FEATURE REVIEW

Molecular studies of major depressive disorder: the epigenetic perspective

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Major depressive disorder (MDD) is a common and highly heterogeneous psychiatric disorder encompassing a spectrum of symptoms involving deficits to a range of cognitive, psychomotor and emotional processes. As is the norm for aetiological studies into the majority of psychiatric phenotypes, particular focus has fallen on the interplay between genetic and environmental factors. There are, however, several epidemiological, clinical and molecular peculiarities associated with MDD that are hard to explain using traditional gene- and environment-based approaches. Our goal in this study is to demonstrate the benefits of looking beyond conventional 'DNA + environment' and 'DNA × environment' aetiological paradigms. Epigenetic factors – inherited and acquired modifications of DNA and histones that regulate various genomic functions occurring without a change in nuclear DNA sequence – offer new insights about many of the non-Mendelian features of major depression, and provide a direct mechanistic route via which the environment can interact with the genome. The study of epigenetics, especially in complex diseases, is a relatively new field of research, and optimal laboratory techniques and analysis methods are still being developed. Incorporating epigenetic research into aetiological studies of MDD thus presents a number of methodological and interpretive challenges that need to be addressed. Despite these difficulties, the study of DNA methylation and histone modifications has the potential to transform our understanding about the molecular aetiology of complex diseases.
Molecular Psychiatry (2007) **12**, 799–814; doi:10.1038/sj.mp.4001992; published online 10 April 2007

Introduction

Major depressive disorder (MDD) is defined by episodes of depressed mood lasting for greater than 2 weeks accompanied by additional symptoms including disturbed sleep and appetite, reduced concentration and energy, excessive guilt, slowed movements and suicidal thoughts.[1] Depression is an extremely common disorder, ranking second in the global burden of disease,[2] with the overall lifetime risk of MDD estimated to be 16.2% in the general US population.[3] The social and economic consequences of depression are huge, eclipsing those of many other mental and somatic illnesses, and as a result funding agencies across the world have invested huge sums of money into aetiological research.

As is the norm for aetiological studies into the majority of psychiatric phenotypes, particular focus has fallen on the interplay between genetic and environmental factors. Using these traditional research paradigms, however, progress in understand-ing the neurobiology of MDD has been slow. We are some distance from our ultimate goal of revealing clear risk factors that can help in the diagnosis, prevention and treatment of major depression. The first studies into the genetics of depression began over 30 years ago.[4–8] Since then it has been repeatedly demonstrated that affective disorders run in families, with certain gene polymorphisms and environmental stressors postulated to increase susceptibility. Despite significant progress in our understanding of the probable causes of MDD, however, we are still some distance from identifying *proven* aetiological risks for depression and understanding the *mechanisms* behind their action.

Contemporary theories about the causes of depression usually separate inherited biological factors from the effect of hazardous environmental exposure. A common assumption is that 'genes', that is, DNA sequence variants, and 'environments' are the only sets of factors influencing susceptibility, and that anything not caused by the former must be owing to the latter. Only very recently have researchers started to move away from these traditional aetiological models, and look beyond the role of simple additive genetic and environmental effects. Cohort-based studies have started to address the problem of *why* only *certain* individuals – carriers of some specific

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genetic variants – exposed to a putative risk environment actually develop depression, finding evidence for interactions between specific environmental factors and genotype.[9]

There are, in fact, numerous clinical and epidemiological peculiarities associated with major depression that are hard to explain – not only in terms of traditional genetic and environmental approaches, but also by gene–environment interactions. If changes to the nuclear DNA sequence, and exposure to certain 'risky environments' is all that matters, why are so many identical twins raised in the same way discordant for the symptoms of depression? Why is the prevalence of MDD in women approximately double that seen in men after puberty? Why does depression follow such a striking developmental trajectory in women, with a sharp rise in prevalence following puberty? Why do some genes appear to increase the risk of developing major depression only if they are inherited from one parent but not the other?

Our goal in this study is to demonstrate the advantages of looking beyond the conventional 'DNA + environment' and 'DNA × environment' aetiological paradigms that dominate research into MDD. We briefly review the current state of traditional molecular genetic and environmental research into MDD and show that (1) it may be impossible to accurately estimate the relative contributions of either genes or the environment in MDD; (2) the boundary between genetic and environmental factors is less clear-cut than is widely believed and the environment may actually be a proxy for a more complex set of phenomenon containing a significant inherited component; (3) the inherited, biological component of MDD susceptibility may comprise of much more than simple DNA sequence variation; and (4) epigenetic factors, that is, inherited and acquired mechanisms regulating gene function occurring without a change in nuclear DNA sequence, can offer new insights about many of the non-Mendelian features of MDD including the discordance of identical twins, sex- and parental origin- effects, as well as some controversial findings in traditional genetic studies (see Table 1). It is our view that by focussing only on the sequence of the 'genome' and the impact of the 'environome', the importance of a third set of aetiological influences, namely those that act upon the 'epigenome', has been largely neglected.

Genes and environments: limitations to the traditional research approach

Genetics and MDD: progress, but few convincing risk loci identified

It is clear that MDD aggregates in families, and a meta-analysis of quantitative genetic studies reveals a relative risk of 2.84 for the first-degree relatives of affected sibs.[10] Conventional twin analyses have concluded that much of this familial clustering is

Table 1 The epigenetic perspective on the aetiological complexities of MDD

Aetiological complexity	The epigenetic perspective
Discordance of MZ twins	Discordance between MZ twins is traditionally attributed to non-shared environmental factors. There is increasing evidence that there are considerable epigenetic differences between MZ twins. Such differences can be stochastic or environmentally induced, and can explain phenotypic differences between genetically identical individuals.
Environmental contribution	Epigenetic factors may account for much of the variability traditionally attributed to non-shared environmental factors, and may explain why it has proven hard to identify many convincing environmental risk factors for MDD.
High heritability of MDD but slow progress in identifying risk genes	It is traditionally assumed that inherited traits result from the transmission of DNA polymorphisms, but it appears that epigenetic marks may not be fully erased during meiosis and can be transmitted intergenerationally. Epigenetic inheritance may partly explain why it has proven hard to identify specific causal gene polymorphisms in apparently highly heritable disorders like MDD.
Gene–environment interactions	A number of gene–environment interactions have been reported for MDD. To date the mechanism behind these interactions is unknown. It is now apparent that the environment can have profound effects on the epigenetic profile of the genome, and that epigenetic marks can directly link environmental factors to gene function.
High female prevalence of MDD	Skewed X-chromosome inactivation is one X-linked epigenetic process that could potentially cause the excess rates of MDD in females, and also explain female MZ twin discordance. There is also evidence that susceptibly to depression may be mediated by the hormone-specific epigenetic modification of certain genes.
Parental origin effects	The most likely mechanism behind parent-of-origin effects is genomic imprinting – the differential expression of the genetic material at either a chromosomal or allelic level depending on whether the genetic material has been transmitted from the paternal or the maternal side.

