr>
<td>Melas et al. 201378</td>
<td>Swedish participants from a longitudinal population study, 23–74 yrs, 100% female</td>
<td>82-93 depression, 83-92 controls (no psychiatric history/treatment)</td>
<td>Depression (including MDD, dysthymia, &amp; mixed anxiety depression); MDI. AD use: not indicated</td>
<td>saliva</td>
<td>NR3C1, 1F, MAOA, exon I</td>
<td>↑ early parental death, mediated by MAOA low activity allele, ↓ depression</td>
<td>Matched for childhood adversity, adjusted age, smoking, type of adversity</td>
</tr>
<tr>
<td>Bayles et al. 201278</td>
<td>Case-control population-based study in Australia, 30–50 yrs, 42% female</td>
<td>4 MDD, 4 controls, 5 before and after treatment</td>
<td>MDD: MINI, HAM-D, BDI ≥ 18. Cases untreated at recruitment, AD: given 12-week treatment</td>
<td>White blood cells</td>
<td>SLC6A2, 2 promoter regions</td>
<td>o MDD, ↑ AD</td>
<td>Excluded participants with medical problems</td>
</tr>
<tr>
<td>Dempster et al. 200679</td>
<td>Post-mortem SMRI brain samples</td>
<td>15 depression, 15 controls (non-psychiatric)</td>
<td>Reported history of depression. AD use: not indicated</td>
<td>cerebellum</td>
<td>COMT, 2 promoter CpG sites</td>
<td>o depression, rs4680, rs737865, rs165599</td>
<td>Not indicated</td>
</tr>
<tr>
<td>Klempan et al. 200990</td>
<td>Post-mortem QSBB samples, 18–55 yrs, 0% female</td>
<td>4 MDD suicide victims, 4 controls with no suicidal behaviour</td>
<td>MDD: proxy SCID-I, clinical history, psychological autopsies. Most cases undiagnosed at death, AD use minimal</td>
<td>cortical &amp; sub-cortical brain regions</td>
<td>QKI (quaking homolog, KH domain RNA binding), promoter &amp; exon I</td>
<td>o MDD and expression</td>
<td>Matched for age; considered a number of potential covariates</td>
</tr>
<tr>
<td>Powell et al. 201391</td>
<td>GENDEP participants, part of a 12-wk pharmacogenetic study, European, 19–72 yrs, 59% female</td>
<td>113 MDD (moderate-severe), randomly selected from cohort</td>
<td>MDD: SCAN semi-structured interview. Depression severity: MADRS. AD response: % change in MADRS from baseline to 12-weeks. All individuals drug-free 2 weeks before study</td>
<td>blood</td>
<td>IL11, CpG island in intron</td>
<td>AD response: ↓ CpG5, ↑ CpG4 (escitalopram), ↓ CpG4 (nortriptyline), ↑ CpG11 &amp; GG of rs1126757</td>
<td>Age, gender, centre, baseline MADRS score, AD, considered previous medication, duration of depression, recent stressful events</td>
</tr>
</tbody>
</table>

(Continued)
### Table 2. Continued

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study characteristics</th>
<th>Methylation analysis, N</th>
<th>Depression diagnosis* and AD treatment</th>
<th>DNA Source</th>
<th>Gene, region</th>
<th>Methylation association</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rotter et al. 2010&lt;sup&gt;82&lt;/sup&gt;</td>
<td>Hospital patients in Germany, 18–75 yrs, 52% female</td>
<td>11 MDD, 18 healthy controls</td>
<td>MDD: SCID-II Depression severity: HAM-D, BDI. AD: patients using treatment</td>
<td>Blood</td>
<td>Orexin A, promoter</td>
<td>o MDD. o expression overall or in specific CpGs</td>
<td>Matched for gender &amp; age; excluded other medical problems, no association between gender or body weight and methylation</td>
</tr>
<tr>
<td>Zill et al. 2013&lt;sup&gt;83&lt;/sup&gt;</td>
<td>Hospital inpatients in Germany, population controls, Caucasian, 19–76 yrs, 64% female</td>
<td>81 MDD, 81 controls</td>
<td>MDD: SCID, HAM-D score ≥ 17. AD use: not indicated</td>
<td>Blood</td>
<td>ACE, promoter</td>
<td>† MDD, † ACE serum levels</td>
<td>Ethnically matched and adjusted for age, gender</td>
</tr>
</tbody>
</table>

*All diagnoses based on DSM-IV criteria, unless indicated.

Methylation association: † = increased levels (hypermethylation); † = decreased levels (hypomethylation); o = no association significant.

