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Methylomics in Psychiatry: Modulation of Gene–Environment Interactions may be Through DNA Methylation

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Fine-tuning of neuronal connections during development is regulated through environmental interactions. Some fine-tuning occurs through changes in gene expression and/or epigenetic gene-specific DNA methylation states. DNA methylation occurs by transfer of a methyl group from *S*-adenosyl methionine to cytosine residues in the dinucleotide sequence CpG. Although CpG sequences spread throughout the genome are usually heavily methylated, those occurring in CpG islands in the promoter regions of genes are less methylated. In most cases, the extent of DNA methylation correlates with the extent of gene inactivation. Other known epigenetic mechanisms include histone deacetylation and chromatin remodeling, RNA inhibition, RNA modification, and DNA rearrangement. Exposure memory expressed as epigenetic DNA modifications allows genomic plasticity and short-term adaptation of each generation to their environment. Environmental factors that affect DNA methylation include diet, proteins, drugs, and hormones. Induced methylation changes may produce altered gene response upon subsequent hormonal stimulation. The gene-specific DNA methylation state may be preserved upon transmission through mitosis and meiosis. An increasing amount of data implicates a role for DNA methylation in multi-factorial psychiatric disorders. For example, L-methionine treatment can exacerbate psychosis; while valproate, a drug producing hypomethylated DNA, reduces such symptoms. Hypermethylation of the promoter region of the RELN gene correlates with reduced gene expression. This gene's protein Reelin, which is necessary for neuronal migration and synaptogenesis, is reduced in schizophrenia and bipolar disorder, suggesting hypermethylation of the promoter region in these disorders. Some evidence implicates methylation of the promoter regions of the *DRD2* and *HTR2A* genes in schizophrenia and mood disorders as well. DNA methylation usually increases with age, although hypomethylation of the promoter region of the amyloid A4 precursor gene during aging may play a role in Alzheimer's disease. More studies are needed to define the role of methylomics and other epigenetic phenomena in the nervous system.
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INTRODUCTION

The human genome is composed of three billion basepairs divided into 23 DNA molecules that make up chromosomes, and 30,000–50,000 genes [Ewing and Green, 2000; Venter et al., 2001] encoded in only 3% of the DNA sequence. The function of 40% of the genes and the remaining non-gene encoding genome is unknown. Some portion of the genome is needed to maintain chromosome structure and regulate gene expression [Sadock and Sadock, 2000].

Potentially, all genes could be expressed in all cells. However, gene function and regulation is modulated by genetic (i.e., DNA base sequence) and epigenetic factors. Epigenetic mechanisms allow short-term adaptation of genomic DNA and cells to the local environment [Hartl and Jones, 2001].

Thirty percent of the human genes, specifying at least 700 different types of proteins, are expressed in the nervous system. Some genes code for enzymes, receptors, and neurotransmitters, while others code for proteins responsible for the formation and structure of the nervous system [Davies and Morris, 1997]. Gene expression is modulated by the expression of other genes, DNA structure, and the environment; i.e., the surrounding cells and molecules [Hartl and Jones, 2001].

The mature adult nervous system, composed of 10^{15} synaptic connections, requires genetic and environmental factors and interactions for proper organization and stabilization [Tasman et al., 2003]. Sensory deprivation experiments demonstrated that the absence of external stimuli leads to decreases in synapses and permanent neuronal atrophy [Sadock and Sadock, 2000]. Hence, sensory deprivation lays a foundation for aberrant responses to the environment that can lead to disordered states, or diseases.

Numerous proteins synthesized in response to an environmental stimulus will activate gene expression in the nervous system. For example, serotonin (5-HT), in a process mediated by c-AMP and c-AMP response element-binding protein (CREB), activates expression of specific genes. Synapses previously activated by 5-HT can utilize the newly synthesized proteins for further growth and differentiation into new structural features of the brain [Kandel, 2001].

DNA methylation, an epigenetic mechanism associated with the modulation of gene expression, is used to imprint chromosomal DNA with a memory of past gene–environment interactions in the brain and other tissues [Bird, 2002]. Other epigenetic mechanisms include histone deacetylation and chromatin modification [Hendrich et al., 1999a,b; Hmadcha et al., 1999; Bird, 2002], RNA interference, RNA editing [Bass, 1997; Couzin, 2002], and DNA rearrangement [Hartl and Jones, 2001]. This article focuses on "methylomics" [Costello and Plass, 2001], or the science of DNA methylation, which is a concept widely appreciated in cancer research but only recently applied to the field of neuroscience.

