Epigenetics in mood disorders

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Abstract Depression develops as an interaction between stress and an individual's vulnerability to stress. The effect of early life stress and a gene–environment interaction may play a role in the development of stress vulnerability as a risk factor for depression. The epigenetic regulation of the promoter of the glucocorticoid receptor gene has been suggested as a molecular basis of such stress vulnerability. It has also been suggested that antidepressive treatment, such as antidepressant medication and electroconvulsive therapy, may be mediated by histone modification on the promoter of the brain-derived neurotrophic factor gene. Clinical genetic studies in bipolar disorder suggest the role of genomic imprinting, although no direct molecular evidence of this has been reported. The role of DNA methylation in mood regulation is indicated by the antimanic effect of valproate, a histone deacetylase inhibitor, and the antidepressive effect of S-adenosyl methionine, a methyl donor in DNA methylation. Studies of postmortem brains of patients have implicated altered DNA methylation of the promoter region of membrane-bound catechol-O-methyltransferase in bipolar disorder. An altered DNA methylation status of PPIEL (peptidylprolyl isomerase E-like) was found in a pair of monozygotic twins discordant for bipolar disorder. Hypomethylation of PPIEL was also found in patients with bipolar II disorder in a case control analysis. These fragmentary findings suggest the possible role of epigenetics in mood disorders. Further studies of epigenetics in mood disorders are warranted.

Keywords Biopolar disorder · DNA methylation · Environment · Epigenetics · Mood disorders · Stress vulnerability

Introduction

It is clear that both genes and the environment confer risk for mood disorders. A relative recent development in the field of biological psychiatry has been the focus on attempts to understand functional outcomes of the additive and combinatorial effects of genes and the environment at the molecular level [1]. As such, the interplay between a relatively fixed genome and an often variable environment involves epigenetic factors.

Epigenetic changes are long-lasting modifications in gene function that do not involve changes in gene sequences. Recent evidence suggests that these changes may occur in both dividing and nondividing cells [2–5] and may be transmitted intergenerationally [6, 7]. Epigenetic mechanisms involve modifications of the functional unit of the genome, the nucleosome, which is composed primarily of an octamer of pairs of H2A, H2B, H3, and H4 histones, around which is wrapped a 147-bp segment of DNA [8]. This configuration allows for the regulation of transcription through the control of access to the gene. Although many epigenetic modifications influence gene regulation, in the context of molecular psychiatric analysis, the most prevalently studied modifications to date are DNA methylation of CpG dinucleotides and acetylation and methylation at the N-terminal tails of histones. DNA is methylated by the transfer of a methyl group from S-adenosyl methionine

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(SAM) by DNA methyltransferase (DNMT) enzymes. Methylation of the promoter region of genes is generally associated with the inhibition of transcription factor binding to *cis*-acting regulatory sequences and the recruitment of repressor complexes, including methyl CpG binding proteins (MBDs), resulting in transcriptional repression [9, 10]. Histone modifications confer what has been called a 'histone code' on the genome, defining parts of the genome that are accessible to transcription in a given tissue type at a given time [11]. For example, acetylation of the ninth lysine residue on histone 3 (H3–K9) is classically associated with active transcription and open chromatin [12]. The role of histone methylation is less clear, as it can either enhance or repress transcription depending on the histone modified [8]. Because enzymes that deacetylate histones (HDACs) are known to recruit transcriptional repressors, such as MBDs, patterns of methylation and acetylation are intimately linked. Pharmacological manipulations, including drugs of abuse, such as cocaine and alcohol, as well as a mood stabilizer, such as valproate, have been shown to modulate chromatin function by influencing the activity of these enzymes. Recent evidence also suggests that chromatin remodeling as a result of environmental perturbations plays a role postnatally in processes that affect behavior.

In this review, evidence suggesting that epigenetic factors might influence the development of mood disorders, such as depression and bipolar disorder, are introduced.