**Notes:** MDD, major depression; AD, antidepressants; HAM-D, Hamilton Rating Scale for Depression; SCID-I, Structured Clinical Interview I for DSM Disorders; BDI/II, Beck Depression Inventory I/II; CIDI, Composite International Diagnostic Interview; CIS, Clinical Interview Schedule; DSM, Diagnostic and Statistical Manual of Mental Disorders; MDI, Major Depression Inventory; MINI, Mini-International Neuropsychiatric Interview; PHQ-9, Patient Health Questionnaire; SADS, Schedule for Affective Disorders and Schizophrenia; SCAN, Schedule for Clinical Assessment in Neuropsychiatry interview; (S)MFQ, (short) mood and feelings questionnaire; SSAGA, Semi-Structured Assessment for the Genetics of Alcoholism; MADRS, Montgomery–Åsberg Depression Rating Scale; HADS-D, Hospital Anxiety and Depression Scale; CBCL, Child Behaviour Checklist; QSB, Quebec Suicide Brain Bank (Canada); SMRI, Stanley Medical Research Institute (USA); MZ, monozygotic twin pair; PBMC, peripheral blood mononuclear cells; EWAS, epigenome-wide association study; MeDIP-seq, methylated DNA immunoprecipitation and high-throughput sequencing; 27K, Illumina Human Methylation 27K Beadchip; 450K, Illumina Human Methylation 450K Beadchip; CHARM, comprehensive high-throughput relative methylation.
(mean age 45, 23% female) relative to controls. Genes subject to differential methylation included CPSF3, LASS2, PRIMA1 and ZNF263, though all failed to replicate in an independent cohort (n = 27). An EWAS of saliva from maltreated children, (n = 190, age range 5-14 years, 58% female), identified three genes that were differentially methylated in 33 abused children with depression, vs controls: ID3, TPPP and GRIN1. In keeping with this latter finding, genetic association studies have previously implicated GRIN2A and -2B in psychiatric disorders.

Dempster et al. performed an EWAS in buccal cells from monozygotic twins (n = 18 pairs discordant for self-reported depression, mean age 16.8 years, 72% female) and found several methylation associations in buccal tissue. Ten of the top-ranked genes were independently investigated in the cerebellum (n = 29, age range 29-68 years, 41% female), with two showing associations with depression: STK32C, which ranked first in the EWAS (average 2% methylation difference, p = 0.03) and DEPDC7, which ranked eighth (average 3% methylation difference, p = 0.03). Another EWAS study of monozygotic adult twins discordant for MDD (n = 27 and 23 pairs respectively; 86% female), failed to find genome-wide significance in whole blood. However, subsequent replication of the four highest ranked differentially methylated genes in an independent cohort (n = 354, 100% female) identified zinc finger and BTB domain containing 20 (ZBTB20) as hypermethylated in patients with major depression (effect size = 28.2%, p = 0.018). ZBTB20 is associated with hippocampus integrity, and plays a role in neurogenesis and neurodevelopment. Deletion of ZBTB20 in mice has previously been associated with impaired memory and neuroplasticity. Recently, the dosage imbalance of ZBTB20 has also been linked to several neurodevelopmental, cognitive and psychiatric disorders. Results of this study support current research suggesting that depression may arise due to structural impairments of neurons, though the association between ZBTB20 methylation and depression has not yet been independently replicated.

Astrocytic abnormalities have been identified in MDD as well as depressive psychopathologies and suicide. In a two-step study, Nagy et al. characterized individuals with astrocytic dysfunction, and then conducted an EWAS comparing the prefrontal cortex of suicide completers with depression and low expression of astrocytic markers (cases), and healthy controls. They found GRIK2 and BEGAIN as the two most differentially methylated regions (DMRs) between suicide completers and sudden death controls. GRIK2 is found in astrocytes, and polymorphisms of GRIK2 have previously been implicated in mood disorders. Furthermore, mood stabilizers were found to lower GRIK2 levels in mouse astrocytes. Methylation analysis revealed that GRIK2 is hypomethylated in cases compared with controls (effect size ± 20% across all sites, range of p < 0.001, 0.01 or 0.05 at nine of 13 CpG sites). The authors postulate that hypomethylation influences alternative splicing of GRIK2. BEGAIN, on the other hand, is hypermethylated in cases, showing a dramatic average 3-fold increase within clusters. This is among the largest reported effect size in the field of psychiatric epigenetics (p < 0.05, 0.01). The differentially methylated region (DMR) of BEGAIN has both gene promoter and enhancer functionalities, and in vitro reporter-based assays indicated that DNA methylation of this region almost completely abolishes these activities. This is interesting, but further studies are needed to determine the generalizability and biological significance. It is also possible that this marker is specific to depression in the presence of astrocytic dysfunction and therefore its relevance to depression more globally requires investigation.

Overall, EWAS studies have provided some interesting insights into the pathophysiology of depression, though results are generally not yet corroborated by independent replication. Despite this, EWAS have the advantage of being able to interrogate information across the genome, potentially identifying new methylation variants and genes associated with disease aetiology. With improving technologies, such studies are becoming increasingly recognized as a valuable discovery approach in psychiatric (and other) disorders. However, several limitations are present in EWAS, particularly with regard to current costs associated with such approaches. that generally limit sample size. Other issues of note include: (i) suboptimal and biased interrogation of the genome, which is an issue for most current epigenetic analyses (see below); and (ii) varying sensitivity and reproducibility associated with different platforms, including batch effects contributing to technical error. Furthermore, the issue of statistical analysis and interpretation of EWAS data remains an important one, with no consensus yet reached on how this should be accomplished. Analysis approaches range from examining individual CpG sites with appropriate adjustment for multiple testing, to data reduction approaches such as investigating regional differential methylation (DMRs), or clustering analysis to identify groups of CpG sites. Another issue to consider includes the possibility of obtaining statistical significance even for very small effect sizes of questionable biological significance. Stringent p-values for EWAS are necessary to minimize the risk of type 1 errors; however this necessitates larger sample sizes, sufficiently powered to detect true associations if they are indeed present. A comprehensive discussion of the main considerations in
the design and analysis of EWAS is reviewed in great detail by Michels et al.97 and Rakyan et al.96 Ongoing attempts to standardize EWAS approaches would be a great step in improving the reliability of results and thus the possibility of replicating findings across different studies.

