

# Pre- and peri-natal environmental risks for attention-deficit hyperactivity disorder (ADHD): the potential role of epigenetic processes in mediating susceptibility

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Attention-deficit hyperactivity disorder (ADHD) is a common childhood neurobehavioural disorder defined by symptoms of developmentally inappropriate inattention, impulsivity and hyperactivity. As is the norm for most psychiatric phenotypes, traditional aetiological studies have focused primarily on the interplay between genetic and environmental factors. It is likely that epigenetic factors, i.e., heritable, but reversible changes to genomic function that are independent of DNA sequence, are also important. It is known that epigenetic processes can be induced following exposure to a range of external factors, and thus provide a mechanism by which the environment can lead to long-term alterations in phenotype. In this article we hypothesise that epigenetic dysregulation may mediate the association observed between early-development environmental insults and ADHD. We propose that understanding the epigenetic processes involved in linking specific environmental pathogens to an increased risk for ADHD may offer new possibilities for preventative and therapeutic intervention. **Keywords:** Epigenetics, DNA methylation, environment, prenatal, attention-deficit hyperactivity disorder (ADHD), genetics, psychiatry, DOHaD, fetal programming.

ADHD is a common childhood neurobehavioural disorder defined by symptoms of developmentally inappropriate inattention, impulsivity and hyperactivity. A recent meta-analysis estimated the worldwide prevalence of ADHD to be 5.29% (Polanczyk, de Lima, Horta, Biederman, & Rohde, 2007), making it the most prevalent psychiatric disorder of childhood. The social and economic costs of childhood ADHD are considerable (Leibson, Katusic, Barbaresi, Ransom, & O'Brien, 2001), and difficulties often persist into adulthood. Children with ADHD are at high risk for developing adjustment problems, antisocial behaviour, substance abuse, other psychiatric disorders, and difficulties in education and work (Harpin, 2005).

As is the norm for the majority of psychiatric phenotypes, traditional aetiological studies have focused primarily on the interplay between genetic and environmental factors. Family, twin, and adoption studies provide overwhelming evidence for an inherited contribution to the pathogenesis of ADHD, with heritability estimated to be about 70% (Faraone et al., 2005). Recent years have seen considerable research effort dedicated towards understanding the genetic basis of ADHD, and replicated associations with polymorphisms in several genes have been uncovered. Despite this, we are still a considerable distance from fully understanding the inherited causes of ADHD, and the ultimate goal of using

genetic information to aid in diagnosis and the development of novel therapeutic strategies remains some distance away. In addition to the large genetic component to ADHD, quantitative genetic studies highlight that environmental factors are likely to be important. Accumulating epidemiological evidence now links exposure to various environmental insults, occurring especially prenatally or early in development, with an increased risk of ADHD. The mechanism(s) by which such environmental factors mediate susceptibility, however, is not well understood. The aim of this article is to highlight the potential role of epigenetic processes in linking the prenatal environment to ADHD-like behaviour via long-lasting changes in gene expression. Epigenetics refers to the heritable, but reversible, regulation of various genomic functions mediated principally through changes in DNA methylation and chromatin structure (Henikoff & Matzke, 1997).

## The prenatal environment and ADHD

A number of comprehensive reviews describing the environmental risks associated with ADHD have been recently published (Banerjee, Middleton, & Faraone, 2007; Williams & Ross, 2007), and so they will not be discussed in detail here. One consistent observation from these studies is that the timing of exposure to environmental insult appears to be important. In fact, exposure to virtually all known environmental risk factors for ADHD occurs early in development – either *in utero* or during the

Conflict of interest statement: No conflicts declared.

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immediate neonatal period. Whilst it can be difficult to tease apart the pathogenic effects of such early-life environmental factors from the effects of correlated parental behaviour occurring via gene–environment correlations (Jaffee & Price, 2007), the available epidemiological data suggests that there may be a direct association between environmental insults at this time and ADHD.

