Epigenetics: what is it and why is it important to mental disease?

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Introduction: The chemical marking of the DNA and surrounding histone proteins represent some of the means by which gene expression is controlled. Many of these epigenetic modifications are pre-programmed and are an important part of the control of development.

Sources of data: There is an accumulating body of evidence from clinical genetics and animal work that suggests some epigenetic processes may also be labile.

Areas of agreement: A number of these studies have demonstrated that the epigenetic status of genes can be altered through environmental events such as in vitro culture of embryos and exposure to toxins, sometimes resulting in disease.

Areas of controversy: More routine variations in life events may also be encoded by changes in the epigenetic status of genes, and as such these processes may provide a mechanism mediating interplay between genes and the environment, including the now recognized idea of gene–environment interactions.

Growing points and areas timely for developing research: The significance of epigenetics for mental disease is becoming increasingly clear. It is important that the techniques developed to analyse the epigenome are now applied to the study of the molecular basis of mental disease to assess the contribution of gene–environment interactions to brain function.

Keywords: imprinted genes/gene expression/DNA methylation/histone modifications/Angelman/Prader–Willi/Rett/gene–environment interaction

What is epigenetics?

For some time now it has been acknowledged that a discrepancy exists between the information provided by the DNA sequence (i.e. number of genes) and what is actually represented at the level of encoded products (messenger RNA and proteins). What is now clear is that, although the DNA sequence provides the basic blueprint for life, this code is subject to a number of ‘epigenetic’ modifications that provide another complex layer of information.

The development of the idea and the first use of the term ‘epigenotype’ occurred as far back as 1942. Conrad H. Waddington suggested the existence of epigenetic mechanisms to explain the control
of gene expression programmes during development. Implicit in the term epigenetics is that these mechanisms are labile and are erased and reset. Over the years the definition of epigenetics has shifted a little, such that these mechanisms are not limited to development only, although there is still an understanding that epigenetic marks controlling gene expression are stably transmitted through cell divisions. For the purposes of this review, epigenetics can be loosely defined as the transmission and perpetuation of coding information that is not based on alteration of the DNA sequence. These coding changes may be mediated via chemical marking of the DNA sequence itself (DNA methylation) and/or chemical tagging of histone proteins that bind DNA and are molecular tools by which gene expression levels are controlled.

Over the past two decades the techniques required to analyse these molecular processes have been developed and refined, and we now recognize the complexity and breadth of epigenetic mechanisms. DNA methylation represents the simplest epigenetic process, and usually increased methylation levels at a gene loci equates to decreased expression of a gene. Most techniques to analyse DNA methylation rely on bisulfite treatment of the DNA. Here, bisulfite effectively deaminates methylated cytosines to uracils, providing a difference that can be detected by direct assessment of the treated DNA sequence. Far more complex are other epigenetic mechanisms related to chromatin modification. Differing histone protein modifications result in altered 3-D chromatin structures (open or closed), meaning the DNA sequences underneath are more or less accessible to transcription enzymes, which in turn leads to more or less gene expression. Applying chromatin immuno-precipitation (ChIP) techniques utilizing recently developed antibodies that recognize specific histone modifications (acetylation, methylation, phosphorylation, etc.) to appropriately processed tissue preparations ‘pulls down’ those stretches of DNA bound by these modified chromatin proteins. The relative abundance of a specific DNA sequence in a ChIP preparation allows the quantification of, for instance, Histone H3 acetylation at a given gene. However, unlike DNA methylation, the histone code is less easy to ‘read’, due to the different types of histone proteins and possible modifications.

Analysis using these techniques has shown, as originally suggested, that epigenetic mechanisms are crucial in determining cell programming and consequently development. It is no surprise therefore that epigenetic processes are implicated when gene expression goes wrong and thus far have been extensively studied in cancer, where several ‘epigenetic’ drugs are currently being developed. It is becoming increasingly clear, however, that epigenetic mechanisms contribute in a major
way to the functioning of many different systems, including the brain, and therefore by extension may be involved in the pathogenesis of mental disease. In this article we review the basic science and clinical literature, and outline the ways by which disruption of epigenetic processes may give rise to abnormalities in brain function and, potentially, mental disease. Firstly, we focus on one category of genes subject to developmentally programmed epigenetic modification, namely imprinted genes, and how disruption of these genes and their control mechanisms can affect brain and behaviour. Second, we discuss an emerging idea that processes associated with epigenetic regulation are labile across the lifespan, and may provide a mechanism by which environmental events can be encoded at the molecular level.