Candidate gene approaches, on the other hand, require smaller sample sizes (less stringent adjustment for multiple testing), making them logistically simpler to perform. However, the clear disadvantage of such approaches is their narrow focus on genes previously implicated in depression, thus prohibiting the discovery of new markers or systems involved in the disorder. As mentioned above, most studies examining epigenetics in depression have taken this approach (summarized in Table 2 according to the candidate gene examined). The most widely investigated genes have logically been those previously implicated in depression through genetic association studies and where preliminary evidence of gene-environment interactions is available. This includes SLC6A4, BDNF and NR3C1,42 all described above.

A total of seven candidate gene studies have investigated SLC6A4 promoter methylation in depression,64–68,70,100 which has been associated with decreased mRNA levels.66,67 Findings are mixed, and likely relate to the differences in tissues, depressive phenotype and consideration of genetic factors (including ethnicity). The earliest paper investigating SLC6A4 found a trend of higher promoter methylation in adults (mean age 40, 50% female) with a history of lifetime depression (n = 68) vs 124 healthy controls.67 However, DNA for this analysis was obtained from virally transformed lymphoblasts cultured in vitro, which has potential to alter DNA methylation profiles.67,101 The same gene region was investigated using DNA extracted from buccal tissue, in a study of adolescent depression (n = 150, depression prevalence 16.7%).66 Although no independent association between SLC6A4 methylation and depression or genetic variants was found, adolescents with both higher methylation and the short (s) allele of the 5HTTLPR polymorphism had a higher risk of persistent depression in stratified analysis (at five out of eight promoter subregions). However, these results were preliminary, as the sample size was too low to satisfactorily model joint effects.66 Okada et al. found that in whole blood (n = 100, age range 21–62 years, 46% female), methylation levels of the promoter region were not associated with either depression or genotype independently, though methylation at one CpG site was positively correlated with depression severity (r = 0.30, p = 0.03).65 Potential gene-methylation interactions were not examined. Furthermore, methylation levels prior to antidepressant treatment were correlated with response to antidepressants (r = 0.36, p = 0.02).65 Importantly, this was corroborated by the results of Domschke et al. who found that SLC6A4 promoter hypomethylation predicted impaired antidepressant response (26% difference in HAM-D % change after 6 weeks, p = 0.005).100 One mechanism which could explain this is that a hypomethylation-related increase in SLC6A4 expression decreases serotonin availability, hence counteracting the serotonergic effects of antidepressants. This is in contrast to a study by Kang et al. involving blood from 108 hospital patients with MDD (mean age 55, 41% female), where no association was found between promoter methylation and antidepressant response after correcting for multiple comparisons.64 Kang et al. also reported positive correlations (r = 0.28, 0.29 and 0.37 with different rating scales, p < 0.003) between depression severity and SLC6A4 promoter methylation in blood, though no association was found between the gold standard rating scale, HAM-D, and methylation.64 Likewise, Zhao et al. have reported positive associations between variation in leukocyte SLC6A4 methylation and depression in monozygotic twins (mean age 55 years), with a 10% difference of methylation levels corresponding to a difference of Beck Depression Inventory scores by 4.4 (p < 0.01).68 However in blood, post-stroke depression was positively associated with methylation at one CpG site (n = 244; effect size = 3.8%, p < 0.01). Depression severity (HAM-D) was also positively associated with methylation though, as with Olsson et al. this was only apparent in individuals carrying the 5HTTLPRs allele (r = 0.313, p < 0.01).70 Discrepancies between results may be attributed to several variations between studies and the low statistical power, with only one study having a sample size greater than 200.102 The selection and definition of cases and controls may also be an important factor influencing the results, with some studies focusing on moderate depression rather than MDD. In addition, few studies have data on antidepressant use. Again, tissue heterogeneity is also a likely contributor to differences across studies.

Studies investigating BDNF methylation have shown more consistent results than SLC6A4, with four of six reporting associations between DNA methylation and depression, depressive symptoms or antidepressant response. Fuchikami et al. investigated CpG islands in the promoters upstream of exon I and IV from 20 hospital patients with MDD and 18 controls, and found an association between depression and promoter I methylation only, with most CpG sites (29 of 35 examined) being differentially methylated (effect sizes ranging from +25.5% to −56.4%).71 The same region was investigated in 41 MDD cases and 44 controls by D’Addario et al. who found positive associations between depression and DNA methylation (controls 24.04% vs depressed 32.52%, p < 0.05).71 Although not
previously implicated in depression, Kang et al. investigated a region within the CpG island near exon IV, and found positive associations ($p < 0.05$) between DNA methylation and suicidal tendencies in depressive patients ($n = 108$, mean age 55, 41% female). All of these studies examined methylation in blood; to date, BDNF methylation in post-mortem brain studies in relation to major depression has not been directly investigated. However, post-mortem brain studies have been conducted in suicide completers, though their psychiatric diagnoses were not taken into account in analyses. Results suggest that BDNF promoter IV is hypermethylated in the Wernicke’s area of the brain of suicide completers. As BDNF is able to cross the blood-brain barrier, DNA methylation in the peripheral tissue may exert effects on neuronal tissue and vice versa, highlighting the potential utility of peripheral BDNF methylation as a biomarker.