This observation of prenatal environmental factors increasing risk of ADHD concurs with findings from many other types of illness, both psychiatric and physiological. A common theme across the biomedical sciences is that exposure to adverse environments, particularly during key *in utero* and postnatal developmental periods, dramatically increases the risk of disease later in life. Such a link is particularly well established for cardiovascular and metabolic disorders such as coronary heart disease, obesity and type 2 diabetes. The developmental origin of health and disease (DOHaD) hypothesis argues that a poor environment in pre- and early postnatal life manifests itself in permanent changes to various metabolic processes in the body. Whilst such changes are initially adaptive, they lead to an increased risk of chronic disease later in life. Epidemiological studies show that reduced birth-weight, which is strongly correlated with fetal under-nutrition, is associated with a range of cardiovascular and metabolic diseases (Barker, 1998; Gluckman & Hanson, 2004). Despite mounting evidence for a role of the prenatal environment in ADHD, and other forms of psychiatric illness, researchers have only just begun to interpret these disorders within the context of the DOHaD hypothesis. One limitation is that the biological mechanisms underlying such fetal programming are not yet well understood, especially with regard to behaviour-related phenotypes.

Examples of specific *in utero* risk-factors associated with ADHD-like deficits include prenatal exposure to nicotine (Linnet et al., 2003), alcohol (Mick, Biederman, Faraone, Sayer, & Kleinman, 2002), and recreational drugs (Accornero et al., 2007; Bandstra, Morrow, Anthony, Accornero, & Fried, 2001), pre- and neo-natal exposure to toxins such as polychlorinated biphenyls (PCBs) (Jacobson & Jacobson, 2003; Stewart et al., 2003) and hexachlorobenzene (Ribas-Fito et al., 2007), prenatal exposure to glucocorticoids (French, Hagan, Evans, Mullan, & Newnham, 2004), maternal stress during pregnancy (O'Connor, Heron, Golding, Beveridge, & Glover, 2002; Van den Bergh, Mulder, Mennes, & Glover, 2005), and a poor maternal diet (Neugebauer, Hoek, & Susser, 1999; Vermiglio et al., 2004). In addition, one overall indicator of a sub-optimal *in utero* environment, i.e., small size at birth, is also one of the strongest 'environmental' predictors of ADHD-like symptoms in childhood (Bhutta, Cleves, Casey, Craddock, & Anand, 2002; Hultman et al., 2007; Kelly, Nazroo, McMunn, Boreham, & Marmot, 2001; Lahti et al., 2006; Nigg & Breslau, 2007). This is an

interesting observation given that fetal growth is known to be an indicator of the environmental exposures that programme fetal development. The impact of the prenatal environment on ADHD may, at least in part, be mediated by genetic factors. For example, the dopamine transporter gene (*DAT1*) interacts with prenatal nicotine and alcohol exposure to increase risk for ADHD (Brookes et al., 2006; Kahn, Khoury, Nichols, & Lanphear, 2003). At present, such findings are hard to interpret because the precise mechanistic routes via which these two sets of aetiological factors interact have yet to be ascertained. Understanding these mechanisms is vital if any diagnostic, therapeutic or preventative benefits are to result.