Epigenetic studies relevant to depression

Clinical evidence suggesting the role of epigenetics in depression

Depression is not only one of the most prevalent causes of mental suffering [13, 14] but also places an enormous economic burden on society [15]. The accumulated evidence from epidemiological studies suggests that genetic predispositions interact with the environment in potentiating depression [16, 17]. Aversive life events potentiate depression in some individuals and resiliency in others. Such interindividual variation may be mediated by variations of neurotrophic and neurotransmitter systems. A polymorphism in the brain-derived neurotrophic factor (BDNF) coding region, producing pro-BDNF with either a methionine or a valine in position 66, has been associated with depression in several populations [18, 19]. With respect to neurotransmitter systems, particular emphasis has been placed on serotonin dysfunction in depression. For example, individuals carrying a common short variant of a repetitive sequence in the serotonin transporter gene in a region controlling transcription (5-HTTLPR, the

serotonin transporter gene-linked polymorphic region) show increased neuroticism or harm avoidance relative to individuals homozygous for the long variant of the 5-HTTLPR. There is some controversy as to whether or not the polymorphic region confers greater risk of depression per se. A longitudinal study found that individuals carrying the short 5-HTTLPR variant showed more depressive symptoms as a function of stressful life events, while the carriers of the long 5-HTTLPR variant did not show increase of depression associated with stress. This result suggested a possible gene environmental interaction [20], although this is still controversial [21]. In the brain, these changes might be reflected in differential functional connectivity between areas, including the hippocampus, as a function of life stress [22].

Finally, there is evidence in a variety of species, including humans, nonhuman primates, and rodents, that early postnatal care (or early life stress) influences the risk for depression in adulthood. In humans, there is considerable evidence that early childhood abuse or neglect increases the risk of depression as well as other psychopathologies [23–25]. Patterns of abuse and neglect may be transmitted intergenerationally from mother to daughter in both humans and in non-human primates [26]. In rhesus macaques, infants cross-fostered from non-abusive mothers to abusive mothers, and infants cross-fostered from abusive mothers to non-abusive mothers showed levels of abuse in adulthood similar to that of their adoptive mothers, suggesting transmission via an epigenetic mechanism that remains to be defined [27]. Other studies have shown that early childhood adversity in humans [17] and non-human primates [28] enhances stress reactivity in adulthood. These data imply that resiliency to stress has a protective effect against depression. Similar studies in rodents have recently shed light on possible molecular mechanisms of these effects.

Epigenetic regulation of stress vulnerability in rodents

The laboratories of Michael Meaney and Moshe Szyf, working with a rodent model of maternal care, were the first to demonstrate a mechanism of epigenetic regulation of stress [12]. In rats, naturally occurring variations in maternal care have been shown to regulate the expression of the glucocorticoid receptor (GR) in the hippocampus of offspring. This effect is stable into adulthood, and recent evidence suggests that it is epigenetically regulated [6]. Rat mothers show large individual differences in licking and grooming (LG) of pups during the first week of life. Pups reared by 'high' LG mothers (at least 1 SD above the mean) show less anxiety-like behavior and a more rapid recovery from stress than do pups reared by 'low' LG (at

least 1 SD below the mean) mothers [29]. Interestingly, these differences appear to be transmitted non-genomically, as female pups exhibit maternal behavior characteristic of their foster mother [30]. In addition, pups from high LG litters cross-fostered to low LG litters, and vice versa, exhibit behavioral and physiological responses to stress in adulthood that are characteristic of their foster environment [30]. These effects are mediated, at least in part, by alterations in the hypothalamic–pituitary–adrenal axis function, including enhanced glucocorticoid negative feedback sensitivity due to an increase in GR in the offspring of high LG mothers. Glucocorticoid receptor expression is regulated at the level of RNA, through splice variation in the $5'$ untranslated region (UTR) of exon 1 [31].