NR3C1, which codes for the glucocorticoid receptor (GR), has been investigated in three candidate gene studies. The untranslated first exon has multiple locations, each mutually exclusive and with its own promoter. Methylation analysis of NR3C1 suggests that in lymphocytes, individual DNA methylation patterns vary across the gene’s numerous alternative promoters. Weaver’s landmark study of GR methylation in rats found an association between hippocampal methylation and maternal grooming in promoter 1F, an orthologue of the human promoter 1F. Promoter methylation was found to have an impact on transcription factor binding. Subsequent studies in depression also investigated promoter 1F, with contradictory results. Alt et al. found no association between 1F methylation and depression in a very small study of post-mortem samples ($n = 12$), though differential expression of alternate GR splice forms in the hippocampus was apparent. However, another study found hypermethylation of two CpG sites in blood samples from MDD patients ($n = 45$) vs healthy controls ($n = 72$), though effect sizes were very small ($< 1\%$, $p < 0.05$). Interestingly, hypermethylation of the 1F promoter was associated with childhood abuse severity, as well as early parental death (effect size $= 5.4\%$ at one CpG site, $p = 0.005$). Future studies of depression should therefore include information on history of childhood abuse or other early life traumatic events, as it is possible that the psychopathology of depressed patients with such a history is different to that of other depressed individuals.

Undoubtedly more studies in this area will appear in the coming years. Care must be taken in the interpretation of findings, and independent replication is of crucial importance. As aforementioned, evidence for associations is often inconsistent, perhaps owing to low effect sizes and small sample sizes (low statistical power). Population demographics also vary, mostly in terms of ethnicity; the age range of participants in most studies is wide, often covering the period from young adulthood to old age, and tools used to diagnose depression vary considerably. The tissue type utilized in analyses is critical when interpreting findings and likely contributes to inconsistencies, as could confounding factors such as underlying genetic variation. These points are discussed in future detail in section 3 below.

**Human studies of histone modification profiles**

Few studies so far have investigated the relationship between histone methylation and acetylation with depression or antidepressants (Table 3). In synapsin variant 1 (SYN1a and b), Cruceanu et al. found a positive association between H3K4 tri-methylation in brain tissue and MDD, though results showed no corresponding change in expression. A post-mortem prefrontal cortex study suggests that antidepressant use was associated with less methylation of lysine H3K27 at the BDNF promoter I, and evidence from another study suggests that this association may be causal; treatment responders show decreased H3K27me in blood after 8 weeks of antidepressant treatment, compared with baseline. This corresponds to a decrease in serum BDNF levels, which is also associated with H3K27me, suggesting an effect of histone methylation in regulating gene expression. Given the key role of histone modifications in regulation of memory and other neurological processes, additional studies will be important to fully understand the role of such processes in depression aetiology.

**Epigenetic changes associated with genetic variation**

Epigenetic variation that arises prior to a disease could be caused by several factors outside environmental influence, including underlying genetic variation and stochasticity (‘noise’). Twin studies have been an invaluable tool for untangling the factors that shape the epigenome. For example, monozygotic twins (genetically identical) consistently show different epigenetic profiles, even at birth, highlighting the importance of non-shared environment apart from heritable factors. Furthermore, dizygotic twins (sharing around 50% of genetic variants) generally show a higher discordance in the epigenome relative to MZ twins, providing evidence for the involvement of the genome in regulating the epigenome, with a recent study showing that the majority of transgenerational similarity in DNA methylation is attributable to heritable genetic effects.
Table 3. Studies investigating the association between depression and/or antidepressant use and chromatin modifications

<table>
<thead>
<tr>
<th>Reference</th>
<th>Study characteristics</th>
<th>Methylation analysis, N</th>
<th>Depression diagnosisa &amp; treatment</th>
<th>DNA source</th>
<th>Gene, region</th>
<th>Methylation association</th>
<th>Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chen et al. 2011</td>
<td>Post-mortem samples, Caucasian, 0% female, further information not given</td>
<td>18 MDD, 9 control</td>
<td>MDD: proxy-based SCID-I. AD: all patients treatment-naive; treatment given to 7 with MDD</td>
<td>Brain (BA10)</td>
<td>BDNF, IV promoter</td>
<td>↓ AD use with H3K27me</td>
<td>Excluded other psychiatric disorders, substance use. No associations with brain pH, post-mortem index, age, &amp; BDNF expression or h3k27me</td>
</tr>
<tr>
<td>Cruceanu et al. 2013</td>
<td>Post-mortem QSBB samples, 20–60 yrs, 30% female</td>
<td>18 MDD and suicide, 14 controls</td>
<td>MDD: proxy-based SCID-I, psychological autopsies. AD use: considered in analysis</td>
<td>Brain (BA10)</td>
<td>SYN (synapsin) variants, promoter</td>
<td>↑ H3K4me3 with MDD at SYN1a + b</td>
<td>No significant difference between groups in gender, age, post-mortem delay, pH and RNA integrity numbers; no effect from AD use</td>
</tr>
<tr>
<td>Lopez et al. 2013</td>
<td>Prospective study, 52% female, further information not given</td>
<td>25 MDD</td>
<td>MDD: HAM-D ≥ 24. AD: all patients treatment-naive; 8-week treatment given</td>
<td>Blood</td>
<td>BDNF, IV promoter</td>
<td>↓ H3K27me in AD responders, ↓ change depression severity with H3K27me, ↓ serum BDNF and H3K27me</td>
<td>Excluded other psychiatric disorders, general medical illness, substance use</td>
</tr>
</tbody>
</table>