Findings linking the prenatal environment to ADHD in human epidemiological studies are supported by animal experiments. For example, animal studies clearly demonstrate an association between *in utero* toxin exposure, maternal stress and malnutrition during pregnancy, and a range of neurochemical and behavioural changes paralleling those seen in humans with ADHD (Hausknecht et al., 2005; Holene, Nafstad, Skaare, & Sagvolden, 1998; LeSage, Gustaf, Dufek, & Pentel, 2006; Paz, Barsness, Martenson, Tanner, & Allan, 2007; Weinstock, 2001). Animal studies have shown that many toxins, including PCBs, nicotine, alcohol, and other recreational drugs, can pass directly and rapidly across the placenta to the developing fetus, where they become highly concentrated and have effects on the developing nervous system (Walker, Rosenberg, & Balaban-Gil, 1999). Prenatal exposure to nicotine, for example, disrupts neuronal path-finding, produces abnormalities in cell proliferation and differentiation, and stunts the development of cholinergic and catecholaminergic systems (Ernst, Moolchan, & Robinson, 2001). *In utero* exposure to alcohol disrupts brain development, and can cause neuronal loss, altered circuitry, and neurodegeneration (Huizink & Mulder, 2006). PCBs are known to have strong neurotoxic effects on the developing brain, altering thyroid functioning, neurotransmitter levels, and the metabolism of dopamine (Tilson & Kodavanti, 1997). It appears that prenatal maternal stress exerts an effect on offspring behaviour via increased corticotrophin-releasing hormone levels and disruption to normal hypothalamic-pituitary-adrenal (HPA) axis functioning (Talge, Neal, & Glover, 2007; Weinstock, 2001). This is interesting because recent data supports a general role for the HPA-axis in mediating the effects of fetal programming on susceptibility to a whole swathe of chronic diseases after birth via the action of adrenal glucocorticoids (Phillips, 2007; Phillips, Jones, & Goulden, 2006). These conclusions are especially pertinent for neuropsychiatric diseases given the strong connections between HPA-axis dysfunction, neurobiological development, and the risk of psychiatric disorders (Teicher et al., 2003).

Whilst a connection between early environmental insults and ADHD has been shown at a statistical level, and we are starting to understand more about the intermediate neurophysiological developmental processes involved in bringing about these effects, a mechanism directly linking the *in utero* environment to postnatal risk is still to be uncovered. It remains unclear how exposure to various toxins early in development produces the altered neurochemistry later in childhood that manifests phenotypically in disorders like ADHD. It is likely that adverse *in utero* environmental factors exert their effect by changing the patterns of gene expression associated with normal neurodevelopment, but the specific molecular processes involved in this chain of events have yet to be described. It is thus pertinent that many environmental toxins, both chemical and psychosocial, have been shown to cause long-lasting epigenetic changes to the genome, directly altering gene expression and phenotypic outcome. The aim of this article is to highlight the potential role of epigenetic mechanisms in linking early-life environmental factors to the risk of developing ADHD later in childhood.

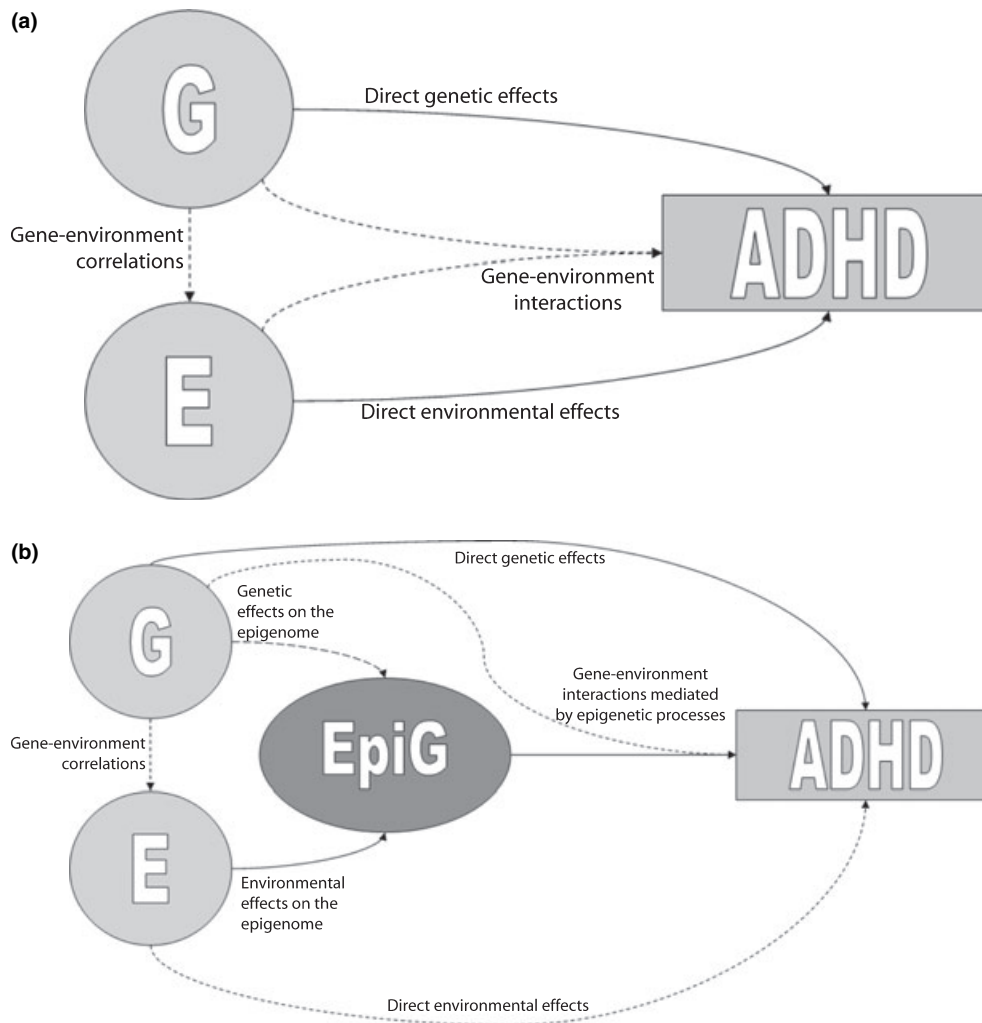
### Epigenetic processes and their relevance to ADHD

