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Abstract

Epigenetic mechanisms regulate gene expression, influencing protein levels and ultimately shaping phenotypes during life. However, both stochastic epigenetic variations and environmental reprogramming of the epigenome might influence neurodevelopment and ageing, and this may contribute to the origins of mental ill-health. Studying the role of epigenetic mechanisms is challenging, as genotype-, tissue- and cell type-dependent epigenetic changes have to be taken into account, while the nature of mental disorders also poses significant challenges

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for linking them with biological profiles. In this chapter, we summarise the current evidence suggesting the role of DNA methylation as a key epigenetic mechanism in major depressive disorder.

Keywords

Epigenetics • DNA methylation • Major depressive disorders • Antidepressants

Major depressive disorder (MDD) is a prevalent mood disorder characterised by persistent low mood accompanied by significant morbidity and mortality. The lifetime prevalence of MDD is 15–20% and women suffer from MDD about twice more often than men [1]. MDD is a complex multifactorial disorder, with both genetic and environmental factors playing an important role in its development. The heritability is estimated to be approximately 37%, and numerous links have been made between genetic variation and clinical depression [2]. However, DNA sequence variations cannot fully explain the susceptibility to MDD as detecting strong and replicable genetic associations with the development and course of clinical depression have proven difficult. Moreover, exposure to known environmental risk factors for MDD such as childhood adversities does not always generate the disorder. Therefore, the development and course of MDD are thought to be explained by gene-environment (GxE) interactions, where the effect of the environment depends on a person's genotype or, equivalently, the effect of a person's genotype depends on the environment [3]. Thus, it can be suggested that individuals genetically vulnerable to mental illness might undergo structural brain changes, imbalances in multiple neurotransmitter systems, alterations in neurotrophic signalling, and neuroendocrine abnormalities when exposed to harmful environmental factors at critical times during neurodevelopment.

A consistent line of evidence in human and rodent studies has shown that environmental factors regulate gene transcription and epigenetic mechanisms emerged as prime candidates for mediating GxE interactions in several brain regions [4]. In this chapter, we focus on DNA methylation, representing the most studied epigenetic mechanisms in psychiatric research. We will first summarise the current stage of knowledge within this field indicating the potential contribution of DNA methylation to MDD and discuss the results of the most replicated human studies on DNA methylation alterations in relation to environmental exposures associated with MDD. Finally, we will discuss the current challenges and perspectives in the field of epigenetic research on MDD.

10.1 DNA Methylation and MDD

Several methods have been applied to investigate MDD-associated and stress-induced alterations in DNA methylation. In the following sections, we will discuss the observed candidate and methylome-wide associations in MDD.

10.1.1 Candidate Gene Studies

Most DNA methylation studies thus far used candidate gene approaches. Impairments in stress response pathways, neurotrophic signalling and monoaminergic systems are well-known processes involved in the pathogenesis of MDD. Therefore, the focus of candidate gene methylation studies was predominantly on the promoter sequences of genes involved in these biological pathways. Here, we will address a selection of the main findings of the first studies on candidate genes implicated in MDD.

10.1.2 Stress Reactivity Genes

Given the role of the hypothalamic-pituitary-adrenal (HPA) axis and early life experiences and the aetiology of MDD, a series of studies investigated the DNA methylation patterns of the NR3C1 gene. NR3C1 encodes the glucocorticoid receptor (GR), which is known for its regulatory role in dampening the activity of the HPA axis. Environmental reprogramming of NR3C1 gene expression has been shown in both the brain and periphery. Both human and rodent studies suggested that early life trauma is associated with significant hypermethylation in the promoter region of the alternate exon 1_F (humans) or 1₇ (rodents) within the hippocampus and, subsequently, reduced GR expression [5, 6]. Increased methylation levels of the NR3C1 promoter have been reported in lymphocytes of newborn prenatally exposed to maternal depression [7, 8]. One study examined a functional association between methylation statuses of NR3C1 gene promoter and cortisol as the end product of the stress pathway. A decreased cortisol response to the dexamethasone/corticotropin-releasing hormone (CRH) test was associated with increased methylation levels exon 1_F NR3C1 gene promoter in leukocytes [9].

FK506-binding protein 5 (FKBP5), a member of the immunophilin protein family, functionally interacts with GRs and is linked to environmental stress exposure and MDD. FKBP5 protein reduces glucocorticoid-binding affinity [10], while cortisol and its binding to GR induce FKBP5 expression [11]. Several studies have shown the interactive effects of polymorphisms in FKBP5 and early life adversities predicting MDD [12]. Decreased allele-specific methylation of FKBP5 has been observed in peripheral blood cells of subjects who have experienced childhood abuse, whereas a similar effect has been observed in a neuronal progenitor cell line after exposure to GR agonists [13].