Methylation association, ↑ = increased levels (hypermethylation); ↓ = decreased levels (hypomethylation); o = no association.

aAll diagnoses based on DSM-IV criteria. MDD, major depression; AD, antidepressants; HAM-D, Hamilton Rating Scale for Depression; SCID-I, Structured Clinical Interview I for DSM Disorders; BA10, Brodmann’s area; 10, QSBB, Quebec Suicide Brain Bank (Canada).
To date, the relationship between genetic variation and DNA methylation remains to be fully elucidated, although a number of important studies in this area are starting to emerge.117,118 The most obvious mechanism by which the genome can exert an effect on the epigenome is the introduction or elimination of CpG sites due to a single nucleotide polymorphism (SNP), which has been documented in type 2 diabetes.119 However, SNPs may also influence DNA methylation indirectly, at nearby (cis effects) or even distal sites (trans effects). Recent studies have begun to examine the association between genetic variants and DNA methylation in both cis and trans, revealing that cis effects predominate.120,121 In cis refers to the CpG methylation site being within 1MB of the genetic polymorphism, whereas in trans refers to distances greater than this. Such associations appear to vary across the genome; some sites may show little to no relation between DNA methylation and genetic polymorphisms.122 Still, strict relationships between genotype and epigenotype have seldom been described; more often, genetic variants affect the probability of DNA methylation rather than being definite causal factors.96,123 Another consideration, however, is how they could combine to modify depression risk. The association between genetic variants and the disorder could be mediated via its ability to influence DNA methylation, and loci with genetic variants that affect DNA methylation are termed methylation quantitative trait loci (eQTL).124 More commonly, DNA methylation status modulates the expression of genes potentially influencing disease risk.

Table 4. A summary of some major limitations in previous epigenetic and depression association studies

<table>
<thead>
<tr>
<th>Main limitations of prior studies in the field</th>
<th>Possible solutions for future research</th>
</tr>
</thead>
<tbody>
<tr>
<td>Limited focus</td>
<td>Almost all studies have explored DNA methylation</td>
</tr>
<tr>
<td>Sample size and statistical power</td>
<td>Sample size calculations are complicated by the lack of generalizable knowledge regarding DNA methylation effect size</td>
</tr>
<tr>
<td>Establishing causality</td>
<td>Widely used cross-sectional studies are generally not amenable to causal inference due to issues of confounding and reverse causation</td>
</tr>
<tr>
<td>Tissue relevance</td>
<td>Post-mortem studies of brain tissue are critical for understanding disease aetiology. Peripheral tissue less relevant to the mechanisms underlying the disorder but readily accessible and could still prove useful as biomarkers</td>
</tr>
<tr>
<td>Genetic variation</td>
<td>Most epigenetic-focused studies have failed to consider the effect of genetic variation on the epigenome in relation to depression</td>
</tr>
<tr>
<td>Replication</td>
<td>Replication is generally lacking due to differences in study methodology, including measures of depressive symptoms, tissue under investigation, timing of sampling, or measurement of epigenetic profile</td>
</tr>
<tr>
<td>Insufficient data concerning participants</td>
<td>Associations could be driven by underlying confounding factors that were not considered</td>
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SLC6A4 methylation, investigators have also examined the 5HTTLPR polymorphism which lies 960 bp upstream of the CpG island. Generally, no association between DNA methylation levels and 5HTTLPR genotype has been found.\textsuperscript{63,65,66,70} However, this variant may modify the association between DNA methylation and depression.\textsuperscript{66,70}

One study, investigating BDNF promoter 1 methylation and post-stroke depression, also assessed the association with the commonly studied BDNF variant rs6265 located over 600bp downstream, but found no evidence that this SNP modified the DNA methylation-depression association.\textsuperscript{69} Likewise, a study of COMT methylation at two CpG sites considered three common variants in this gene, all located more than 15kb away.\textsuperscript{79} They found no association between genetic variation and DNA methylation levels, nor any evidence of an interaction to modify depression risk.\textsuperscript{79} Finally, interesting findings from Melas et al. suggest that the association between methylation of NR3C1 (located on chromosome 5) and depression appears to be mediated by the u-VNTR polymorphism of the X-linked MAOA gene,\textsuperscript{28} though this has not been replicated.