Epigenetic processes are essential for normal cellular development and differentiation, and allow the regulation of gene function through non-mutagenic mechanisms. The primary focus of this article 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 (Jaenisch & Bird, 2003). The methylation of these CpG sites, over-represented 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, another epigenetic mechanism mediating gene expression, affects chromatin structure via the processes of histone acetylation, histone methylation, and histone phosphorylation (Jenuwein & Allis, 2001). 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 (Jones et al., 1998). Transcriptionally competent chromatin is generally enriched with acetylated histones, but transcriptionally silent chromatin is normally deacetylated. Recently, a third epigenetic system involving small interfering RNA (siRNA) has

been described (Hamilton, Voinnet, Chappell, & Baulcombe, 2002). It has been shown that siRNA can suppress the activity of specific genes via targeted RNA interference (RNAi), a mechanism likely to be integral to the developmental regulation of gene expression.

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 tissue-specific, developmentally regulated and potentially highly dynamic, even within an individual. As will be discussed below, it is known that environmental factors can alter epigenetic processes, but in addition random stochastic and developmental epigenetic changes are also important. 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 (Riggs, Xiong, Wang, & LeBon, 1998; Ushijima et al., 2003). Because epigenetic processes are integral in determining when and where specific genes are expressed, such epigenetic metastability, environmentally or stochastically induced, may have important phenotypic effects. Understanding the heritability and mutability of epigenetic marks is critical if we are to comprehend how epigenetic processes may mediate developmental phenotypic changes in response to the environment.

The dynamic nature of the epigenome offers new insights about several non-Mendelian features of ADHD, unexplainable using traditional gene- and environment-based approaches (see Figure 1). It has been argued that epigenetic factors make an important contribution to disease susceptibility for a number of complex psychiatric phenotypes (Mill & Petronis, 2007; Petronis, 2004). Epigenetic differences could account for the incomplete monozygotic (MZ) twin concordance rates often observed for ADHD-related behavioural traits (Lehn et al., 2007). This notion is supported by mounting evidence for DNA methylation differences between genetically identical individuals (Fraga et al., 2005; Mill et al., 2006). Epigenetic processes may also cause the highly skewed sex ratio observed in ADHD – prevalence rates in males are approximately five times higher than those observed in females. It has been shown that sex hormones can alter DNA methylation around specific loci in the genome (Saluz, Jiricny, & Jost, 1986; Yokomori, Moore, & Negishi, 1995), controlling gene expression in a sex-specific manner. Epigenetic mechanisms may also be behind the parental-origin effects observed in molecular genetic association studies of ADHD (Hawi et al., 2005). One explanation for such effects is genomic imprinting – the differential expression of genes depending on