10.1.3 Neurotrophic Signalling Genes

Brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family and has been shown to regulate the development, plasticity and survival of dopaminergic, cholinergic and serotonergic neurons. The neurotrophic hypothesis of depression suggests that low levels of BDNF, concomitant with reduced neuronal

and synaptic plasticity, are associated with clinical depression [14]. The BDNF protein is abundant in the brain and periphery and known to be able to cross the blood-brain barrier [15]. Chronic stress-induced decreases in BDNF and, in particular, antidepressant-induced increases in BDNF have been extensively studied in relation to the development and course of MDD [16]. Therefore, the correlation between BDNF methylation status and MDD has already been a focus of interest for years [17]. Differential regulation of BDNF exons I, IV and IX expression has been reported repeatedly, accompanied by changes in (allele-specific) DNA methylation of the corresponding promoters within various brain regions as well in the blood of patients with MDD [18]. The majority of studies have found increased methylation levels at different loci within the BDNF gene in MDD patients compared to controls in both brain and the periphery [19]. However, the direction of the effects is not always congruent [20].

To explore the functional relevance of DNA methylation variation and MDD, some studies examined the association between DNA methylation at the BDNF gene and structural changes in the brain of MDD patients. As such, in patients with MDD, the prefrontal and occipital cortices have been indicated as regions in which BDNF promoter hypermethylation is associated with reduced cortical thickness [21]. Another study showed a correlation between BDNF promoter hypermethylation and reduced white-matter integrity in individuals with MDD [22]. Significant associations have been found between BDNF promoter VI hypermethylation and a history of suicidal attempts and suicidal ideation [23]. In two subsequent studies, the same group found a significant association between BDNF promoter VI methylation levels and late-life depression [24], as well as depression related to breast cancer [25].

Methylation status of BDNF promoters has also been studied as a predictor for antidepressant treatment. Differential methylation of BDNF promoter I was observed when comparing responders and nonresponders to electroconvulsive therapy (ECT) [26]. Likewise, the methylation state of a CpG site within the exon IV promoter region of BDNF has been suggested to predict responses to antidepressant pharmacotherapy [27].

10.1.4 Monoaminergic Transporter Genes

The monoamine hypothesis of depression highlights the importance of serotonin (5-HT) turnover and transmission in synaptic cleft in the pathophysiology of major depression [28]. In particular, genetic variation in SLC6A4, the gene encoding the serotonin transporter (5-HTT or SERT), is a well-known candidate gene studied in MDD. 5-HTT is responsible for the reuptake of 5-HT into the presynaptic neuron and the primary target of antidepressants to normalise 5-HT levels and, consequently, the clinical symptoms of depression. SLC6A4 polymorphisms and their interaction with environmental stress have been studied extensively in association with MDD [29]. However, studies on the link between genetic variation of SLC6A4, life events and MDD are characterised by

inconsistent findings, which may be partly explained by the additional and possibly interdependent role of epigenetic variation. Hypermethylation in the promoter region of *SLC6A4* in lymphoblast cell lines was accompanied by lower gene expression and associated with vulnerability to MDD, concomitant with a complex interaction with genetic variation in *SLC6A4* [30]. Clearly, these findings await replication.

Increased buccal cell methylation levels have been observed in short-allele carriers of the *SLC6A4* gene in association with depressive symptoms [31]. In addition, increased *SLC6A4* promoter methylation status was significantly associated with childhood adversities, a family history of depression, but not antidepressant treatment outcomes [32]. However, another study showed that lower average *SLC6A4* CpG methylation was associated with an impaired antidepressant treatment response [33]. Thus far, all studies with regard to the association between DNA methylation within the *SLC6A4* gene have been performed on peripheral blood tissues. A number of studies have examined the association between *SLC6A4* promoter methylation and structural and functional changes in the brain. Using positron emission tomography (PET), decreased 5-HT synthesis in the orbitofrontal cortex was shown to be correlated to increased *SLC6A4* promoter methylation [34]. In addition, increased methylation of the *SLC6A4* promoter has been shown to be associated with increased hippocampal volume assessed by voxel-based morphometry [35]. Increased *SLC6A4* methylation has furthermore been associated with childhood trauma and decreased hippocampal volume [36].