**Developmentally programmed epigenetic modification: imprinted genes**

In a small subset of mammalian genes one of the two inherited parental copies of a gene (alleles) in the offspring gets switched off depending on whether it has come from the mother or the father. These idiosyncratic loci are known as imprinted genes and are subject to a form of developmentally determined epigenetic control that leads to selective silencing of alleles depending on whether they pass through the egg or sperm. Though relatively small in number—there are currently approximately 80 known imprinted genes—they have major influences on physiology. This was clearly demonstrated by the original experiments pointing to the existence of imprinted genes, in which parthenogenetic (two copies of maternal DNA) and androgenetic (two copies of paternal DNA) mouse embryos were produced and failed to develop beyond early gestation. Since these initial experiments, individual-imprinted genes have been identified, many of which are found to be expressed in brain where they are increasingly recognized to influence neurodevelopment and behaviour.

The silencing of one of the parental alleles of imprinted genes, which is established in the early post-implantation embryo, is known to involve a complex combination of methyl groups being added to DNA at key ‘imprinting control regions’ (ICRs), modification of core histones surrounding the genes and regulation by non-coding RNAs. Differential epigenetic marking of the parental chromosomes results in differential reading by the transcriptional machinery, and as a consequence gene expression is predominantly from one parental allele. However, it is becoming increasingly clear that there is a high degree of control of expression of imprinted genes, such that some genes only
show imprinted expression (i.e. monoallelic) in particular tissues and in other tissues show non-imprinted (biallelic) expression. For instance the maternally expressed gene UBE3A is imprinted in brain, but non-imprinted in other tissues where it is expressed.11

Imprinted genes and mental disease

Given their involvement in a number of important physiological processes, it is no surprise that imprinted genes are involved in a number of key diseases.12 Two of the first syndromes linked with genomic imprinting were the neurodevelopmental disorders Angelman (AS) and Prader–Willi (PWS); AS is characterized by severe learning difficulties, ataxia, seizures and EEG abnormalities, PWS is characterized by a failure to thrive in infancy, mild learning difficulties and on emerging from infancy a grossly abnormal satiety response to food intake. Although phenotypically very different, both are caused by disruption of the cluster of imprinted genes on human chromosome 15q11–q13, which contains a number of paternally expressed genes and brain-specific small nucleolar RNA species, and two maternally expressed genes (Fig. 1A). The key point however is that despite being due to disruptions of the same region, AS and PWS have distinct molecular genetic underpinnings. AS is due to a lack of expression of maternally derived genes (in particular UBE3A), caused by maternally derived de novo deletions of the cluster, chromosome 15 paternal uniparental disomy (pUPD) and disruption of the imprinting mechanism itself. Conversely, PWS results as a consequence of lack of expression of paternally derived genes, caused, again, by de novo deletions of the cluster (this time paternally derived), chromosome 15 maternal uniparental disomy (mUPD) (Fig. 1B and C) and ICR mutations.

In addition to the main phenotypic characteristics, both AS and PWS display a high incidence of neuropsychiatric abnormalities. Individuals with PWS in particular are prone to an affective disorder, including mood instability, non-psychotic depression and psychosis.13 What is emerging is that different genotypes, all resulting in the core deficits in PWS (Fig. 1B and C), can give rise to different patterns of mental illness.13,14 Specifically, those individuals with PWS as a result of maternal chromosome 15 mUPD genetic sub-type in particular, are far more prone to experiencing a psychotic episode than the paternal 15q11–q13 deletion sub-type. These data allow us to start assigning specific gene(s) to particular parts of the phenotype; for instance, the predicted over-dosage of maternally expressed UBE3A and ATP10C gene products due to the presence of two maternal copies in the
mUPDs suggests that these gene products are important in the aetiology of psychosis in PWS.\textsuperscript{15,16}

In addition to the specific examples provided by these two syndromes, more general imprinted gene effects (if not actual genes) have been assigned to various psychiatric illness including Tourette syndrome, Bipolar disorder, schizophrenia and autism.\textsuperscript{17} The degree of evidence for these varies and comes from the association of areas of the genome known to contain imprinted genes with a particular psychiatric condition, to biases (maternal or paternal) in the inheritance pattern of these illnesses. However, the region of chromosome 15 associated with AS and PWS appears to be a particular ‘hot-spot’, with a strong maternally derived association with autism.\textsuperscript{18,19}

Disruption of the epigenetic control mechanism: Rett syndrome

In addition to physical changes in the DNA encoding imprinted genes (mutation, deletion, duplication of the DNA sequence), disruption of...
the epigenetic regulation itself can also result in abnormalities. A striking example of this is when there is a mutation in one of the genes encoding part of the regulatory cascade, as occurs in Rett syndrome.