**Figure 1** Incorporating epigenetic factors into aetiological models of ADHD. a) Traditional aetiological approaches see susceptibility only in terms of genetic and environmental factors. These factors are generally studied independently, but there is increasing emphasis on gene–environment interactions, whereby the phenotypic effect of genotype is mediated by the environment. b) The epigenetic theory of complex disease includes a third set of aetiological factors, namely those that impact upon the epigenome. Epigenetic dysfunction, mediated via processes such as DNA methylation and histone modifications, may act directly but are also likely to interact with both genetic and environmental factors. Increasing evidence suggests that the phenotypic effects of environmental factors, especially during key developmental periods, may be mediated by epigenetic processes.

whether they are inherited from the paternal or the maternal side. Genomic imprinting is fundamental to normal mammalian development and growth, and plays an important role in brain function and behaviour (Davies, Isles, & Wilkinson, 2005).

It was traditionally believed that epigenetic profiles are reset 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 (Rakyan, Preis, Morgan, & Whitelaw, 2001). It appears that such meiotic transmission of epigenetic alleles, or ‘soft inheritance’, may be a fairly common phenomenon in a number of eukaryotic organisms (Richards, 2006). 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, especially if epigenetic processes are environmentally labile, and may partly explain why researchers are finding it hard to pinpoint specific causal gene polymorphisms in apparently highly heritable disorders like ADHD.

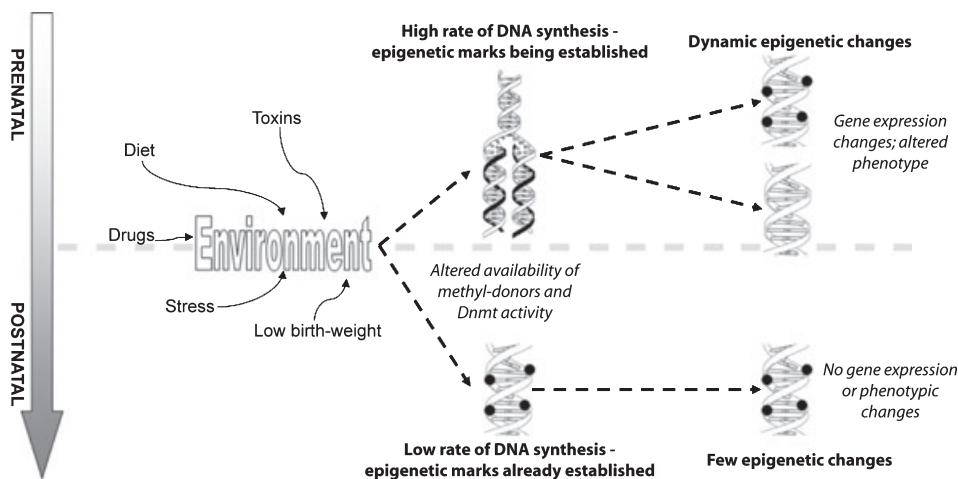
### Epigenetics – linking the environment to phenotype

There is mounting evidence to suggest that epigenetic processes can be induced following exposure to a

range of environmental insults. DNA methylation, for example, has been shown to vary as a function of numerous nutritional, chemical, physical, and psychosocial factors. Because such epigenetic changes are inherited mitotically in somatic cells, and can have important effects on normal gene function, they provide a mechanism by which the environment can lead to long-term alterations in phenotype. A recent study by Fraga and colleagues examined DNA methylation and histone acetylation in 80 pairs of MZ twins, ranging from 3 to 74 years of age, using a combination of global and locus-specific methods (Fraga et al., 2005). They found that one-third of MZ twins had a significantly dissimilar epigenetic profile, with older twins and those with a history of non-shared environments being the most disparate, suggesting that environmental factors may shape the epigenome over the life-course. While this study highlighted mounting epigenetic discordance with age, a recent study of the *COMT* gene also found significant DNA methylation differences within MZ twin-pairs detectable in very young children (Mill et al., 2006). In fact, given the predominantly prenatal environmental factors associated with increased risk of ADHD, it is notable that the epigenome appears to be particularly labile during a number of key developmental periods (Waterland & Michels, 2007). Figure 2 illustrates how this is particularly the case during embryogenesis; during this period, the rate of DNA synthesis is high and the epigenetic marks needed for normal tissue differentiation and development are being established (Dolinoy, Weidman, & Jirtle, 2007). At this time,