Alterations in maternal behavior are associated with alterations in the expression of GR, including differences in the expression of the exon 1_7 transcript [31, 32]. DNA methylation of the $GR1_7$ promoter has recently been shown to be greater in the offspring of low LG mothers than in those of high LG mothers [6]. These differences in DNA methylation emerge during the first week of life in parallel with differences in maternal behavior, and they are remarkably stable into adulthood. Nevertheless, this epigenetic programming is reversible by pharmacological manipulations later in life. Specifically, infusion of Trichostatin A (TSA), an HDAC inhibitor, eliminated the hypermethylation of $GR1_7$ in rats from low LG litters [6], whereas central infusion of the methyl donor L-methionine enhanced the methylation of $GR1_7$ in rats from high LG litters [33]. These data provide a first example of epigenetic programming by the social environment and suggest that DNA methylation may be malleable in postmitotic neurons.

Role of histone modification in antidepressive treatment action

A complete understanding of the mechanisms of action of common physiological interventions used to treat depression, including monoamine oxidase (MAO) inhibitors, tricyclic antidepressants, such as imipramine, and electroconvulsive shock (ECS) therapy has remained elusive, in part because of the relative stability of symptoms and the delayed behavioral response to treatment with these methods [8]. All antidepressants are known to increase levels of monoamine neurotransmitters at the synapse [34]. In addition, several treatments have recently been implicated in chromatin remodeling. For example, the histone demethylase BHC110/LSD1, which bears a strong sequence homology with MAO, was shown to target dimethyl Histone 3 at lysine 4 (H3K9) for demethylation in vitro [35]. Furthermore, transcriptional activity at genes targeted by BHC110 was enhanced by increases in

dimethyl H3K9. These results implicate epigenetic mechanisms in the activity of MAO inhibitors.

Eric Nestler and colleagues have experimental documentation of the associations between histone modifications and changes in behavioral function in response to antidepressant treatment and ECS in the hippocampus of rodents, a brain region implicated in depression [8, 36, 37]. In mice subjected to chronic social defeat stress, chronically administered imipramine produced a selective hyperacetylation of histone H3 at the BDNF III and BDFN IV promoters as well as increased H3K4 dimethylation at the BDNF III promoter [37]. These changes were concomitant with an enhancement of BDNF transcription in these mice. In contrast, such hyperacetylation was not observed in nondefeated control mice. In addition, levels of the histone deacetylase HDAC5 decreased and HDAC9 increased with social defeat stress in imipramine-treated mice relative to controls, whereas drug treatment or stress alone had no effect. Finally, the overexpression of HDAC5 blocked the effect of imipramine in the social defeat paradigm. Thus, although BDNF expression increased in both the control and the socially defeated mice, the observed histone and HDAC modifications with imipramine treatment occurred only in the context of social defeat stress. The data provide a new explanation for the delayed onset of action of antidepressants in the treatment of depression. It should be noted that an observed increase in dimethylation of H3K27 as a result of social defeat stress was not reversed by imipramine.

In another study, Tsankova et al. [36] examined histone modification 30 min, 2 h and 24 h after acute or repeated ECS in rats. These researchers observed significant differences in the levels of H4 acetylation in both the c-Fos and cAMP regulatory element binding protein (CREB) promoter regions, and a significant decrease in acetylated H4 after 24 h in the chronic ECS only, together with decreases in expression. Electroconvulsive shock leads to H3 phosphoacetylation in the c-Fos promoter in both the acute and chronic conditions, but only in the chronic condition for the BDNF. The regulation of BDNF also differed between chronic and acute ECS conditions. For the BDNF II promoter, acetylated H4 decreased significantly 24 h after chronic ECS treatment, whereas during the same time interval there was an increase in acetylated H4 in the acute condition. However, there was no change in the levels of acetylated H4 in the BDNF II promoter. In addition, in the chronic condition only, phosphoacetylated H3 increased in the BDNF II promoter but decreased in the BDNF III promoter. These changes accompanied increased transient levels of mRNA in the acute condition and 24 h after the chronic condition. Interestingly, acetylated H3 was observed only in the chronic ECS condition for both the BDNF II and BDNF III promoters. These differences in