Abbreviations: MDD, major depressive disorder; MZ, monozygotic.

due to inherited factors. A recent meta-analysis of five twin studies, for example, concluded that the heritability of depression is 37%,[10] with severe, recurrent and early-onset forms of the disorder demonstrating an elevated genetic contribution. Another way to tease out the contribution of genetic and environmental factors in the aetiology of MDD is to examine the prevalence of depression in the biological and adoptive relatives of adoptees with MDD compared to matched unaffected adoptees. Only a couple of adoption studies have been performed for depression, and while these suffer certain methodological problems,[10] they provide additional evidence to indicate a strong genetic aetiology in MDD (e.g. Wender PH et al.[11]). Although behavioural genetic studies generally report similar heritabilities for males and females, several authors report significantly higher heritability in females (see e.g. Bierut et al.[12] and Kendler et al.[13]), a potentially interesting observation given the significantly higher prevalence of MDD in women. Also of note is the considerable familial comorbidity observed between MDD and other affective disorders, including anxiety and bipolar disorder, suggesting that these conditions may be aetiologically related and perhaps share common inherited risk factors.[14]

The apparently clear contribution of inherited factors to MDD led to early optimism among the psychiatric genetics research community that loci involved in aetiology would be identified with ease. As was the case with research into other forms of psychopathology, perhaps most notably schizophrenia, considerable effort has been expended on genetic linkage and association studies with researchers around the world being swept along by the tide of enthusiasm spawned by the rapid progress of the Human Genome Project in the late 1990s. It soon became apparent that no major MDD gene was going to be identified using the classical 'candidate gene' or 'whole genome linkage' approaches that were commonly employed in these studies. As with other psychiatric conditions, the widely accepted aetiological doctrine now sees the role of genes as much more complex, involving numerous loci of small effect interacting epistatically with each other and with a range of environmental pathogens. Although some progress in identifying these risk loci has undoubtedly been made, we are still a long way from being able to definitively prove the role of specific molecular factors in MDD aetiology.

Despite the publication of numerous genetic linkage scans and a plethora of association studies, progress in identifying risk loci for MDD remains slow. Results from a number of genome-wide linkage studies for MDD have highlighted a swathe of potential susceptibility regions, although there are numerous inconsistencies between studies (for a comprehensive review see Camp and Cannon-Albright[15]). Perhaps the most thorough study for MDD to date investigated linkage in 110 large extended pedigrees, comprising of 1890 individuals, with a strong family history of major depression.[16]

Significant linkage to major depression in males was identified at marker D12S1300 (multipoint heterogeneity LOD score 4.6; $P = 0.00003$) suggesting the existence of a sex-specific predisposition gene to major depression at 12q22–q23.2. Other studies, however, comprising of smaller sample sets, have highlighted a number of different chromosomal regions, with the overall picture looking highly complicated. In short, all that can be taken from the research effort expended on linkage studies in MDD to date are a number of large chromosomal regions that exhibit relatively low LOD scores, but show some degree of overlap between studies (e.g. 1p, 2q, 3centr, 8p, 12q, 15q and 18q). Although these regions may be candidates for future dissection by positional cloning, the interpretation of the available linkage data is difficult because of the considerable heterogeneity between studies resulting from differences in study design, sample recruitment and analytical methods employed.

Association studies of a priori candidate genes in MDD research have primarily focussed on genes involved in the serotoninergic system. The rationale behind this approach is that considerable evidence implicates dysregulation of this system as being pivotal in the development of depression. Of particular note is the effectiveness of selective serotonin reuptake inhibitors (SSRIs), which block the reuptake of serotonin at the synapse, in the treatment of depression. Studies have also demonstrated considerable impairment in serotoninergic function in a number of brain regions in individuals with major depression.[17] SLC6A4, located on chromosome 17q, encodes the serotonin transporter, the primary target of SSRIs and has been implicated in the aetiology of MDD by several studies. Replicated evidence exists for an association between polymorphic variants in SLC6A4 and MDD, in particular with the short allele of a repeat polymorphism in the promoter region of the gene. A meta-analysis including data from several thousand MDD patients demonstrates that the overall effect of this polymorphism is relatively small, with an overall odds ratio in individuals homozygous for the short allele being 1.16.[18] Paradoxically, this association with the short SLC6A4 allele in depression counteracts what would be predicted from functional studies of the polymorphism. The short allele is generally associated with reduced transporter activity and lower SLC6A4 expression[19] – precisely the functional effect of the SSRIs used to treat depression – although a recent study investigating allelic expression in the serotonin transporter gene found no correlation between expression levels and the promoter polymorphism.[20] Other genes that have been putatively associated with MDD include those encoding tryptophan hydroxylase,[21] brain-derived neurotrophic factor,[22,23] catechol-O-methyl transferase,[24] phospholipase A2,[25] the glucocorticoid receptor[26] and the serotonin receptor 1A,[27] although these findings still await convincing replication in other samples (see Levinson[28] a recent review).

Environmental influences: modest evidence for direct causal effects

Given that the heritability estimates for MDD are well below 100%, most quantitative geneticists have argued that, in addition to genetic factors, environmental influences are likely to be important in the aetiology of the disorder.[10] Indeed, there is circumstantial evidence to link exposure to a range of specific psychosocial environmental pathogens with the development of depression. These include exposure to stressful life events, the death of a spouse or close relative, prolonged medical illness and injuries, disability and functional decline and social isolation.[29] One caveat of studies into the environmental causes of MDD relates to the actual overall contribution that the environment makes. A major tenet of quantitative genetic theory argues that phenotypic variation not attributable to genetic factors must be environmental in origin. In this way, it is argued, all the phenotypic differences observed between monozygotic (MZ) twins, who have their entire genome in common, must be owing to non-shared environmental factors. Given that the proband-wise MZ concordance for MDD is only 31% for men and 48% for female MZ twins,[30] this theory proposes a very large non-shared environmental contribution to the aetiology of MDD. However, a recent review of numerous behavioural studies measuring the environments of twins and non-twin siblings, and relating them to differences in their developmental outcomes, has shown that whereas over 50% of phenotypic variance is accounted for by factors attributed to be 'non-shared environment', actual measured environmental variables account for only a very small proportion of this variability.[31] Furthermore, several other studies allude to the notion that behavioural differences observed between MZ twins may not be entirely environmental in origin. For example, normal MZ twins reared together show similar correlations for various behavioural characteristics as do MZ twins reared apart.[32,33] Adoption studies also indicate that the rearing environment may be secondary to inherited factors in mediating susceptibility to MDD.[11] This is certainly the case for other psychiatric disorders, often related aetiologically to depression, in which better controlled studies have been performed – for example, it has been shown that the risk of schizophrenia does not decrease if a child born to an affected parent is raised in a healthy family.[34]

Another problem is that while certain environmental factors such as serious illness, divorce, violent crime and sexual assault are strongly correlated with MDD, it is not clear whether they are directly causal in their action. As a result, teasing their effect apart from that of genes may be more difficult than is often realized. It has been demonstrated that individual exposure to environmental stressors is itself strongly influenced by genetic factors, that is, predisposed individuals may choose 'risky' environments.[35,36] The inherited risk factors for some of the environmental factors linked to MDD, in particular stressful life events, are actually strongly correlated with those influencing susceptibility to depression itself[37] indicating that the same genes are likely to be involved in both. So while exposure to stressful life events and the development of depression are almost definitely related, we can conclude relatively little about the causality behind this relationship. Another example is urbanicity, an 'environmental' factor commonly associated with MDD, schizophrenia and other forms of psychopathology.[38] The prevalence rate of depression is clearly higher in individuals residing in urban areas with numerous studies reporting a supposedly clear cause–effect relationship between city living and depression, an observation often attributed to social isolation, increased stress, poverty and a reduced role of the family unit.[39] It now appears, however, that where people live can't always be considered a true 'environmental' factor. Recent research suggests that amongst adults in Australia there is actually a clear inherited contribution to where people choose to live.[40] It is interesting to note that this finding is not universal, and may be not applicable to some densely populated countries such as the Netherlands[41] highlighting the context-dependent complexities of aetiological research.