environmentally induced epigenetic changes are more likely to be propagated via mitosis leading to long-term changes in gene expression and phenotype, and potentially increasing susceptibility to disorders such as ADHD after birth. Later in development, when epigenetic marks are already established and the rate of DNA synthesis is much lower, environmental factors are likely to have a much smaller influence on epigenomic lability.

There are several mechanistic routes via which the environment may influence epigenetic processes to bring about changes in gene expression and phenotype. First, a major external influence on DNA methylation involves the availability of methyl-donors and co-factors, usually contained in the diet, which are required for the formation of S-adenosyl-methionine (SAM). SAM, in turn, acts as a methyl-donor for the methylation of cytosine DNA residues, a reaction catalyzed by a family of enzymes called DNA methyltransferases (Dnmts). *De novo* DNA methylation is initiated by Dnmt3a and Dnmt3b, and maintained in mitotic lineages by Dnmt1 (Bestor, 2000). Examples of dietary factors required for the formation of SAM include folate, methionine, choline, vitamin B12, vitamin B6, and vitamin B2. Given the role of DNA methylation in coordinating the correct pattern of gene expression during embryogenesis and development, it can be postulated that exposure to a diet lacking such components at specific developmental time-points could have detrimental phenotypic effects. Extreme folate deficiency, for example, is known to cause depletion of SAM, resulting in genome-wide DNA hypomethyl-



**Figure 2** Environmental insults during certain key developmental periods may have important long-term pathogenic effects that are mediated by epigenetic processes such as DNA methylation. Influences such as the availability of methyl-donors in the diet are likely to be particularly important during embryogenesis and the immediate postnatal period when the rate of DNA synthesis is high and the epigenetic marks needed for normal tissue development are being established. At this time, environmentally induced epigenetic changes are more likely to be propagated via mitosis leading to long-term changes in gene expression and phenotype, and potentially increasing susceptibility to disorders such as ADHD after birth. Later in development, when epigenetic marks are already established and the rate of DNA synthesis is much lower, environmental factors are likely to have a much smaller influence on epigenomic lability

ation, the activation of oncogenes, and cancer (Duthie et al., 2004). Interestingly, it has been shown that a dietary deficiency of vitamin B12 and folate is linked to impaired central nervous system development and a number of psychiatric conditions, including behavioural disturbances in childhood (Reynolds, 2006). Second, environmental factors may also impact on gene expression by regulating DNA methyltransferase activity – a folate deficient diet, for example, has been shown to alter the expression of Dnmts and cause epigenetic gene silencing across certain genomic regions (Ghoshal et al., 2006). Third, it is likely that environmental-epigenetic interactions operate in the other direction; i.e., the epigenetic status of a gene may actually influence the physiological response of a cell to a specific environmental pathogen and thus make it more (or less) vulnerable to its pathogenic effects.