histone modifications accompanying acute versus chronic ECS may be instructive because the stimulus was identical and differed only in the frequency of its application. Much as is the case of antidepressant medication, a significant response to ECS treatment in depression depends upon chronic administration in patients. The findings demonstrate that several long-lasting histone modifications occur in response to chronic ECS conditions, leading to long-term effects on gene regulation. In addition, the histone modifications that accompanied ECS were idiosyncratic – not only to the type of ECS treatment but also dependent on the gene promoter analyzed. These results provide further credence to the aforementioned notion that a histone code of specific modifications determines the regulation of a specific gene (and of specific promoters within a given gene [11]. The characterization of this histone code for other genes affected by depression should lead to further advances in treatment.

Epigenetics in bipolar disorder

Genomic imprinting

In the genomic imprinting phenomenon, a maternally or paternally transmitted allele is inactivated by DNA methylation and hemiallelic expression is observed. Many imprinted genes are related to development. Transmission patterns of genetic diseases caused by the mutation of imprinted genes are complex, because their influence on offspring depends on the gender of the parent who transmitted the mutated allele. Such gender differences of transmission observed in the transmission of diseases caused by mutations of imprinted genes are referred to as a parent-of-origin effect (POE) [38–40].

Bipolar disorder may be transmitted from a mother more often than from a father [40]. When bipolar disorder is transmitted from the father, the offspring tends to have a more severe form of the illness [41] and an earlier age of onset [42] than when it is transmitted from the mother; in addition, there is a higher prevalence with the former [43]. Based on evidence suggesting the role of genomic imprinting in bipolar disorder, Gershon et al. [44] performed a linkage analysis in which they assessed the gender of the transmitting parent. These researchers found a linkage with chromosome 18 only in paternal transmission. Nothen et al. [45] confirmed that the linkage of bipolar disorder with 18p11.2 could only be seen in paternally inherited pedigrees, and McInnis et al. [46] reported that they observed linkage with 18q22 only in paternally transmitted pedigrees.

These pieces of evidence prompted the search for imprinted genes on chromosome 18. Corradi et al. [47] found that GNAL at 18p11.2, which encodes a G protein alpha subunit, may be a candidate of an imprinted gene. Although they showed that the promoter region of this gene is methylated, no evidence of allele specific methylation was shown.

Recent linkage analyses have used software to calculate the linkage based on the assumption of paternal or maternal imprinting. Such studies have detected several additional linkage loci suggestive of imprinting: 13q12, 1q41 [46], 2p24-21, 2q31-q32, 14q32, and 16q21-q23 [48]. Transmission disequilibrium tests in trio samples also revealed the association of several genes when the gender of the transmitting parent was taken into consideration [49, 50]. None of these linkages or findings in association studies suggestive of imprinting have as yet been validated by molecular biological experiments to show allele-specific expression or methylation. Apparent POE does not always represent genomic imprinting but can be caused by several mechanisms [40]. Indeed, although we found the association of polymorphisms of HSPA5 at 9q33-34.1 with bipolar disorder only in paternal transmission, this gene showed biallelic expression in the brain, which ruled out the possibility that HSPA5 is imprinted in the brain, at least in the prefrontal cortex [51].

Using a machine learning approach, Luedi et al. searched for imprinted genes in the mouse genome and reported that several genes on the candidate loci of bipolar disorder (13q13 and 18q22) might be imprinted [52]. However, this prediction also awaits experimental validation. In addition, DNA methylation status may differ between mice and humans.

In summary, genomic imprinting in bipolar disorder has only been suggested by statistical genetics and, to date, there has been no molecular biological evidence to support this possibility.

None of the findings in linkage analysis and genetic association studies in bipolar disorder have been consistently replicated. One possible explanation for the lack of consistency is false positive findings due to multiple statistical testings. It should be cautioned that an analysis considering the possibility of genomic imprinting not only provides a clue to understanding the pathophysiology of the illness but also increases the probability of false positive results, if the results are not adequately validated by experiments.