A major dilemma facing epidemiologists investigating the impact of environmental pathogens on the development of complex disorders is that it is exceedingly difficult to initiate a well-designed, fully controlled study. The huge number of confounding variables means that the investigation of specific environmental factors, particularly in human subjects, is hard. The most powerful method with which to assess an environments' contribution to MDD aetiology would be via longitudinal randomized trial experiments in which subjects are randomly exposed to risk factors and their outcome followed up compared to controls. For obvious ethical reasons, such approaches are not viable in the investigation of variables such as severe stressful life events, and thus firm empirical evidence directly linking exposure to outcome is not available. Although animal research has given us valuable insight into the neurochemical response to environmental stressors, the degree to which these findings can be extrapolated from the laboratory rodents to real human subjects is not known.

Gene–environment interactions: causal mechanisms needed

Most behavioural geneticists would concur that the traditional idea of 'nature' and 'nurture' as distinct entities is outdated, with neither genes nor the environment likely to act in isolation to increase susceptibility. The study of gene–environment interaction effects are still in their infancy, with the first direct evidence for such interplay in the aetiology of depression being reported by Caspi et al.[9] who found that a polymorphism in the serotonin transporter gene regulates the effect of stressful life events on susceptibility to depression. Citing evidence demonstrating

that the serotonin transporter moderates the biological response to stressful experiences, they found that the effect of stressful life events on depression was significantly stronger in individuals carrying at least one 'short' allele of the *5-HTTLPR* polymorphism in a large, epidemiologically ascertained birth cohort sample. Similar findings have been subsequently reported by other groups (e.g. Kendler *et al.*,[42] Wilhelm *et al.*[43]), although both studies differ in a number of ways from the original report by Caspi *et al.*[9]

Although reports of gene–environment interactions have breathed new life into aetiological research, a number of important caveats should be considered when interpreting the results from these studies. First, given the current high level of interest in gene–environment interaction analyses and the potential for false-positive findings, credence should only be given to carefully planned hypothesis-driven research. The replication of gene–environment interaction findings is needed before firm conclusions can be drawn, but will prove extremely difficult given the unique nature of the samples used to accurately detect these effects. In this regard, it is important that studies reporting gene–environment interactions are scrutinized for proper scientific rigour, and follow a clearly defined set of strategies such as those set out by Moffitt *et al.*[44] Second, as mentioned above, it has been shown that genes may influence the risk of developing depression by mediating an individuals' sensitivity to stressful life-events. Given the likely genetic contribution to environmental exposure, it is plausible that at least some observed gene–environment interactions are actually the result of gene–gene interactions, with the environment in question being a proxy for inherited factors. In other words, the role of the environment in gene–environment interactions may be overplayed and conclusions regarding environmental intervention strategies may be premature. Third, it is important to acknowledge that statistical evidence of an interaction between a measured gene and a measured environment does not provide any clues about the molecular mechanisms behind the interaction. Elucidating the precise mechanism(s) through which gene–environment interactions operate is vital if any diagnostic, therapeutic or preventative benefits are to result. Although progress in identifying the causes of depression has no doubt been enhanced by investigating interactions between genes and environments, it is our belief that the relatively unexplored area of epigenetics can provide new insights and new experimental strategies to uncover the molecular mechanisms behind MDD (see Figure 1).

Epigenetics: from basic mechanisms to aetiology of human disease

Epigenetics refers to the heritable, but reversible, regulation of various genomic functions mediated principally through changes in DNA methylation and
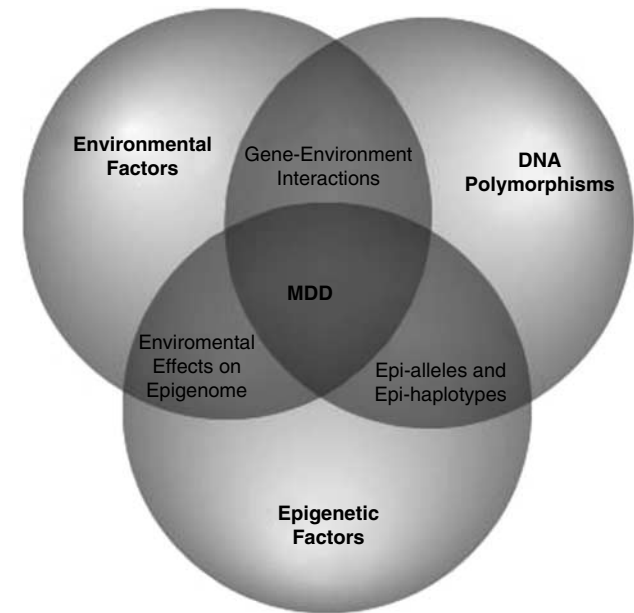

Figure 1 MDD results from a combination of interacting genetic, environmental and epigenetic factors.

chromatin structure.[45] Epigenetic processes are essential for normal cellular development and differentiation, and allow the regulation of gene function through non-mutagenic mechanisms. Of particular interest is the phenomenon of cytosine methylation, occurring at position 5 of the cytosine pyrimidine ring in CpG dinucleotides. This process is intrinsically linked to the regulation of gene expression, with many genes demonstrating an inverse correlation between the degree of methylation and the level of expression.[46] The methylation of these CpG sites, overrepresented in CpG islands in the promoter regulatory regions of many genes, disrupts the binding of transcription factors and attracts methyl-binding proteins that are associated with gene silencing and chromatin compaction. Histone modification, the other major type of epigenetic mechanism mediating gene expression, affects chromatin structure via the processes of histone acetylation, histone methylation and histone phosphorylation.[47] Interestingly, these two broad types of epigenetic mechanism are not mutually exclusive and interact in a number of ways. The methyl-binding protein MeCP2, for example, binds specifically to methylated cytosines, attracting histone deacetylases, which hypoacetylate histones.[48] Transcriptionally competent chromatin is generally enriched with acetylated histones, but transcriptionally silent chromatin is normally deacetylated.[49]

Like the DNA sequence, the epigenetic profile of somatic cells is inherited from maternal to daughter chromatids during mitosis. Unlike the DNA sequence, which is stable and strongly conserved, epigenetic processes are highly dynamic even within an individual: they can be tissue-specific, developmentally

regulated, and induced by exposure to a range of environmental factors. In addition, stochastic factors are important in determining the epigenetic milieu of the genome. Experiments tracking the inheritance of epigenetic marks through generations of genetically identical cells in tissue culture have indicated that there is considerable infidelity in the maintenance of methylation patterns in mammalian cells, and that *de novo* methylation events are fairly common during mitosis.[50] The fidelity of the methylation pattern varies across the genome, with unmethylated regions showing a higher error rate than predominantly methylated regions, particularly in areas of the genome outside promoter regions.[51] In conclusion, whereas DNA sequences exhibit nearly complete interclonal fidelity, the epigenetic profile of a cell lineage can display substantial intergenerational differences. Because processes like DNA methylation are integral in determining when and where certain genes are expressed – the precise coordination of gene expression is crucial to the correct development of any organism – this epigenetic metastability could have profound effects. While the DNA sequence of an organism dictates the physical structure of proteins, epigenetic mechanisms control the quantity, location and timing of gene expression.