Finally, environmental mediation of the epigenome may go some way towards providing a mechanism for the gene-environment interactions that are being uncovered in ADHD (Brookes et al., 2006; Kahn et al., 2003). For example, genetic polymorphisms may alter the ability of a specific region of the genome to be epigenetically altered in response to an environmental pathogen. Alternatively, the pathogenic effects of a polymorphism associated with disrupted gene function may be exaggerated if the expression level of that specific gene is directly influenced by certain environmental factors via processes such as DNA methylation. The interplay between the genome, the environment, and epigenetic processes may be further complicated by the fact that some DNA alleles and haplotypes are themselves associated with a specific epigenetic profile. Of relevance to ADHD, given that the serotonin 5-HT<sub>2A</sub> receptor gene is a promising functional candidate for the disorder (Norton & Owen, 2005), is evidence that the C102T polymorphism in this gene is methylated specifically on the C allele (Poleskaya, Aston, & Sokolov, 2006). 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 such as ADHD, and suggests that in addition to investigating environmental effects on the epigenome, a comprehensive epigenetic analysis of candidate SNPs and haplotypes is also warranted.

### Environmental mediation of the epigenome – specific examples that could inform studies of ADHD

There are now several specific examples of environmentally induced epigenetic lability. Of particular interest are DNA methylation changes at so-called ‘metastable epialleles’; loci that can be epigenetically

modified to produce a range of phenotypes from genetically identical cells (Rakyan, Blewitt, Druker, Preis, & Whitelaw, 2002). Many of these loci have been shown to be environmentally sensitive, particularly in response to the nutritional environment of the developing fetus. Several studies now support the notion that maternal methyl supplementation during pregnancy can alter DNA methylation, gene expression, and phenotype at a number of these metastable epialleles (Cooney, Dave, & Wolff, 2002; Dolinoy, Weidman, Waterland, & Jirtle, 2006; Waterland et al., 2006).

One classic example is that of the agouti viable yellow allele ( $A^{vy}$ ) inbred mouse strain, which demonstrates a range of coat-colour phenotypes, depending upon the epigenetic state of a transposable element upstream of the agouti gene. The transposon contains a cryptic promoter, which expresses a phenotype characterised by yellow fur and various detrimental metabolic phenotypes such as diabetes and obesity. When the transposon is methylated, this phenotype is not expressed; the mice have brown fur and are metabolically healthy. Interestingly, DNA methylation across this region, and thus phenotype, can be manipulated in offspring by altering the diet of pregnant mothers (Cooney et al., 2002; Dolinoy et al., 2006). Enriching the maternal diet with methyl-donor supplements increases offspring DNA methylation across this region, leading to gene expression changes associated with brown fur and metabolic health. A similar effect is seen for another transposon-associated metastable epiallele in *Axin Fused* mice that produces a kinky tail phenotype directly correlated to the degree of DNA methylation across the region (Waterland et al., 2006). Supplementing the maternal diet with methyl donors increases transposon DNA methylation in offspring, dramatically reducing the tail-kink phenotype (Waterland et al., 2006). Increasing evidence suggests that epigenetic dysfunction may be the cause of the metabolic phenotypes resulting from a poor *in utero* environment, thus providing a mechanism behind the effects described by the DOHaD hypothesis. Feeding a protein-restricted diet to pregnant rats, for example, causes hypomethylation of the hepatic glucocorticoid receptor in their offspring, a process mediated by reduced Dnmt1 expression, and results in increased metabolic risk (Lillycrop et al., 2007). Such strong links between nutrition and phenotype, mediated by epigenetic processes, are interesting given the evidence linking adverse dietary factors, both prenatally (Neugebauer et al., 1999; Vermiglio et al., 2004) and during childhood (Burgess, Stevens, Zhang, & Peck, 2000; Stevenson, 2006) with ADHD-related symptoms.

Various other external environmental factors have been shown to epigenetically alter gene expression in animals. Of interest given the recent link between prenatal hexachlorobenzene exposure and ADHD