DNA METHYLATION

In humans and other higher organisms, the vast majority of DNA methylation involves the addition of a methyl group to the cytosine ring carbon in position 5 (5-mC) in the dinucleotide sequence 5′CpG3′ of DNAs [Sadock and Sadock, 2000]. This reaction is catalyzed by multiple methyltransferases, including DNMT1, DNMT2, DNMT3A, and DNMT3B, which are encoded on different chromosomes [19p13.2, 10p15.1, 2p23, and 20q11.2, respectively; Bestor, 2000; Kim et al., 2002]. Methyl groups are provided by *S*-adenosyl methionine [Kim, 2000]. Recent data have also demonstrated triggering of DNA methylation by histone methylation [Lachner et al., 2001; Li, 2002]. In Table I, we have shown the precise chromosomal location of some genes involved in DNA methylation.

In the human genome, approximately 4–8% of Cs are methylated, and 5-mC constitutes only about 1% of the total residues that make up the genome [Attwood et al., 2002; Bird, 2002]. Potentially, there are 100 million CpG dinucleotide methylation targets in the diploid mammalian genome [Petronis et al., 1999]. The dinucleotide CpG occurs once per 80 dinucleotides throughout 98% of the genome; however, the frequency is increased in "CpG islands" located within and around the regulatory regions of genes [Costello and Plass, 2001].

There are approximately 29,000 CpG islands in the human genome, and 50–60% of all genes contain a CpG island [Singal and Ginder, 1999; Bird, 2002]. In vertebrates, 70% of CpGs are methylated, while the promoter regions of active genes, in general, are less than 30% methylated. Usually CpG islands of expressed genes have low levels of methylation [Naveh-Many and Cedar, 1981; Kress et al., 2001]. For example, the gene coding for the dopamine receptor D2 gene (*DRD2*) is fully methylated in intronic CpG rich areas in the frontal lobe (Mostafavi et al., in press), however, it is sparsely methylated in the promoter area in the striatum. Interestingly, in the human brain, the ratio of 5-mC:C at the DRD2 locus is significantly higher in the right vs. left striatum [Popendikyte et al., 1999]. Perhaps DNA methylation or other epigenetic factors control brain hemisphere laterality, which may be under epigenetic and developmental influences.

DNA METHYLATION AND SPECIFIC GENE EXPRESSION

Four proteins—MeCP2, MBD1, MBD2, and MBD3 (encoded on chromosomes X, 18q21, 18q21, and 19, respectively)—bind specifically to methylated DNA [Hendrich et al., 1999a]. Concurrently, histones are

TABLE I. Genes Involved in DNA Methylation

Genes	Chromosomal location
DNMT31	21q22.3
DNMT3a	2p23
DNMT3b	20q11.2
DNMT1	19p13.2
DNMT2	10p15.1
MBD1,2	18q21
MBD3	19p13.3
MBD4	3q21-q22
NOS1	12q24.2-q24.31

TABLE II. Some Genes Silenced by Promoter CpG Island Methylation

Gene	Protein
CD4	CD4 antigen
CD8	CD8 antigen
CDKN2A	Cyclin-dependent kinase inhibitor 2A
HBB	Hemoglobin beta
IFNG	Interferon, gamma
IL4	Interleukin 4
OXTR	Oxytocin receptor

deacetylated and the chromatin structure of the chromosome is modified [Hendrich et al., 1999b; Bird, 2002; Geiman and Robertson, 2002] reducing transcription [Attwood et al., 2002; Kim et al., 2002]. This process has been reviewed previously for tumor suppressor genes [Esteller, 2002] and some other genes [Attwood et al., 2002], which are summarized in Table II.

Dense DNA methylation is associated with irreversible silencing of gene expression, while partial methylation, a more frequent occurrence, marks genes that can be reactivated [Russo et al., 1996]. In spite of evidence indicating the presence of an active demethylation process [Szyf et al., 1995], no specific demethylating enzyme has yet been identified. However, some proteins and hormones, acting as transcription factors and binding to DNA, may have such a role [Costello and Plass, 2001], and some methyl CpG-binding proteins such as MBD2 could have demethylase activity as well [Detich et al., 2002].

Although demethylation is gene specific, in most instances changes are regionally rather than dinucleotide specific. However, a change in the methylation status of a few CpG dinucleotides is associated with a change in gene expression at a few loci. For example, differences in methylation at specific CpG dinucleotides in the cAMP-like response sequence element in several hormonal gene promoter regions is associated with changes in gene expression [Ngo et al., 1996].

Experiments in 1975 [Galfaian et al., 1975] demonstrated that dexamethasone treatments increased 5-mC content in rat brain by approximately 25%. These changes correlated with changes in functional activity and RNA synthesis. Based on these findings, the authors suggested that DNA methylation might be regulating transcription.

DNA methylation is believed to be important where the timing and location of gene expression are important [Russo et al., 1996; Kress et al., 2001]. Tissue-specific methylation modulation is a continuous process that allows gene expression levels to change in specific cells at specific times [Kress et al., 2001], especially during critical periods of development [Futscher et al., 2002]. For example, lactation and gestation change the level of DNA methylation and the level of gene expression of prolactin- and growth hormone-producing genes in the pituitary gland [Kumar and Biswas, 1988]. Other agents that induce DNA methylation changes include folic acid [McKinley et al., 2001], nitric oxide [Hmadcha et al., 1999], estradiol [Russo et al., 1996], estrogen [Kulig et al., 1992], prolactin [Reddy and Reddy, 1990], and steroid hormones [Galfaian et al., 1975; Thomassin et al., 2001]. Also, the effects of hormones are related to methylation of hormone-responsive genes, as has been shown for thyroid hormones [Adylova et al., 1993].