It has been traditionally believed that epigenetic profiles are reset and erased during gametogenesis, thus preventing the meiotic transmission of epigenetic information between generations. Evidence is mounting, however, that the epigenetic marks of at least some mammalian genes are not fully erased during meiosis and can thus be transmitted from generation to generation.[52] It appears that such meiotic transmission of epigenetic alleles, or 'soft inheritance', may be a fairly common phenomenon in a number of eukaryotic organisms.[53] While the process of epigenetic inheritance is clearly less stable than DNA sequence inheritance, it provides another, often ignored, mechanism via which information can be passed on transgenerationally. This has obvious ramifications for traditional approaches to disease gene mapping in which all inherited traits are assumed to result from the transmission of DNA sequence changes, and may partly explain why researchers are finding it hard to pinpoint specific causal gene polymorphisms in apparently highly heritable disorders like MDD.

Epigenetic research indicates that the regulation of gene activity is critically important for normal functioning of the genome. Genes, even the ones that carry no mutations or disease predisposing polymorphisms, may be useless or even harmful if not expressed in the appropriate amount, at the right time of the cell cycle or in the right compartment of the nucleus. Cells operate normally only if both DNA sequence and epigenetic components of the genome function properly.[46] In this regard, epigenetic abnormalities have been well characterized in several rare diseases such as Prader–Willi, Angelman and Beckwith–Wiedemann syndromes.[54] Other syndromes

postulated to have an epigenetic aetiology include: immunodeficiency, centromeric region instability, facial anomalies syndrome (ICF) which is caused by mutations in the DNA–methyltransferase 3B (DNMT3B) gene;[55] Rett syndrome, a neurological disorder that occurs in females and is caused by mutations in the methylcytosine-binding protein (MeCP2) gene;[56] X-linked α-thalassaemia/mental retardation (ATRX) Syndrome, caused by mutations in the X-chromosome gene *ATR-X* which result in the hypomethylation of repeat sequences;[57] and Fragile-X syndrome, which is caused by a combination of both genetic and epigenetic mechanisms.[58] It is becoming increasing clear that epigenetic processes are important in the development of cancer. In various cancers there is some degree of epigenetic misregulation, including both global genome-wide hypomethylation and the CpG island promoter hypermethylation of tumour suppressor genes.[59] Complex non-malignant diseases such as MDD, however, have hardly been investigated from an epigenetic perspective. As will be discussed below, the partial stability of epigenetic signals during meiosis, and their partial instability during mitosis resulting from a host of developmental, environmental and stochastic events, makes epigenetics an attractive aetiological candidate for such diseases (see Figure 2).

Relevance of epigenetics to aetiological studies of MDD

Discordance of MZ twins and environmental impact

Twin studies have strongly implicated genetic factors in the aetiology of MDD, but as is the case for all complex psychiatric conditions, MZ twin concordances are observed to be considerably less than 100%. In the classical twin-study approach, in which MZ twins are assumed to be genetically identical, any discordance between MZ twins is attributed solely to 'non-shared' environmental factors. An alternative explanation, however, is that some of the observed phenotypic differences between MZ twins may be the result of epigenetic factors.[60,61] A recent study by Fraga *et al.*[62] has demonstrated that fairly profound epigenetic differences across the genome do arise during the lifetime of MZ twins, highlighting the dynamic nature of epigenetic processes. Interestingly, MZ twin methylation differences have been reported for CpG sites in a number of specific genes that have been associated with psychiatric illness including the dopamine D2 receptor (*DRD2*) gene[63] and the catechol-*O*-methyltransferase (*COMT*) gene.[64] Similarly, genetically identical inbred animals have been shown to demonstrate considerable epigenetic differences that may be linked to gene expression differences resulting in marked phenotypic variation.[65] It is becoming increasingly apparent that many of the observed epigenetic differences between MZ twins and inbred animals may be the result of random stochastic events.[60] It can be envisaged that such stochastic epigenetic 'mutations' may accumulate
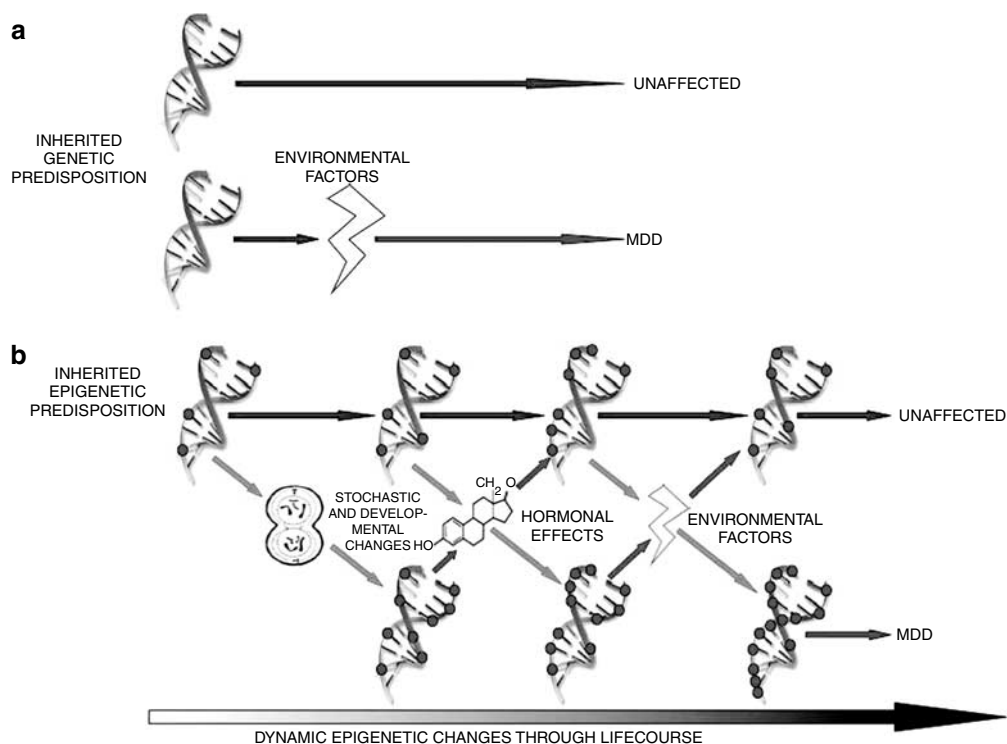
Figure 2 The epigenetic model of MDD. (**a**) Traditional aetiological models of MDD have considered only genetic polymorphisms and environmental factors. (**b**) The epigenetic model of MDD postulates that epigenetic changes can result from environmental, hormonal and random stochastic factors to increase susceptibility to MDD.

over the millions of mitotic divisions occurring during the lifetime of two MZ twins, and could lead to profound gene expression alterations if present in regulatory regions of the genome. It has been proposed that such stochastic epigenetic variation may be more important in complex psychiatric disorders than is currently recognized, perhaps accounting for some of the risk currently attributed to environmental factors.[66]

Of course, the fact that epigenetic differences exist between MZ twins does not rule out the role of environmental factors in disease susceptibility. In fact, there is growing evidence that the environment can itself influence the epigenetic status of the genome – either globally or at specific loci.[67] It is becoming increasingly apparent, for example, that a range of environmental toxins, both chemical and psychosocial, can lead to long-lasting alterations to the epigenetic profile of the genome or specific genes through processes such as the modification of DNA and histones. The fact that epigenetic marks can directly link environmental factors to gene function makes them attractive targets for a mechanistic role in the gene–environment interaction effects that are increasingly being uncovered in aetiological studies of complex diseases. Many environmentally induced epigenetic changes appear to be related to dietary intake (for a detailed review see Waterland and Jirtle[68]). It appears that DNA methylation can be affected by the dietary levels of methyl-donor compo-

nents, for example folic acid, and that maternal dietary methyl supplements can increase DNA methylation and alter methylation-dependent phenotypes in mammalian offspring. One classic example is that of the epigenetic state of the *agouti viable yellow* locus in mice, which contains a gene determining coat colour and can be manipulated by altering the diet of the pregnant female.[69] Certain drugs may also modify epigenetic regulation – the use of metamphetamine, for example, linked to psychiatric illness in humans, alters the DNA methylation profile of genes expressed in the brain.[70] Intriguingly, and perhaps more pertinent to the aetiology of depression, it appears that the psychosocial environment may also mediate gene expression epigenetically. Research by Meaney and colleagues has shown that postnatal maternal care in rats, as measured by increased pup licking, grooming, and arch-backed nursing, leads to epigenetic modification of a NGF1-A transcription factor binding site in the promoter region of the glucocorticoid receptor gene (*NR3C1*).[71] This finding is of particular relevance to the study of depression given the likely role of both the early rearing environment and the HPA axis in the aetiology of affective disorders.