In summary therefore, a number of factors interact to influence depressive phenotype, including the independent and joint influence of genetic polymorphisms, the environment and the epigenome, as illustrated in the simple schematic in Figure 1. More research in this area is needed to have a better understanding of how these factors come together to influence an individual’s risk.

Limitations in Epigenetic Studies

Despite the considerable increase in studies examining epigenetic mechanisms in depression over the past few years, there are a number of limitations to these studies that must be considered when interpreting the findings. Many of these limitations also apply to the broader field of epigenetic epidemiology, and a number of excellent more general reviews have been published recently.\textsuperscript{96,125,126}

Causality

Although studies have reported associations between epigenetic markers and depression, identifying causal processes proves difficult due to the relatively ‘plastic’ nature (both spatially and temporally) of much of the epigenome. This is commonly acknowledged in the broader field of epigenetic epidemiology\textsuperscript{126} where the vast majority of studies are cross-sectional. This is also true for depression, with epigenetic markers assessed at a single point in time making reverse causation an issue.\textsuperscript{125,127} Even the few prospective studies to date that have examined changes in depression scores over time, or response to antidepressant treatment over a given period, do not have longitudinal epigenetic data at these different time points. It therefore remains unclear to what extent the reported associations are driving the disorder, or are merely a consequence, or a combination of both. Some animal studies have obtained epigenetic information before and after experimentally-induced exposure (Table 1), therefore ruling out reverse causation. Similar experimental designs are simply not possible in humans, although longitudinal cohorts with multiple biospecimens would help provide stronger evidence towards causation. A prospective design, for example, could collect biospecimens on a large non-depressed cohort at baseline, and then follow the individuals over time to track depression incidence. Alternatively among a depressed cohort, a study could examine depression remittance over time with biospecimens collected at baseline and after remittance. The gold standard for establishing causation would involve a longitudinal study with biospecimens collected early in life prior to depression onset and followed up at multiple time points throughout the disease course, including progression or remittance. Although limited by cost and logistic factors, a number of such studies are currently under way.\textsuperscript{128}

In addition to features of the study design, with temporality being the key to inferences of causality, there are other methods that could be employed to help infer estimates of causation although these are not without their own limitations. Probably the most widely used method to ascertain causality in epidemiological studies is the Bradford Hill criteria,\textsuperscript{129} where the strength of evidence for a causal relation is weighted against a series of criteria. These include but are not limited to effect size, consistency and specificity in findings, temporality and dose-response effects. Sophisticated analysis approaches have also been developed to help test causal hypotheses, including structural equation modelling.\textsuperscript{130} A two-step Mendelian
randomization strategy to infer causation based on cross-sectional data has also been proposed, which involves first using a genetic variant as a proxy for an exposure to establish whether the exposure causes DNA methylation, and then using another genetic variant as a proxy for methylation to establish causality between DNA methylation and a disease phenotype or outcome (i.e. depression). Advantages and limitations of this method are detailed further in the paper by Relton et al.

Distinguishing whether epigenetic modifications are a cause or consequence is important for the understanding of the function of depression-associated epigenetic marks, furthering knowledge on the aetiology of the disorder as well as the identification of diagnostic and therapeutic tools. Depression is known to be associated with an increased risk of comorbidity, including cognitive, inflammatory and cardiovascular problems. Epigenetic markers identified through cross-sectional studies are therefore highly likely to reflect disease processes co-occurring with depression, even if such markers are present before depression diagnosis. However, causation arguably presents less of an issue if solely in terms of a biomarker for depression. Even without being directly involved in aetiology, a biomarker with high sensitivity and specificity for depression would improve diagnosis, allowing for accurate and early detection of cases; consequently, therapeutic interventions could potentially be introduced at an earlier time point. Likewise, this would also be the case for a strong prognostic biomarker in those diagnosed with depression.

Tissue relevance and heterogeneity
Since the epigenome is thought to drive cell-specific biology, the importance of tissue type in epigenetic studies cannot be underestimated. As the brain plays a central role in depression, it would seem an obvious choice for investigation. However the epigenome will also vary across different regions of the brain, and the choice of specific brain tissue to investigate is thus an important one. Further still, specific brain regions themselves comprise difference cell populations (astrocytes, neurons, microglia, oligodendrocytes). Use of post-mortem brain tissues, both human and animal, has provided several insights about the nature of epigenetic modifications in relation to mood disorders. Still, post-mortem studies are relatively uncommon, largely owing to the difficulty of obtaining samples. Several limitations when using post-mortem tissue are also present: methods of tissue preservation can alter the quality of biomolecules obtained, and must at least be considered during analyses; untangling real depression-related effects from confounders (such as antidepressants and other medications) can be challenging; and results obtained from post-mortem analyses will always just be descriptive associations, examined at a single point in time.

With improving technology, technical challenges in preserving brain tissue can largely be overcome, and in some cases detailed medical histories of patients can be obtained, providing insight on potential confounders. One currently insurmountable limitation in using brain tissue, then, is the obvious fact that tissue samples cannot be collected from living patients. This renders longitudinal studies focusing on the brain inconvenient, if not impossible, and establishing causal relationships with brain markers would thus prove challenging. Furthermore, one of the keys aims in behavioural epigenetic studies is the search for biomarkers of psychiatric disorders, which could lead to improvements in diagnosis and options for early intervention therapies, and these biomarkers must derive from easily obtainable tissue.