Rett syndrome is caused, in 80% of cases, by a disruption of the gene MeCP2, situated on the X-chromosome. It is a severe, progressive neurodevelopmental syndrome, affecting only girls (the mutation is lethal in males), which demonstrates a number of overlapping clinical features with autism and AS. This latter observation has been strengthened by recent molecular analysis of the role of methyl CpG binding protein 2, the product encoded by MeCP2. MeCP2 is a nuclear protein that binds specifically to methylated DNA, of crucial importance in the epigenetic control of imprinted expression.4 It is thought to act as an expression silencer, binding to methylated regions of DNA and altering the chemical tagging of the surrounding histones. This possibly also results in changes in the 3-D conformation of the chromatin, reducing the access of the expression machinery to the DNA sequence.20 Although not essential for the control of all imprinted genes, MeCP2 appears to have a special regulatory relationship with at least two: DLX5 and the AS candidate gene UBE3A. Studies of MeCP2-deficient mice have demonstrated that this protein is involved in subtle epigenetic control at the UBE3A locus. While DNA-methylation of this interval is unchanged, the absence of MeCP2 results in histone changes associated with the ICR, that in turn eliminates the expression of UBE3A,21 as occurs in AS. Given these molecular links, and the overlap in clinical features between AS, Rett and autism, we can begin to ascribe aspects of brain abnormalities to genes and underlying neurobiology, in this case for instance, synaptic dysfunction.22

**Disruption of the epigenetic control mechanism: assisted reproductive technologies**

A number of animal studies have established that *in vitro* culture of early stage embryos disrupts the normal developmentally programmed epigenetic processes. These can have pronounced phenotypic effects, particularly in ruminants,23 and may provide an explanation for the foetal over-growth and other problems seen in cloned animals.24 Although not exclusive to imprinted genes,25 this class of genes will obviously be particularly sensitive to any alteration in epigenetic status that occurs through *in vitro* culture.

It is against this background that attention has recently turned to examining the consequences of assisted reproductive technologies (ART) on imprinted-gene-related disorders. There is an accumulating body of evidence suggesting that this is indeed the case, although it must be stressed that as many imprinted gene disorders are in
themselves quite rare, the absolute risk is extremely low.\textsuperscript{25} A number of studies have suggested increased incidence of offspring born with AS following ART, and in all cases there was loss of methylation at a key ICR, demonstrating that alteration of the epigenetic status was causal (as opposed to a \textit{de novo} gene deletion).\textsuperscript{26,27} However, the extent to which epigenetic changes at imprinted (and other) genes occur with ART remains to be seen—for instance two recent studies have failed to find an increased incidence of PWS.\textsuperscript{28,29} Nevertheless, taken together, animal studies and initial clinical work do suggest that the epigenetic code may change with embryo culture \textit{in vitro}. Furthermore, this demonstrates quite nicely the fact that unlike the far more stable DNA code, the information provided by the epigenome is less fixed.

\section*{Labile epigenetic mechanisms in the brain}

Although many epigenetic modifications are developmentally determined, there is also an inherent lability in some, as has been demonstrated by the effects of ART, described above, and also of toxins and environmental insults.\textsuperscript{30} A striking example of the epigenome’s ability to alter has recently been shown by examining changes in DNA methylation and histone modification at the genome level over time. In monozygotic twins, where the DNA code is identical, the epigenome of twins diverged with age,\textsuperscript{31} suggesting that this may be a molecular mechanism by which differing life experiences are encoded. These data set up the possibility that epigenetic marks may have evolved as an additional molecular response mechanism, speeding up the process of adaptation to a changing or less predictable environment.\textsuperscript{32}

In some circumstances, these changes to the epigenome are also ‘heritable’. A good example of this is provided by the epigenetic inheritance of maternal behaviours. Variations in care given to pups by female rats has long-lasting consequences for the development of those offspring, including effects on cognition and response to stress.\textsuperscript{33} The levels of care can be characterized and easily measured in the experimental setting, by the frequency of licking and grooming of the pup by the mother. These variations in maternal care are transmitted across generations, such that low licking and grooming (LG) mothers beget low LG mothers. Fascinatingly, this inheritance of maternal care is non-genomic.\textsuperscript{34} Recently, a possible molecular basis of this behavioural difference has been identified.\textsuperscript{35} Individuals raised by high LG mothers, demonstrate higher expression of the oestrogen receptor (ER)-\textalpha in the medial pre-optic area of the hypothalamus, a gene and brain region associated with maternal care. The opposite effect is seen with individuals raised by low LG mothers. Correlative changes in
DNA-methylation at key promoter regions of the ERα gene probably control this expression, and in turn the manifestation of maternal behaviour. These molecular changes are probably not heritable themselves, rather the behaviour of the mother sets up key molecular changes that last into adulthood affecting subsequent brain function. However, these mechanisms do appear to provide a ‘soft’ inheritance system that parallels that of the hard-wired genome.36