Proband sex effects
As discussed earlier, the prevalence of MDD is approximately doubled in females compared to males, and several studies suggest that the heritability

of the disorder is significantly higher in women.[12,72] Furthermore, it appears that the genetic aetiology of MDD may differ between the sexes with the overlap of putative genetic factors involved in the disorder being incomplete.[72] Following the investigation of X-linked Mendelian diseases that exhibit sexual dimorphism, one potential explanation is that the overrepresentation of depression in women results from a MDD risk gene on the X chromosome. Several segregation analyses have suggested involvement of X-chromosome genes in MDD (see e.g. Vaillant *et al.*[73]) and certain X-linked genes, for example, *GPR50,* have been associated with the disorder,[74] although such conclusions are not ubiquitous (see e.g. Faraone *et al.*[75]) and no firm conclusions can yet been drawn about the involvement of X-linked sequence variants in depression.

Skewed X-chromosome inactivation is one X-linked *epigenetic* process that could potentially cause the excess rates of MDD in women, and also explain female MZ twin discordance.[76] X-inactivation silences gene expression and is initially instigated by expression of the *XIST* gene and then maintained via DNA methylation and histone modifications. The role of X inactivation is to compensate for the greater dosage of X-linked genes in women (who have two X chromosomes) compared to men (who have one X chromosome). It is generally assumed that in most women X inactivation is stochastic for each cell lineage – either the maternally or paternally inherited X can be silenced – but is maintained throughout subsequent cell divisions. There is, however, increasing evidence that in some cases the selection process is not random. It has been demonstrated, for example, that approximately half the female carriers of X-linked mental retardation exhibit skewed X-inactivation where the activation ratio between the two X chromosomes is 80:20% or higher.[77] Non-random X-chromosome inactivation has been observed in a number of disorders, including several X-linked immunodeficiencies, Lesch–Nyhan disease, incontinentia pigmenti, focal dermal hypoplasia and adrenoleukodystrophy.[78] In the normal population of women without a family history of X-linked disorders, 5–20% of women have constitutional skewing of X inactivation.[79] According to other authors, 30–40% of females exhibit ratios of 60:40% or more, and 10% of normal females demonstrate even more extreme ratios.[78] In studies of MDD, therefore, we propose that the 'skewedness' of the X chromosome should be thoroughly investigated, and if present taken into account when performing genetic studies because the results of linkage and association studies may be mediated by the proportion of females with skewed X-inactivation. Skewed X-inactivation also provides a mechanism for phenotypic discordance between female MZ twins. Male MZ twins will only express X-linked genes inherited from their mothers, but females can express either maternally or paternally inherited genes. Loat and colleagues.[80] have argued that given the random nature of X inactivation, female MZ twin pairs do not necessarily inactivate the same X chromosome and will thus show higher levels of discordance than male MZ twin pairs. To our knowledge, no study has yet tested this theory on individuals with MDD, but analyses on MZ twins assessed at ages 2, 3 and 4 suggests that this pattern is observed for a number of behavioural traits closely related to depression.[80] Another epigenetic phenomenon that may confound X-linked gene studies in MDD is the existence of genes escaping X inactivation. Not all genes on the X chromosome are inactivated, with as many as 15% showing evidence of expression from both X chromosomes.[76,81] Genes escaping X inactivation are obvious candidates for explaining sexual dimorphism in disease prevalence, especially if no Y-chromosome homologue for those genes exists.

Sex effects have also been recently detected in genetic linkage and association studies of autosomal genes. For example, genome-wide linkage scans of MDD families provide evidence for male-only linkage on chromosome 12q22–q23.2[16] and evidence for female-only linkage on chromosome 2q33–35.[82] There are numerous other examples across the spectrum of psychiatric diseases where autosomal loci exhibit apparently clear-cut sex differences. Sex hormones are the usual 'culprit' used to explain such gender effects in complex disease, based in part on the myriad of data associating hormonal differences with disease states, and their critical involvement in numerous regulatory processes. Little is known, however, about the underlying mechanisms of how sex hormones predispose to or protect from a disease. The gender-specific effects observed in genetic linkage and association studies suggest that chromosomal regions and individual genes may be the target of sex hormone action, but the molecular mechanisms behind these interactions are yet to be elucidated. Although sex hormones cannot change DNA sequence, it is known they can be potent modifiers of epigenetic status and gene expression. There are several reports of the female sex hormone oestrogen altering the chromatin configuration of certain genes.[83,84] Furthermore, it has been proposed that sex hormones may act to alter the DNA methylation profile of specific loci in the genome,[85,86] controlling gene expression in a sex-specific manner. It is thus plausible that susceptibly to depression is mediated by the hormone-specific epigenetic modification of genes contained within nominated linkage regions. This may explain some of the sex effects observed widely in genetic linkage and association studies: a specific allele or haplotype may be a risk factor only after epigenetic modification by some aspect of the endocrinological millieu.

Parental origin effects

Another non-Mendelian feature that is commonly observed in studies of psychopathology is a parent-of-origin effect, where disease susceptibility is mediated by parental factors in a sex-specific manner. Parent-

of-origin effects are apparent at both a phenotypic level, where the risk to offspring depends on the sex of the affected parent, and also at a molecular level when risk alleles only confer increased susceptibility if they are transmitted from the mother or the father. These effects have been observed in a number of psychiatric phenotypes, including several closely related to major depression. For example, it has been shown that the risk of developing bipolar disorder is higher in offspring with affected mothers than in those with affected fathers.[87] Genome-wide linkage-scans and candidate–gene association studies have also provided some preliminary evidence for parent-of-origin effects in depression. Zill et al.[88] for example, find evidence for a parent-of-origin effect with the GOLF gene, and Schiffer and Heinemann[89] report preferential maternal transmission of a GluR7 gene risk allele to MDD patients. Many other examples, however, have probably gone unnoticed because parent-of-origin effects are not routinely tested in traditional genetic studies. In fact, it is likely that parental origin effects are a much wider phenomenon in psychiatric diseases than it is generally thought given that linkage analyses, as a rule, are performed in a sex-averaged way.