Pharmacology

Lithium is the best established mood stabilizer, having antimanic, antidepressive, and prophylactic effects on bipolar disorder. Valproate is the next most widely used mood stabilizer, having robust antimanic effects, putative prophylactic effects, but no antidepressive effects.

Although the mechanism of action of valproate on bipolar disorder remains controversial, HDAC inhibition is proposed as one of mechanisms [53]. Neuroprotective effects are common to these two major mood stabilizers, lithium and valproate [54], and the neuroprotective effect of valproate may be mediated by HDAC inhibition [55, 56]. Valproate also enhances neuronal differentiation in neural progenitor cells by HDAC inhibition [57]. These findings suggest a possible role of histone acetylation, which is coupled with DNA methylation, in the pathophysiology of bipolar disorder. However, the role of other mechanisms, for example inositol depletion and the increase of bcl-2 on the mitochondrial membrane, have also been suggested, and it is still not known whether HDAC inhibition is crucial for the effect of valproate on bipolar disorder.

S-adenosyl methionine supplies a methyl residue in a DNA methylation reaction. Many studies have found SAM to have antidepressive effects [58]. Interestingly, Carney et al. [59] reported that nine of 11 patients with bipolar depression treated with SAM switched to mania, suggesting a specific effect of SAM on bipolar depression. As mentioned above, central infusion of L-methionine, a precursor of SAM, increased DNA methylation of the promoter of the GR gene. Methionine treatment was found to abolish the effect of a high LG mother on the offspring, as shown by the decreased DNA methylation status of GR and the inhibition of behavioral despair [33]. The fact that SAM, which similarly enhances DNA methylation, is effective in the treatment of depression is apparently contradictory to the effect of methionine. S-adenosyl methionine is a methyl-residue donor not only for the DNA methylation reaction but also for other enzymatic reactions. For example, creatine is produced from SAM and guanidinoacetate, and SAM treatment increases the phosphocreatine level in the brain [60]. This effect may also contribute to the antidepressive effect of SAM because decreased phosphocreatine levels have been reported in bipolar depression [61].

The antimanic effect of valproate, which inhibits HDAC and decreases DNA methylation, and the antidepressant effect of SAM, which increases DNA methylation, together indirectly suggest a role for DNA methylation in the symptoms of mania and depression in bipolar disorder. However, this is still a preliminary hypothetical mechanism, and to date there has been no study focusing on the role of DNA methylation in mania and depression.

DNA methylation analysis in postmortem brains

Abdolmaleky et al. recently examined the DNA methylation status of the promoter of membrane-bound catechol-O-methyltransferase (COMT) [62], an enzyme which regulates the level of dopamine and which is regarded as a candidate gene in bipolar disorder. A methionine to valine substitution at site 158 (Val158Met), which alters enzyme activity, was reported to be associated with bipolar disorder [63, 64], although this result is still controversial [65]. The COMT gene has two promoters, each generating its own mRNA isoform: the membrane-bound isoform (MB-COMT) and the soluble isoform (S-COMT), respectively. Abdolmaleky et al. [62] examined the methylation status of the MB-COMT promoter in the prefrontal cortex (Brodmann's area 46) by means of a methylation-specific PCR analysis. Although this region of the genome was predominantly unmethylated, a weak methylation signal could be detected. While 60% of 35 controls in the Stanley Microarray Collection samples showed some PCR product obtained from the methylated allele, only 29% of 35 patients with bipolar disorder and 26% of 35 patients with schizophrenia showed a methylation signal. This difference was statistically significant. Subjects with a methylation signal showed significantly lower expression levels of MB-COMT than those not showing a methylation signal in postmortem brain samples obtained from the Harvard Brain Tissue Resource Center [62]. This study suggested the possible role of hypomethylation of the promoter of MB-COMT in bipolar disorder and schizophrenia.