10.2 Methylome-Wide Association Studies

Methylome-wide association studies (MWAS) using different platforms have shown distinguished patterns of DNA methylation in MDD (see Table 10.1). A study comparing post-mortem frontal cortex tissue from MDD patients and healthy controls identified 224 differentially methylated regions (DMRs). These regions were highly enriched for genes involved in neuronal growth and development. However, the technique that has been used to measure the methylation levels for this study could not provide information at a single CpG resolution [37]. Another MWAS on the prefrontal cortex of suicidal completers with depression and sudden death controls showed 115 DMRs mainly related to astrocytic functioning [38]. Findings from MWAS performed on blood samples of individuals with a lifetime history of depression compared to nondepressed controls provided evidence for the involvement of inflammatory pathways previously implicated in depression [39]. Another genome-wide DNA methylation profiling of peripheral leukocytes of medication-free MDD patients identified 363 hypomethylated CpG sites [40]. In this study, three CpG sites residing in *DGKH*, *GSK3B* and *SGK1* genes have been previously implicated in MDD, and they could show a significant inverse correlation between *GSK3B* promoter DNA methylation and expression. A methylome-wide study that incorporated an environmental risk factor reported an association

Table 10.1 Summary of MWAS and twin studies

References	Study characteristics	Tissue type	Platform	Main findings
[39]	33 lifetime history of MDD, 67 C	Peripheral blood	HM27	– Increased methylation in genomic regions related to brain development while decreased methylation in regions related to lipoprotein
[37]	39 MDD, 26 C	Post-mortem frontal cortex	CHARM (3.5 million CpGs)	– 224 candidate regions with methylation differences more than 10%. PRIMA1: the best replicated and validated
[38]	Discovery 76 MDD, 45C; replication cohort: 22 MDD, 17 C	Prefrontal cortex	MBD2-Sequencing	– GRIK2 and BEGAIN as the two most differentially methylated regions (DMRs) between suicide completers and sudden death control
[40]	20 medication-free patients with MDD and 19 C Replication: 12 medication-free patients with MDD, 12 C	Peripheral leukocytes	HM450	– A significant inverse correlation between the GSK3B promoter DNA methylation and expression was observed
[41]	94 maltreated children (35% with MDD), 96 C	Saliva	HM450	– 3 genome-wide-significant predictors of depression: ID3, GRIN1, TPPP
[45]	50 MZ pairs discordant for MDD (27 UK, 23 Australia) Case-control replication: 118 MDD, 236 C	Whole blood	MeDIP-Sequencing	– Hypermethylation within the coding region of ZBTB20 – Replicated in an independent cohort of 356 case-control individuals
[43]	18 MZ pairs discordant for MDD Case-control replication: 14 MDD, 15 C	Buccal cells Post-mortem brain (cerebellum)	HM450	– STK32C hypomethylation associated with MDD – Validated in post-mortem brain case-control individuals
[44]	17 MZ twin pairs (four concordant, six discordant, seven healthy)	Peripheral blood	HM450	– Differentially DNA methylated in the WDR26 gene associated with MDD

Table 10.1 (continued)

References	Study characteristics	Tissue type	Platform	Main findings
[51]	171 MZ twin pairs	White blood cells (100) Prefrontal cortex (71)	8.1 K CpG island microarrays	– Differentially modified regions exhibited a highly significant number of overlaps across the tested tissues and previous studies

HM27 Illumina Infinium Human Methylation 27, *HM450* Illumina Infinium Human Methylation 450 Beadchip, *CHARM* comprehensive high-throughput arrays for relative methylation, *C* control, *MDD* major depressive disorder, *MBD2* methylation-binding domain-2, *MeDIP* methylated DNA immunoprecipitation

between a history of childhood maltreatment and changes in saliva DNA methylation within three genes (*ID3*, *GRIN1* and *TPPP*), of which the degree of methylation predicted depressive symptoms. These genes are known to be involved in stress-related neuroendocrine pathways and neural plasticity [41]. A recent genome-wide profiling of cortical brain regions (*BA11* and *BA25*) from MDD suicide cases compared to controls identified a DMR, upstream of the *PSORS1C3* noncoding gene, which is consistently hypomethylated across both cortical brain regions in MDD patients [42].