The majority of epigenetic association studies therefore utilize peripheral tissue, with most studies still choosing blood over buccal tissue or saliva samples. Given that depression appears to have more global effects on an individual’s health, with physiological changes observed in tissues other than the brain, the utility of peripheral tissue for this research cannot be discounted. None of the studies reviewed here have directly investigated the relationship between brain and peripheral epigenetic landscapes in association with depression. More generally, there have been some studies reporting gene-specific correlations between blood and brain DNA methylation profiles, though a large degree of difference is also present. Correlations across tissues are likely to be gene specific, with some demonstrating good brain-peripheral concordance, whereas others may be very weak. It has also been reported that with the exception of blood-based disorders, buccal tissue may prove more informative as a surrogate tissue than blood in EWAS. A possible reason for this relates to the developmental origins of buccal epithelial cells which, similar to the nervous system, largely derive from the ectoderm. By contrast, blood cells are mesodermally derived in early development. Given the non-invasiveness, ease and relative safety with which buccal and salivary samples can be obtained, it is likely that these will be attractive primary sources of DNA for future large cohort studies. If quantity of DNA is paramount however, blood samples still provide a much higher yield than that from either buccal tissue or saliva.

For most tissues, however, cellular heterogeneity remains an issue, one which current studies are only starting to consider. Disease-related epigenetic effects may be obscured by variation in cellular composition, especially if such effects are localized only to a small proportion of cells. Furthermore, the disorder itself could alter cellular
composition, which could bias the results. An obvious example of this in depression is inflammation, which can alter the profile of cells sampled in the blood. To overcome this, methods of adjusting for DNA methylation variability associated with cell composition differences have been developed for blood and brain, which could be incorporated in statistical models for adjustment.\(^{137,138}\) Separating tissues into homogeneous cellular constituents may also be an option in some instances.\(^ {139}\)

**Phenotypic assessment**

Variability in the way in which the depression phenotype is assessed is likely to play a role in the interpretation and harmonization of findings across different studies. The most comprehensive method of depression diagnosis is through a structured diagnostic interview using DSM\(^ {140}\) criteria and administered by a psychiatrist (i.e. the Structured Clinical Interview, SCID). However, given the cost involved, most studies rely on a structured diagnostic interview administered by a lay person, each with their own advantages and disadvantages. Depression rating scales across different instruments are generally well correlated\(^ {141}\) and, with the exception of one study which used a self-rated measure of stress rather than depression per se,\(^ {74}\) this should have little influence on the overall results. Discrepancies may still arise due to variable severity and specificity of symptoms and the presence of comorbidities; for example, Kim et al. focused on post-stroke depression which likely has a different pathophysiology.\(^ {69}\) Differences could also be due to heterogeneity in the severity and chronicity of depression across studies. Depression severity has been shown to determine disease progression and outcomes,\(^ {142}\) and severe depression (MDD) may indeed involve pathophysiological changes that may not manifest in mild or moderate depression.

**Confounding factors**

Whereas genetic association studies are usually hindered only by low statistical power, epigenetic studies can be confounded by spatial and temporal and other effects—demographic, environmental, age and genetic origins. Cellular heterogeneity, as mentioned above, is one such confounder. Other potential confounders include comorbidities, medications and dietary and lifestyle factors (such as smoking). A number of the studies described in this review adjusted for age and gender in their analysis and sampled from an ethnically homogeneous population; however, a number of analyses are still lacking these basic controls. Ageing has been shown to exert an effect on the epigenome in both in vivo and in vitro studies.\(^ {143,145,146}\) and DNA methylation levels may differ between men and women, although this finding has yet to be confirmed.\(^ {147-149,150}\) Furthermore, as the prevalence rates of depression in women are almost double those of men, with different symptoms and even different risk factors being identified, this may further highlight the need for gender-specific epigenetic association studies.

Only a few of the studies mention adjustment for lifestyle factors such as smoking and alcohol consumption, although both are more prevalent in people with depression and have themselves been associated with epigenetic modifications.\(^ {151-152,153-154}\) This extends to before birth; for example, maternal alcohol consumption may affect offspring epigenetic profile, which may persist into adulthood.\(^ {155}\) However, the correlations between neonatal and adult epigenetic profiles are incompletely understood. Comorbidities may also confound epigenetic analyses, especially considering the small effect sizes of epigenetic change in mood disorders. Incorporating the various confounding factors in epigenetic analysis will also become an important feature of future longitudinal studies which track changes in epigenetics over time and relate this to the disorder. Ageing will be an important component of the models, as will monitoring changes in covariates across these periods.