The most likely mechanism behind such parent-of-origin effects is genomic imprinting – the differential expression of the genetic material at either a chromosomal or allelic level depending on whether the genetic material has been transmitted from the paternal or the maternal side. Genomic imprinting involves the epigenetic marking of chromatin, via the modifications of DNA and histones, which results in expression of only one allele at that locus. Genomic imprinting is fundamental to normal mammalian development and growth, and plays an important role in brain function and behaviour.[90] Genomic imprinting is traditionally viewed as an all-or-nothing phenomenon that occurs in only small, clustered regions of the genome. In these imprinted regions only maternally or paternally inherited genes are turned on, but not both, resulting in monoallelic expression, for example, at both H19 and IGF2. It is estimated that there are between 100 and 200 imprinted loci in the human genome (see http://igc.otago.ac.nz/home.html). Recent evidence, however, suggests that imprinting may be more widespread and less clear-cut than originally believed, and this could have important ramifications for our understanding of the aetiology of complex disorders such as depression. Preis et al.[91] used nine transgenic mouse lines to uncover a much more subtle form of imprinting in the murine genome. They found that a high proportion of transgenic lines displayed lower expression of a reporter transgene when it was passed through the maternal line although paternal alleles were expressed as well. It is proposed that these observations may be representative of imprinting patterns across all mammalian genomes, with perhaps the majority of genes demonstrating some degree of skewed allelic expression in a parent-of-origin

dependent manner. Conversely, it has also been shown that genes previously believed to exhibit total monoallelic expression as a result of imprinting may actually show considerable heterogeneity. A study examining the allelic expression of three imprinted genes (SNRPN, IMPT1, IGF2) in the normal population found evidence for significant biallelic expression indicating that functional variation at imprinted loci may be a common feature of the mammalian genome.[92]

Association of DNA sequence variation with epigenetic variation – epi-alleles, epi-genotypes and epi-haplotypes

As discussed above, few convincing associations between specific polymorphisms and susceptibility to MDD have been revealed by candidate gene studies. One major problem is the heterogeneity apparent in the data generated from these analyses. The pattern of association observed is rarely consistent between studies, and it is not uncommon for different alleles and haplotypes to be associated with the disorder in different study populations. One possible explanation for this heterogeneity comes from the observation that genetic–epigenetic interactions may be commonplace across the genome. 'Epi-alleles' and 'epi-haplotypes' that combine both DNA sequence and epigenetic information may thus be better predictors of the risk for disorders like depression than any of the two components analysed separately. There is increasing evidence that some DNA alleles and haplotypes tend to be associated with a specific epigenetic profile. Of particular interest in this regard is increasing evidence that DNA methylation profiles may be associated with specific DNA alleles in genes putatively linked to mood disorders. For example, the T allele of the C677T polymorphism in the methylenetetrahydrofolate reductase gene (MTHFR), recently implicated in depression,[93] is associated with an increased risk of imprinting defects in the Prader–Willi syndrome/Angelman syndrome region of 15q.[94] Furthermore, the C102T polymorphism in the serotonin 5-HT2A receptor gene (5HT2AR), which has been associated with several psychiatric disorders including depression, was found to be methylated specifically on the C allele.[95] Dempster et al.[96] found evidence that methylation in the promoter region of the COMT gene, another locus implicated in numerous psychiatric phenotypes, was associated in a dose-dependent manner with genotype at the val158met polymorphism, with val158 homozygotes exhibiting lower levels of methylation than met158 homozygotes. Finally, a recent epigenetic study of a C/G single nucleotide polymorphism (SNP) in the CDH13 gene in male germline cells revealed that C alleles are predominantly unmethylated, while G alleles are predominantly methylated.[97] The notion that epigenetic changes may be associated with specific DNA sequence changes sheds new light on the inconsistent genetic association studies observed in complex

diseases, and suggests that a comprehensive epige-netic analysis of candidate SNPs and haplotypes is warranted.

Epigenetic studies in MDD: technological and methodological complexities

While epigenetics provides a new perspective on the aetiology of major depression, it would be naïve to expect that applying epigenetic theory to molecular-based studies of MDD psychopathology is a straight-forward task that can be achieved without encounter-ing a number of technological and methodological complexities. The study of epigenetics, especially in complex diseases, is a relatively new field of research, and optimal laboratory techniques and analysis methods are still being developed. It is vital that laboratory approaches are refined and verified before sweeping conclusions are drawn from experimental data. This is particularly important because epige-netic experiments need to overcome a number of inherent complexities that are not encountered when investigating simple DNA sequence variation. Poten-tial problems surrounding the epigenetic studies can be subdivided into several groups, which will be

discussed briefly in the sections below: (1) the technological limitations in identifying epigenetic variation; (2) sample- and target tissue-related issues; (3) the confounding effects of DNA sequence varia-tion; and (4) the interpretation of a cause-effect relationship between epigenetic changes and disease aetiology.

Technological limitations

Whilst it is easy to *theorize* about the role of epigenetic processes in mediating susceptibility to psychiatric disorders, actually investigating these modifications at a *molecular* level is not so straight-forward. To date, only a few empirical studies have been performed, and it is clear that a number of methodological difficulties need to be overcome before large-scale epigenetic studies can be viably executed.

A number of approaches to investigate DNA methylation have been developed (see Figure 3). Currently, the 'gold standard technique' for fine mapping of methylated cytosines is based on the treatment of genomic DNA with sodium bisulfite, which converts unmethylated cytosines into uracils (and subsequently, via polymerase chain reaction
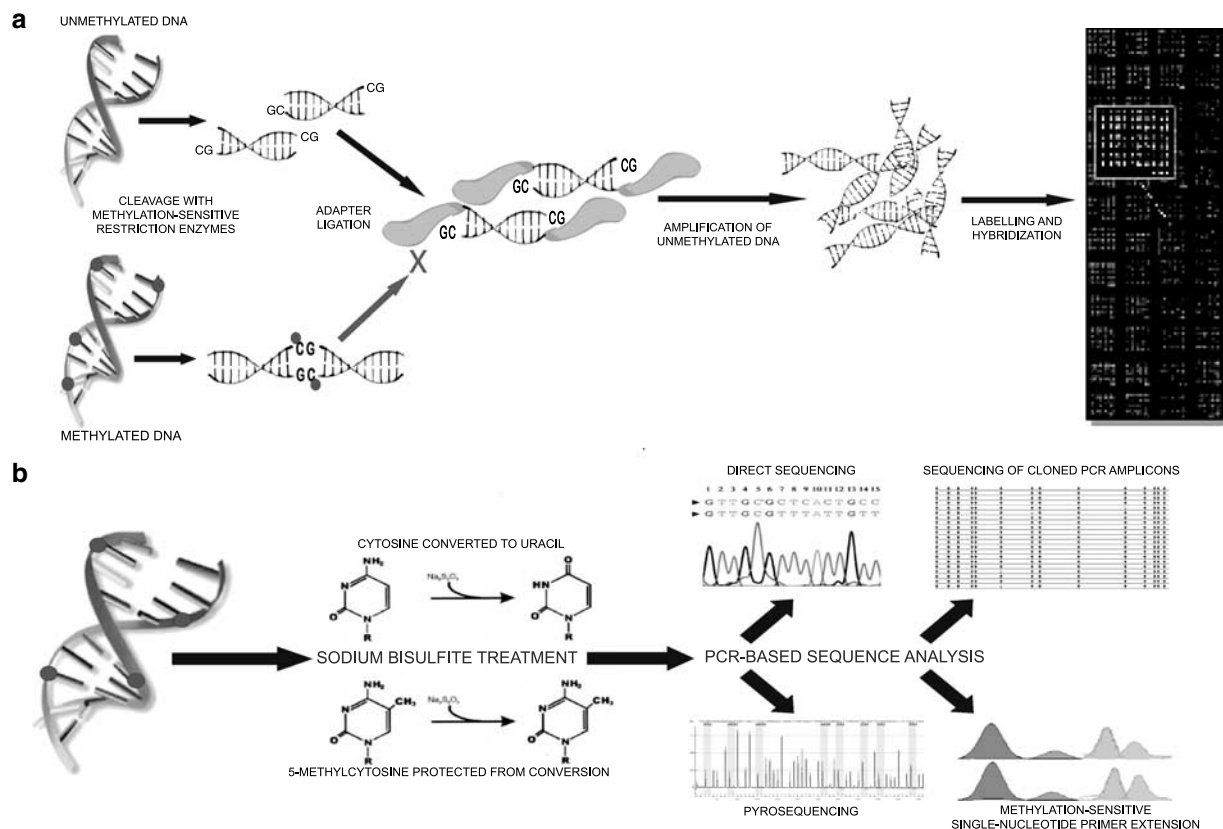