10.2.1 Twin Studies

Phenotypic discordance in monozygotic (MZ) twin pairs provides a valuable tool to differentiate genetic from non-genetic causes of diseases. Utilising MZ twin designs allows ruling out DNA sequence variations as a confounding source for epigenetic studies. While the genomic content in MZ twins is almost identical, the discordance in phenotypes can be attributed to non-shared environmental and stochastic factors. It has been suggested that different levels of DNA methylation at specific loci within MZ pairs measured as differentially methylated positions (DMPs) can be linked to the environmental causes of disease, while the changes in methylation variance, measured as variably methylated probes (VMPs), may be related to stochasticity. There is increasing evidence suggesting that epigenetic variation between MZ pairs plays a role in the aetiology of MDD. However, using buccal and blood cells, studying MZ pairs discordant for MDD has not identified any methylome-wide significant loci after correcting for multiple testing, which is most likely the result of a lack of statistical power. Interestingly, several of the most differentially methylated genes have previously been associated with the pathogenesis of MDD (see Table 10.1) [43, 44]. A meta-analysis of 50 MZ pairs, all female discordant for MDD, using 8.1 K human CpG island microarrays, identified 17 DMRs with genome-wide significance, and some of these genes have previously been associated with MDD (see Table 10.1) [45].

10.3 DNA Methylation and Antidepressant Treatment

There is increasing evidence suggesting that the effectiveness of antidepressants might be associated with DNA methylation status of certain genes. One example is the finding that decreased DNA methylation of SLC6A4 promoter region was associated with impaired antidepressant treatment response [33]. On the contrary, another study showed that less improvement in clinical symptoms of depression was correlated with higher methylation percentage at SLC6A4 gene promoter region [32].

Epigenetic mechanisms have also been proposed to mediate the mechanism of action of antidepressants. Alterations in DNA methylation and enzymes catalysing the methylation processes have been suggested to be involved in therapeutic effects of tricyclic antidepressants (TCA), selective serotonin reuptake inhibitor (SSRI) and valproate (VPA) [46, 47].

A number of MWA studies investigated the methylation changes in newborns exposed to maternal antidepressant use during pregnancy. Exposure to antidepressants, regardless of the medication family, was shown to be associated with differential methylation of CpG sites in the TNFRSF21 and CHRNA2 genes [48]. Another genome-wide methylation study identified increased methylation levels at CpG sites in CYP2E1, EVA1 and SLMAP in SSRIs exposed neonates [49]. Moreover, various studies compared the methylation changes associated with maternal use of antidepressants in candidate genes such as NR3C1, BDNF and SLC6A4 [27, 50]. However, results were not conclusive and none have been replicated in genome-wide array-based approaches.

10.4 Discussion and Future Perspectives

Over the past few years, a considerable number of epigenome-wide and candidate gene studies have been performed to identify a relation between DNA methylation and the pathogenesis of MDD. Current state of evidence on the link between DNA methylation and MDD indicates limited overlap between the identified genes in different studies. Moreover, the significant associations identified in candidate gene studies have not been observed in MWAS. These inconsistent findings can be explained by a broad range of methodological concerns in the field of epigenetic psychiatry. Evidently, limited access to human brain tissue is a major challenge in studying the specific epigenetic patterns in mental illnesses. Moreover, DNA methylation is characterised by the addition of a methyl or hydroxymethyl group to the C5 position of cytosine. DNA hydroxymethylation can both act as a stable functional epigenetic mark and may also serve as an intermediate mark in the active process of DNA demethylation. However, the large majority of current commercially available techniques that have been used to study epigenetic variation in MDD do not distinguish between DNA methylation and other related epigenetic marks, e.g. DNA hydroxymethylation, limiting the power of such approaches. Furthermore, true epigenetic variations can be influenced by state- or disease-specific differences in the cellular composition of both brain and peripheral blood

tissues. In addition, significant findings remain vulnerable to confounding factors such as age, gender and smoking, which might cause false association discoveries between MDD and DNA methylation marks. Similarly, disease-related factors like medication, heterogeneity in diagnostic tools, the onset and course of MDD in different studies might explain the observed inconsistency in findings. Moreover, thus far, no MWAS of MDD has been integrated with genomic data. As such, genetic variations known to predict variations in the methylome should be taken into account in future approaches. Larger sample sizes and meta-analytical approaches can be utilised to boost statistical power in order to identify methylome-wide significant genes in association with MDD or the effects of antidepressants. Evidently, heterogeneity between studies, though strengthening the value of observed converging evidence, at the same time challenges the consolidation of less robust findings within individual datasets. Replication studies of epigenetic findings are therefore highly necessary. In addition, it will be crucial to move from associations and correlations towards examining causality. For instance, *in vivo* epigenetic editing of identified targets, as well as using longitudinal study designs in human cohorts would significantly improve our understanding about the causal relationship between changes in DNA methylation and the development and course of MDD.

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