In contrast, Dempster et al. [66] analyzed the DNA methylation status of the promoter of S-COMT using Pyrosequencing in 60 postmortem brain samples obtained from the Stanley Neuropathology Consortium. These researchers analyzed two CpG sites, site 1 and site 2, corresponding to cytosine 27 and 23, respectively, of an earlier study [67] because these two sites, among the six sites studied, were found be partially methylated in many brain regions. Site 1 showed 45.4% methylation, while site 2 showed 34.5% methylation. Dempster et al. [66] found that although the methylation status of the two CpG sites analyzed showed a high correlation ($r = 0.8$, $P < 0.001$), there was no difference in DNA methylation status between diagnoses, and DNA methylation status was not correlated with the mRNA level of COMT.

DNA methylation differences between discordant twins

In spite of extensive linkage and association studies of bipolar disorder during the past two decades, the results are still not conclusive. No causative gene nor genetic risk factor has been established for bipolar disorder. In an attempt to identify the molecular pathogenesis of bipolar disorder, we have been focusing on monozygotic twins discordant for bipolar disorder.

Within this framework, we performed gene expression analysis of lymphoblastoid cell lines obtained from two

pairs of monozygotic twins discordant for bipolar disorder and found that 17 genes, including XBP1, HSPA5, ECGF1, and ATF5, were commonly down-regulated in both of the twins. Because XBP1 is a endoplasmic reticulum (ER) stress response-related transcription factor which regulates HSPA5, we focused on XBP1 [68]. Although we initially reported that a functional polymorphism of XBP1 was associated with bipolar disorder, this association was not replicated in subsequent studies [69, 70]. We also reported a weak but significant association of bipolar disorder with HSPA5 [51]. The induction of XBP1 upon ER stress was diminished in lymphoblastoid cells derived from patients with bipolar disorder [68]; this result was recently replicated in larger number of samples [71], supporting the role of the ER stress pathway in bipolar disorder.

More recently, Matigian et al. [72] performed a gene expression analysis in three pairs of monozygotic twins discordant for bipolar disorder. These researchers suggested that the WNT pathway is altered in bipolar disorder. While they did not demonstrate the down-regulation of XBP1 and HSPA5 in these three pairs of monozygotic twins discordant for bipolar disorder, they did show the down-regulation of ECGF1 and ATF5 [72]. ATF5 may also be related to the ER stress pathway and interact with DISC1, the most established causative gene for schizophrenia and mood disorders. However, our single nucleotide polymorphism (SNP) analysis did not support the association of ATF5 with bipolar disorder [73].

We postulated that the observed differences in gene expression between twins might be caused by a difference in DNA methylation status [74]. Although it has been reported that differences in DNA methylation were observed between monozygotic twins discordant for schizophrenia [75–77], there have been no such studies in bipolar disorder. The DNA methylation status of XBP1 did not differ between twins [68]; therefore, we began a comprehensive search for genes showing differential DNA methylation patterns between discordant twins. To this end, we employed a molecular biological technique developed by Ushijima and colleagues [78], called MS-RDA (methylation-sensitive representational difference analysis), which was initially developed to search for genes differentially methylated in cancer tissue. This method, which consists of digestion by methylation-sensitive restriction enzyme and subsequent subtraction, is able to selectively amplify differentially methylated genomic regions. This method had been used for the detection of differentially methylated genes from a pair of genomic DNAs obtained from one individual, one sampled from cancer tissue and the other from neighboring normal tissue. These DNAs had the same genomic sequences but different DNA methylation statuses. This method cannot be used for a case control analysis because DNA sequence differences complicate the

results. However, this method can be used for the analysis of monozygotic twins having the same genomic sequences. Applying this method to a pair of monozygotic twins discordant for bipolar disorder, we isolated ten DNA fragments derived from CpG islands or putative promoters [79]. Among these ten fragments, DNA methylation differences in four regions was confirmed by bisulfite sequencing between the bipolar twin and control co-twin. Fraga et al. [80] reported that DNA methylation differences between monozygotic twins increase with age. Consequently, the differences in DNA methylation in discordant twins may not always be related to the pathophysiology of the illness.