Figure 3 Experimental approaches to investigate DNA methylation. (**a**) Microarray-based methylation profiling using microarrays can investigate DNA methylation changes at a genome-wide level. In this example the unmethylated fraction of genomic DNA is enriched using methylation-sensitive restriction enzymes and adaptor-ligation PCR and subsequently hybridized on microarrays (see Schumacher *et al.*[103]); (**b**) locus-specific methylation analysis using sodium bisulfite treatment and PCR-based sequence analysis.

(PCR), to thymidines), whereas methylated cytosines are resistant to bisulfite and remain unchanged.[98] After sodium bisulfite treatment, one crude method to examine DNA methylation is via methylation-specific PCR (MSP). In this approach two PCR reactions are performed on bisulfite-treated DNA with primer sets specific to (i) methylated DNA and (ii) unmethylated DNA. Such an approach can only investigate methylation at a very small number of CpG sites and gives no quantitative information about the degree of methylation. Furthermore, data from experiments utilizing MSP should be treated with caution given the numerous confounding factors that can affect PCR efficiency on bisulfite-treated DNA. A more rigorous method to investigate DNA methylation is bisulfite genomic sequencing. DNA regions of interest are amplified and sequenced to identify $C > T$ transitions or stable C positions, respectively corresponding to unmethylated and methylated cytosines in the native DNA. Typically, PCR amplicons are either sequenced directly to provide a strand-specific average sequence for the population of DNA molecules, or cloned and sequenced to provide methylation maps of single DNA molecules.[98,99] An alternative to the laborious and expensive sequencing of cloned bisulfite-PCR products is the use of pyrosequencing to accurately quantify the degree of CpG methylation. Pyrosequencing is a sequencing-by-synthesis method that relies on the luminometric detection of pyrophosphate release upon nucleotide incorporation via an enzymatic cascade. Because there is a direct correlation between sequence data and the amount of nucleotides incorporated during the reaction, it provides an accurate method for detecting the precise methylation level at any CpG site giving truly quantitative data that are not achievable using standard bisulfite-sequencing protocols.[100] A major problem with the sodium bisulfite approach is that it results in considerable DNA degradation, and large quantities of genomic DNA material are needed if numerous genomic regions are to be profiled – obviously an issue if valuable, relatively inaccessible tissues such as the brain are to be profiled. One potential method to overcome problems associated with tissue availability is the use of whole-genome amplification of bisulfite-treated DNA.[101] In addition, no consensus has yet been reached on how to best statistically analyse bisulfite-sequence data. Such analyses are unlikely to be straightforward, especially if the differences observed across individuals are subtle as would be expected for disorders such as MDD. One possibility is to use an approach based on epigenetic distance, which reduces bisulfite-sequencing data from cloned PCR products to a binary code for each DNA molecule, and allows patterns of 'epigenetic drift' to be easily ascertained.[102]

These traditional, locus-specific techniques limit the number of the CpG sites that can be feasibly assessed, and thus exclude the possibility of performing a genome-wide 'epigenome-scan' to identify novel regions of importance. In recent years, a number of novel, high-throughput microarray-based methods have been developed, which should enable future epigenetic studies to overcome many of these issues.[103,104] At present these methods have not been widely used and are relatively untested. Two general methods exist for treating and enriching DNA samples before array hybridization: the digestion of genomic DNA with restriction-sensitive enzymes, and the use of antibodies to specifically pull-down methylated cytosines. The enzyme approach cannot be used to interrogate every potential methylated cytosine in the genome, whereas the antibody approach can only examine the methylated fraction of the genome, which is far less informative than the unmethylated fraction.[103] As we have learnt from the use of gene expression microarrays in psychiatry, differences in sample preparation, microarray type, sample labeling and data analysis procedures pose a tremendous challenge to the field,[105] and some degree of standardization is needed across epigenetic studies. A major hurdle to the widespread use of methylation microarrays is cost – for example it is currently prohibitively expensive to scan the entire genome using DNA from a large sample of MDD patients and controls, especially if tissue-specific effects are important and numerous brain regions need to be assessed. Finally, until recently, the resolution of the available low-density arrays has been relatively crude, and the available methodology has not been particularly sensitive to subtle methylation differences. Current genome-wide methylation studies have been biased by the fact that current microarray designs focus primarily on cDNA, promoters, and 5′ regulatory sequences of known genes only. As array technology advances, however, with the development of commercially produced whole-genome 'tiling' arrays containing millions of features allowing an unbiased assessment of the entire genome, microarray studies have the potential to rapidly advance our understanding of the epigenetic mileau of the genome, and provide novel insights into the aetiology of MDD.

This article has been primarily dedicated to the role of DNA methylation in MDD aetiology. This focus partly reflects the fact that methods to examine other epigenetic processes such as histone modifications in psychiatric disorders are a step behind those currently available for DNA methylation analysis. The method used to examine the epigenetic state of histones is chromatin immunoprecipitation (ChIP). One limitation to this approach is the large number of cells needed for each experiment, although recent methodological advances may permit ChIP analysis on as few as 100 cells.[106] Another major problem with ChIP analysis is that, compared to DNA methylation marks, histone modifications are far less stable in post-mortem tissues. In this regard, specific immunoprecipitation protocols applicable to post-mortem brain tissue need to be developed, and the impact of potential confounds such as autolysis time or tissue pH need to be thoroughly examined. Some promising

data come from recent investigations that suggest the immunoprecipitation of micrococcal nuclease-digested tissue extracts is a feasible approach to profile histone methylation at defined genomic loci in *post-mortem* brain.[107] Once histone-stability problems have been addressed, the development of powerful microarray-based 'ChIP-on-chip' methods, in which DNA isolated from ChIPs is hybridized to a microarray chip, should allow genome-wide analysis of histone modifications and provide additional epigenetic information to complement data from genome-wide DNA methylation studies.

Sample and target tissue
To identify epigenetic changes associated with depression, the ideal experiment would investigate *prospectively* the dynamics of genome-wide epigenetic changes in the brains of individuals who eventually become affected with MDD compared to unaffected control individuals. At the present time, however, limitations with our current range of epigenetic methodologies render such an approach impossible. As it is not yet possible to perform *in vivo* epigenetic studies, particularly for tissues like the brain, only retrospective study designs using *post-mortem* brain samples are viable.