Gene–environment interactions

These and other studies strongly suggest that modification of DNA-methylation at key regulatory regions and/or chromatin-surrounding genes may be a way encoding life events at a molecular level. But how are we to understand this code? There are upwards of 35 000 genes in people, on top of this there are eight core histones, which may be modified in at least 25 different ways, each of which could theoretically encode differing information. There are moves to decipher this code,37 but in the first instance in order to establish the idea is indeed valid, it may be necessary to centre the epigenetic decoding on a limited set of genes.

One way of doing this is by focusing on gene variants that give rise to disease. A number of gene candidates have now been identified that predispose individuals to neuropsychiatric disorders. Recently it has been shown, not unexpectedly, that these do not automatically result in a disease, but that life events in combination with certain gene variants give a far higher likelihood of developing the disorder. For instance, a polymorphic variant of the gene encoding the monoamine oxidase A (MAO-A) that results in higher expression of this neurotransmitter metabolizing enzyme protects individuals from developing violent tendencies after experiencing maltreatment as children.38 However, individuals who have the low-expressing version of the MAO-A gene and are maltreated as children are more likely to go on and become violent themselves. Similarly, the influence of stressful life events (such as maltreatment as a child) on developing depression is in part moderated by the presence an allelic variant of the serotonin transporter (5-HTT), with a single copy of the ‘short’ variant increasing this risk.39,40 Crucially, those genetically predisposed and who experience maltreatment as a child may yet be prevented from developing depression, as environmental intervention, in the form of social support, moderates the risk yet further.40

Thus far studies of these kinds are few and far between. However, it is tantalizing to think that the effects of these life events are mediated via DNA-methylation and/or histone modifications at the candidate loci. For instance, one hypothetical scenario could be that experiencing
maltreatment results in increased DNA-methylation at the MAO-A gene, therefore decreasing its expression. Those individuals with a high-expression genetic polymorphism are spared any effects, but those with the low-expression variant have MAO-A levels that dip below a key threshold, resulting in a tendency to be violent in later life. Nevertheless, at present these ideas remain untested and are purely theoretical.

**Discussion**

In this review we have begun to address the contribution of epigenetic mechanisms to brain function, and consequently dysfunction in the form of neuropsychiatric disorders. In the classical sense epigenetics describe the machinery and process involved in regulating gene expression, particularly during development. In this respect we have outlined a sub-group of genes, namely those subject to genomic imprinting, for which the epigenetic control is very important and when this goes wrong can result in clinical conditions. Although it is not entirely clear at present, work from animal studies suggests that imprinted genes play an important role in the brain and may well contribute more generally to neuropsychiatric disorder in humans.7,8,15

In addition to pre-programmed gene regulation, there is a growing body of evidence suggesting that epigenetic mechanisms may also provide a molecular memory of environmental experiences. This has been clearly shown in animal models35 and indicated in studies of human twins.31 In some circumstances the epigenetic changes simply reflect long-term changes in gene expression levels. However, alterations to the epigenetic code may not result in gross changes in gene expression per se, but provide an additional level of molecular information. A good example of this has been provided by studies of acute and chronic cocaine use in rats. These drug regimens give rise to subtly different histone modifications around the promoter of the FosB gene,41 but not differences in expression per se. Instead, it is thought that this may underlie the fact that expression of the FosB gene variant, ΔFosB only partially desensitises to chronic cocaine treatment.

Although clearly epigenetic mechanisms are of potential clinical relevance, a key question is whether they are accessible to pharmacological intervention. There are a number of histone deacetylase (HDAC) inhibitors and DNA methyltransferase inhibitors that are currently being used and/or tested as anticancer drugs.3 However, how applicable these are to treating neuropsychiatric disorders is not clear—although valproate has HDAC inhibitor properties and may, in part, exert its action through epigenetic effects on the schizophrenia candidate gene Reelin.42 Currently a problem exists between the
specificity of the epigenetic changes that may occur (i.e. specific modifications, key time points and discrete brain regions\textsuperscript{11,35,41}), and the general influence of HDAC and DNA methyltransferase inhibitors on gene expression. It is hoped that the ongoing epigenome project\textsuperscript{37} and the development of more specific drugs may address these issues.

**Acknowledgements**

ARI is supported by the Beebe Trust Research Fellowship, and LSW is LINK Professor of Behavioural Genetics at the Department of Psychological Medicine, School of Medicine, and School of Psychology, Cardiff University, Cardiff, UK.

**References**