To test the pathophysiological significance of DNA methylation, Kuratomi et al. [79] performed a case control analysis using Pyrosequencing and found an altered DNA methylation status of spermine synthase (SMS) in female patients with bipolar disorder. However, DNA methylation had increased in the bipolar patients, while it had decreased in the bipolar twin. On the other hand, this case control analysis also found a decreased methylation status of PPIEL (peptidylprolyl isomerase E-like) in the affected twin and patients with bipolar II disorder. The DNA methylation status of PPIEL was significantly correlated with its mRNA expression level ($R = -0.81$) and also with the DNA methylation levels in peripheral leukocytes ($R = 0.41$). Because PPIEL is a primate-specific gene, further analysis is not easy. However, we found that mRNA levels of PPIEL were lower in the frontal cortex and hippocampus and highest in the substantia nigra and pituitary gland [79]. These findings suggest that this gene may be involved in dopaminergic neurotransmission and/or neuroendocrine systems. Although it is not known whether the reduced DNA methylation status of PPIEL is a causative factor for bipolar II disorder or the result of the disease, it may in some way be related to the pathophysiology of the illness.

Discussion

As summarized above, epigenetic studies of bipolar disorder have just begun. The results of clinical genetic studies carried out to date suggest the role of genomic imprinting, but no study has yet been reported that directly tests this hypothesis. Pharmacological studies imply that pharmacological manipulation of DNA methylation status might alter mood states, but this has also not been tested experimentally in humans. There have been several studies recently that directly examine the DNA methylation status in samples obtained from patients.

In the case of schizophrenia, the results of DNA methylation analyses of RELN (reelin gene) in postmortem brains are not concordant. Two groups reported increased methylation in schizophrenia [81, 82], but two groups did

not [83, 84]. This discrepancy could be caused by methodological problems, such as the process of sodium bisulfite treatment, PCR bias, or cloning bias. Several studies used the methylation-specific PCR method, which has been successfully used for other applications, such as the diagnosis of imprinting [85] or the detection of hypermethylation in cancer [86]. Although methylation-specific PCR would be useful to analyze such "all-or-none" phenomena, it might not be ideal for the quantitative analysis of DNA methylation in the study of psychiatric illness. For the quantitative analysis of a large number of clinical samples, Pyrosequencing analysis of bisulfite-treated DNA [66, 79] or real-time PCR quantification of DNA digested by a methylation-sensitive restriction enzyme [83] would, in practical terms be useful approaches. Although none of these studies have used serially diluted standard samples to calibrate the DNA methylation levels, such approach may also be useful to obtain reliable and reproducible results in future studies.

The nature of the tissue used for DNA methylation analysis is also critical. In the case of lymphoblastoid cell lines, the DNA methylation status can be potentially affected by transformation by the Epstein-Barr virus; consequently, results in lymphoblastoid cells should be interpreted with caution. In the case of DNA methylation analysis in the brain, tissue heterogeneity might potentially affect the results [87] because brain tissue contains many cell types, including neurons and glia. The ideal approach would be to separate specific cell types in the brain, but the amount of DNA required for DNA methylation analysis currently hampers such approaches. Further technical development is necessary for future studies.

Conclusion

Epigenetic studies focusing on mood disorder are very recent developments. The results of several initial findings, however, seem quite promising; such as alterations in DNA methylation of the GR gene associated with maternal care and stress vulnerability, altered histone modification associated with antidepressive treatments, altered methylation status of COMT in the brains of patients with bipolar disorder and altered DNA methylation status of PPIEL in lymphoblastoid cells of patients with bipolar disorder. Further studies are needed to clarify the biological basis and pathophysiological significance of these findings.

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