Given the role of epigenetics in mediating when and where genes are expressed, an obvious confounding factor in epigenetic studies is tissue heterogeneity. Because different cell types exhibit quite different epigenetic profiles across different genomic regions, specific tissues – that is, the primary sites of disease manifestation – are deemed preferable for aetiological studies. It is likely that for studies of psychiatric disorders such as MDD, the primary focus of aetiological studies will be the numerous brain regions implicated in the disorder. Even within specific-tissue types, however, there is considerable cellular heterogeneity. Tissues like the cortex, for example, consist of numerous different types of neuronal and glial cells, and the detection of cell-type specific epimutations is likely to be difficult unless the epigenetic profile of specific cells can be investigated. This point is highlighted by a recent study identifying epigenetic changes occurring specifically in cortical interneuron cells, suggesting that different cell populations within a single tissue-type can have quite distinct epigenetic profiles.[108] An increasingly popular solution in the epigenetics research field is to apply 'Laser Capture Microdissection' technology, which enables the isolation of specific cell types or single cells from whole tissue avoiding the confounding effects of cell type variation.[109]

Despite the problems of tissue specificity, because the availability of fresh brain tissue from well-phenotyped individuals with disorders like MDD is non-existent, one compromise is to use other, more accessible tissue sources like leukocytes for epigenetic profiling in the hope that the patterns observed will reflect those of the real tissue of interest. There is

increasing evidence that many epimutations are not limited to the affected tissue or cell type, but can also be detected in other tissues. Good examples are the epimutations at *IGF2*[110] in lymphocytes and *MLH1* in sperm cells,[111] observed in colon cancer patients and epimutations at *KCNQ1OT1* in the lymphocytes and skin fibroblasts in Beckwith–Wiedemann syndrome.[112] In fact, peripheral blood-based studies may be useful in revealing epigenetic changes resulting from early embryogenesis, or even highlighting inherited epigenetic variation. The study of peripheral cells may even have some advantages over the use of brain tissue in that they are likely to accumulate fewer epigenetic changes induced by disease-related external factors, such as medications, recreational drugs and stress, which could all be confounding factors to epigenetic studies. The use of peripheral cells would also allow the longitudinal study of epigenetic changes throughout the lifecourse of individuals diagnosed with MDD, providing information about the epigenetic factors associated with the development, remission and relapse of the disorder. Finally, because there have been no thorough epigenetic studies in MDD to date, it is not known how large a sample needs to be investigated. At this stage the degree of any potential epigenetic differences between cases and controls is unknown, although the effects observed are likely to be small.

Defining a 'normal' epigenetic profile and proving a cause–effect relationship between epigenetic differences and MDD
Despite the current interest in the possible role of epigenetic processes in disorders like MDD, very little empirical research has been performed to characterize what actually comprises a 'normal' epigenome. Before we can systematically investigate the epigenetic changes associated with disease, we must first catalogue the genome-wide DNA methylation patterns in all major tissues. This is one of the targets of the Human Epigenome Project (www.epigenome.org), which aims to map all methylation variable positions in the genome.[113] Producing a reference map of the entire human epigenome is going to be a gargantuan task, especially given the huge epigenetic differences that are likely to occur both between tissue/cell types, and within a specific tissue/cell type over the course of development. To date, this private/public consortium has released DNA methylation profiles of the major histocompatibility complex[114] and chromosomes 6, 20 and 22.[115]

As is the case for all non-DNA sequence based (neurochemical, pathophysiological and environmental) studies, proving a direct causal link between epigenetic factors and disease is not straightforward. For example, the observation of a different epigenetic profile between depressed and non-depressed individuals does not necessarily indicate a causal relationship between these epigenetic changes and depression. It is possible that any epigenetic changes

in depressed individuals are not a cause of, but actually the result of MDD-induced changes in the brain. Given that several exogenous chemicals have been linked with processes like DNA methylation, it is also possible, for example, that the medications used to treat depression may cause epigenetic changes. In this regard, tissues that are not the disease site or directly affected by antidepressant medications, for example peripheral blood cells, could be very useful in elucidating inherited epigenetic changes associated with MDD. The ideal study design to identify causal relationships between epigenetic factors and MDD would be a prospective longitudinal study in which individuals at risk for depression are investigated before they develop the disease.

Confounding effects of DNA variation

A major issue in epigenetic studies is that putative epigenetic differences can be masked by the thousands of DNA sequence variants that exist between individuals. Owing to the nature of the technology currently employed to examine epigenetic changes, DNA sequence polymorphisms are not always easy to differentiate from epigenetic differences. This problem applies to all DNA methylation technologies currently utilized in aetiological studies. Cut sites for methylation-sensitive restriction enzymes, for example, can be created or removed by sequence changes. Sodium-bisulfite conversion reduces methylation status to standard sequence information, and can thus be confounded by SNPs. Two possible study designs can be used to address these problems. First, methylation differences in MZ twins discordant for MDD can be examined. MZ twins have identical genomes, so phenotypic differences cannot be accounted for by DNA sequence changes. However, a potential limitation of using MZ twins is that unaffected co-twins may also be carriers of epigenetic changes that may not be too far from a hypothetical 'threshold' of epigenetic misregulation, above which the twin would present with a clinical diagnosis of MDD. Typical microarray experiments are based on relative differences between two individuals, rather than the absolute size of a putative epigenetic change. It is possible that this difference, between an affected twin (a little bit above the 'threshold') and an unaffected co-twin (a little bit below the 'threshold') may be relatively small and difficult to differentiate from experimental errors. A second approach is to instigate a longitudinal study of epigenetic changes during the course of disease in MDD patients, using DNA taken from easily accessible tissue (e.g. peripheral blood cells). In such an experiment, the epigenetic profile of DNA samples taken at various timepoints could be compared (e.g. at first diagnosis, remission and relapse). A similar approach has been suggested as a substitute to twin studies in pharmacogenetic investigations.[116] Such a design is powerful as it can inform us about the extent that epigenetic status fluctuates in peripheral blood cells, and may provide an insight into relapse-associated epigenetic changes.

Epigenetics and MDD: vogue, hassle or necessity?

So what is the role of epigenetics in aetiological studies of MDD: vogue, hassle or necessity? The answer is probably a bit of all three. Largely neglected until very recently, the study of epigenetics has become increasingly 'mainstream'. There is a certain buzz amongst the molecular psychiatry research community about the potential role of DNA methylation and histone modifications in a range of psychiatric phenotypes. It is now generally accepted that these epigenetic factors may sit alongside DNA sequence polymorphisms and environmental factors in mediating susceptibility to disorders like MDD. In this article we have discussed the benefits of including epigenetic theory into models of disease aetiology, highlighting how epigenetic mechanisms can account for the many features of MDD that are unexplainable using traditional genetic and environmental approaches (see Table 1).

Theoretical notions about a role for epigenetic processes in the development of MDD do not *prove* such a link, however. There is a need to move from theory into empirical laboratory-based research, and this is where the current 'vogue' for epigenetics becomes more of a 'hassle'. The study of epigenetics, especially in complex diseases, is a relatively new field of research, and optimal laboratory techniques and analysis methods are still being developed. We should be under no illusion that such experiments are going to be easy. If we are to really fulfill the novel heuristic opportunities that epigenetics provides, the development of novel high-resolution genome-wide methods to scan the epigenome for DNA methylation and histone modifications are vital.

Despite these difficulties, it is also clear that studies investigating epigenetic factors in MDD (as well as various other complex diseases) are a 'necessity'. Years of research effort have been spent attempting to identify the specific genes and environmental factors that were traditionally believed to determine individual susceptibility to MDD. While some progress has been achieved, our focus on only genes, environments and the interactions between them, has left many questions unanswered. Although it would be premature to conclude that epigenetics will lead to revolutionary discoveries in non-Mendelian biology, the study of DNA methylation and histone modifications has the *potential* to transform our understanding about the molecular aetiology of complex diseases.

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