(Ribas-Fito et al., 2007) is the observation that the offspring of pregnant rats exposed to the endocrine disruptor vinclozolin, another agrochemical used as a fungicide in crops, have altered DNA methylation profiles that correlate with adverse phenotypic changes (Chang, Anway, Rekow, & Skinner, 2006). It is not just the chemical environment that can cause long-lasting epigenetic changes – evidence suggests that even the psychosocial environment during key developmental periods early in life can epigenetically mediate gene expression (Szyf, Weaver, & Meaney, 2007). This is potentially interesting given studies linking early psychosocial adversity to an increased risk of developing ADHD (Biederman et al., 1995). Research by Weaver and colleagues (Weaver et al., 2004), for example, has shown that immediate 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, directly affecting gene expression and stress-related phenotypes in offspring. Interestingly, like the other examples discussed above, these epigenetic changes only occur during a specific critical period – in this case immediately after birth. Subsequent transcriptomic studies by the same group identified over 900 genes in the hippocampus that are stably regulated by maternal care (Weaver, Meaney, & Szyf, 2006), suggesting an even more widespread effect of the early social environment on gene expression through the life-course.

### Conclusions – implications for aetiological studies in ADHD

Exposure to a number of chemical and social environmental toxins early in development, either directly or via interactions with various genetic factors, appears to increase the risk of childhood ADHD. These observations concur with the DOHaD hypothesis, increasingly supported in relation to chronic metabolic disorders such as diabetes and coronary heart disease, which postulates that the environment during various critical developmental periods is crucial in determining the onset of diseases later in life. To date, however, the precise mechanisms linking such environmental risks to susceptibility have yet to be described. In this article we have demonstrated that exposure to various environmental toxins, both chemical and psychosocial, can lead to long-lasting alterations to epigenetic processes, which directly alter gene expression and phenotypic outcome. 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. However, we are now at a stage where it is feasible to start investigating the ways in which environmental

factors act upon the genome to bring about epigenetic changes in gene expression and behaviour, especially using animal models as in the examples described in this article. Understanding the epigenetic processes involved in linking specific environmental pathogens to an increased risk for ADHD may offer new possibilities for preventative and therapeutic intervention. In particular, the dynamic nature of the epigenome means that unlike pathogenic DNA sequence mutations, epigenetic disruption is potentially reversible. Furthermore, given the high number of mothers who continue to expose their unborn infants to potential risk factors for ADHD – approximately 20% of pregnant women continue to drink alcohol (Meschke, Holl, & Messelt, 2003) and over 25% of pregnant women who smoke continue to do so during pregnancy (Coleman, 2004) – finding a clear mechanistic link between prenatal toxin exposure and the risk of such psychiatric disorders should inform public health policy related to toxin exposure during pregnancy.

### Acknowledgements

This work was supported by a Canadian Institutes of Health Research (CIHR) Postdoctoral Fellowship to JM. Thanks to Dr Emma Dempster for critically reading the manuscript.

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Manuscript accepted 14 February 2008

## Glossary of key terms

Term	Definition	Key references
The developmental origins of health and disease (DOHAD) hypothesis	The notion that a poor <i>in utero</i> environment manifests itself in permanent changes to various metabolic processes in the body. The biological mechanisms underlying such fetal programming are not yet well understood, but are thought to involve epigenetic processes.	Gluckman & Hanson (2004)
Epigenetics	The heritable, but reversible, regulation of various genomic functions that occur independently of the DNA sequence. Epigenetic regulation is primarily mediated by DNA methylation, physical changes to chromatin structure, and the action of siRNA molecules.	Henikoff & Matzke (1997)
DNA methylation	The addition of a methyl group at position 5 of the cytosine pyrimidine ring in CpG dinucleotides. DNA methylation disrupts the binding of transcription factors and attracts methyl-binding proteins that are associated with gene silencing and chromatin compaction.	Jaenisch & Bird (2003)
Genomic imprinting	An epigenetic process that alters the expression of genes in a parent-of-origin specific manner. Genomic imprinting is fundamental to normal mammalian development and growth.	Davies et al. (2005)

### Glossary of key terms (Continued)

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Term	Definition	Key references
Epigenetic inheritance	Epigenetic signals are transmitted mitotically through cell lineages, but are generally assumed to be reset during gametogenesis and thus not transmitted meiotically. Evidence is mounting, however, that the epigenetic marks of at least some mammalian genes are not fully erased during meiosis suggesting the possibility of transgenerational epigenetic inheritance.	Richards (2006)

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