One important confounder, especially when dealing with depression, is the use of antidepressants. This can be a source of bias in analysis, due to confounding by indication. Depressed people prescribed antidepressants are likely to be substantially different from those who are not, with perhaps more severe symptoms and a willingness to seek treatment. Furthermore, animal models have shown that antidepressant treatment per se can alter epigenetic marks in the brain.\(^ {156}\) Epigenetic variability has also been observed in peripheral tissue from patients administered treatment.\(^ {157,158}\) Currently, several studies have investigated whether associations between DNA methylation and antidepressants occur; the majority of studies found no associations, though this may be limited by sample size or methodology.\(^ {58,65,78}\) In a study of chromatin modifications, Lopez et al. found that H3K27 methylation at BDNF promoter IV was decreased after 8 weeks of antidepressant treatment in patients who responded. BDNF expression also increased, which is associated with the decrease of depression severity.\(^ {110}\) However, the authors cite that this study was preliminary, awaiting results from a larger sample size (\(n = 25\) in the preliminary study). Clearly, additional research is necessary; prospective studies of antidepressant treatment on epigenetics, especially DNA methylation, are rare. Generally speaking, the degree to which antidepressants alter the epigenetic profile remains unknown, though one study using blood found no
association ($n = 108$, mean age 54.9 years). Another important reason to gather information on antidepressant use is the potential for treatment to mask symptoms and thus the underlying disorder. Patients who respond well to treatment may present with no depressive symptoms during study recruitment, and be inadvertently assigned to a control group, obviously introducing classification bias into the analysis. The use of medications must therefore be accounted for, and tested directly in animal models, where possible. Studies may opt to present exclusion criteria, incorporate the effect of antidepressants on both mood disorder phenotype and epigenetics into their statistical models or consider participants taking antidepressants as a separate experimental group. Detailed medical histories of participants are often useful and necessary, though they can be difficult and laborious to obtain.

Other considerations in study design

Unlike genetic information, which typically does not vary between tissues, methylomic information varies across tissues, cells, alleles and, very rarely, DNA strands. This, along with the reportedly small effect sizes of epigenetic change in psychiatric illness, especially when compared with the epigenetic changes that can be observed in *in vitro* studies of cancer, provides a challenge for the accurate measurement of DNA methylation. There is also uncertainty about what level of difference could constitute a biologically meaningful effect. Adding to the difficulty, factors such as cost and lack of resources hinder sample size and, by extension, the statistical power to detect subtle changes in epigenetic profile. Large EWAS studies are still very expensive and the offset between cost and sufficient power needs to be carefully weighted, as detailed in a recent review by Rakyan et al. A consequence of underpowered studies is the low reproducibility of results, which is a pervasive problem across biological research in general. Results from underpowered studies are less likely to generate true differences, often exaggerating effect size. Yet estimating sample sizes required for future studies presents a real challenge in the field. Power calculations can only be made with some knowledge of the degree of epigenetic variation across groups, and to date relatively few data on this exist. This is compounded further by the tissue, gene, region, context and disease-specific nature of epigenetic variation across the genome. Recommendations concerning sample size will continue to evolve as new data become available, and Bayesian inference may become more widely used in this context. Therefore, replication remains crucial in behavioural epigenetics, with sample sizes adequately powered to detect at least the effect sizes observed in the original study they are trying to replicate. Such replication studies finding no associations must also be as likely to be published as studies reporting significant findings, to avoid the ever-present publication bias. Finally, further studies examining DNA methylation should also consider 5-hydroxymethylcytosine (5hmC), which is derived from 5-methylcytosine through the addition of a hydroxyl group. 5hmC is often found in the gene bodies of highly transcribed genes and is found in abundance in the brain. It has been implicated in neurodevelopment and could be particularly important in neuropsychiatric disorders like depression, thus warranting further investigation.

Conclusions

There is a significant gap in understanding of the mechanisms by which epigenetic changes modify phenotype, particularly in association with common disorders in humans. Despite the sharp peak in research in this area, our understanding of the role of the methylome in regulating human health and disease remains in its infancy. This is particularly true in the field of psychiatric disorders and depression. The brain is an extremely complex organ, and subtle epigenomic changes in one area may indeed be able to cause profound system-wide effects. The field of psychiatric epigenetics is expanding at an exponential rate, but care must be taken not to over-interpret current findings, especially in light of the lack of clear replication to date. The field of epigenetics will waste years of research, as the field of genetics has, if more rigorous criteria are not applied to current studies.

Replication is paramount. Approaches to epigenetic studies vary, and no single approach can adequately address every outstanding issue present in epigenetic epidemiology. Both animal models and human studies are critical; animal models are useful in untangling causation and environmental exposures, whereas human studies allow insight into the unique physiological processes of human-specific biology. Many of the current epigenetic studies are quite focused, and placing the findings within the broader context of mood disorder pathophysiology may prove challenging. Furthermore, future studies must also consider genetic variation, given the now well-documented role of underlying genetic variation in determining the DNA methylation status of many genomic loci. Despite this, the potential to identify biomarkers for depressive disorder remains a tantalizing possibility, especially given the potential for large well-controlled longitudinal studies with multiple biospecimens collected over time, as well as the continued advances in epigenetic technology.
underlying depression. In addition to considering genomics, as described above, the integration of epigenomic data with transcriptomic, proteomic, metabolomic and other ‘omic’ data will enable a greater understanding of the functional significance of inter-individual differences observed, and the interactions between different biological systems in influencing depression risk.

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References


106. Rakyan VK, Chong S, Champ ME et al. Transgenerational inheritance of epigenetic states at the murine Axin(Fu) allele occurs after maternal and paternal transmission. *Proc Natl Acad Sci USA* 2003;100:2538–43.