GENE MEMORY AND DNA METHYLATION

In some cases, DNA methylation changes appear to be transmitted through meiosis [Russo et al., 1996]. For example, in mice, transplantation of a nucleus from an early embryo into an egg cell led to DNA methylation increases in some genes, changes in genes expression, and a reduction of the body weight of the offspring. Furthermore, these phenotypes are transmitted to the next generation of offspring [Roemer et al., 1997]. Hence, “Lamarckian” inheritance may be transmitted through DNA methylation or other epigenetic effects [Monk, 1995].

In rat liver, glucocorticoid-induced DNA demethylation of the tyrosine aminotransferase gene is stable after hormone withdrawal and chromatin remodeling. In addition, the demethylated gene has a greater response to subsequent hormone stimulations, providing a potential mechanism for cell memory [Thomassin et al., 2001]. Apparently, there are critical periods during which transcription patterns can be permanently committed to developmental memory.

It is proposed that during early development, binding of gene-activating factors in promoter regions and recruitment of multiple transcription regulatory proteins to different sites that make up gene promoter regions may prevent methylation and lead to increased expression thereafter [Bird, 2002; Tasman et al., 2003]. Moreover, successive bindings of transcription factors to a gene’s regulatory area changes nucleosome structure, which is associated with a decreased methylation rate and more capability for gene expression [Murray et al., 2000].

DNA METHYLATION AND DEVELOPMENT

Global regulation of genomic methylation appears to be restricted to embryonic life. DNA in primordial germline cells is generally unmethylated; however, sperm DNA is highly methylated, whereas egg DNA appears to contain the adult methylation pattern [Haaf, 2001]. During early embryonic life (blastocyst stage), a period marked by large amounts of DNA replication, the paternal DNA is demethylated, but becomes methylated at implantation. During differentiation, the promoter regions of inactive genes become methylated [Oligny, 2001; Futscher et al., 2002]; although, in some cases, CpG methylation is associated with active genes [Costello and Plass, 2001].

The mechanism by which the genome globally demethylates soon after fertilization is not well known. In humans, remethylation begins at about the seventh day after fertilization [Hartl and Jones, 2001]. In mice, demethylation starts in the myoblastic stage and remethylation occurs after birth, indicating that both the pre- and post-natal periods are important stages for establishing adult methylation patterns [Tawa et al., 1990]. In summary, there is a resetting of methylation

patterns in early life and a subsequent dynamic demethylation, coinciding with developmental critical periods [Singal and Ginder, 1999; Thomassin et al., 2001]. Interference in the methylation process may result in developmental defects or even cell death [Watson and Goodman, 2002].

The methylation status of various CpG islands is geographically specific [Shen et al., 2002]. This suggests that environmental factors can disrupt methylation and cause endemic disease syndromes, perhaps through dietary or other effects. DNA methylation can be affected by the dietary level of methyl-donor precursors, such as folic acid. For example, in pregnant mice, supplements of methyl-donor precursor affect DNA methylation and related phenotypes in offspring [Wolff et al., 1998; Cooney et al., 2002]. The growth of granule cells in culture medium under deficient nutritional conditions leads to a reduction in DNA methylation and to apoptosis [Bertino et al., 1996].

DNA methylation is known to be involved in parental imprinting of gene alleles. A few hundred genes are imprinted [Hartl and Jones, 2001] with a memory of their parental origin by methylation [Watson and Goodman, 2002]. Most imprinted genes are involved in embryonic and placental growth. Here, imprinting may provide a mechanism to separate competing maternal and paternal interest in the growth of the embryo [Tilghman, 1999; Hartl and Jones, 2001]. For instance, imprinting is associated with expression of only the paternal insulin-like growth factor-2 gene (*IGF2*) and only the maternal IGF2-receptor in the embryo. A large fetus promoted by high amounts of IGF2 will increase the chance of survival of a single child even at the expense of the mother's health; however, maternal control of the IGF2-receptor allows rationing of the mother's resources and health, preserving the female's ability to have more children.

While the expression of most imprinted genes is inherited, sometimes these genes have biallelic expression during early development or in selected tissues. For example, in peripheral tissue, the paternal allele of the IGF2-receptor is methylated and inactive. In the central nervous system, both alleles (maternal and paternal) are unmethylated and biallelic expression occurs. The tissue-specific pattern of methylation and gene expression, and the fact that 5-azacytidine (a demethylating agent) removes IGF2-receptor gene methylation, suggests that imprinting can be affected by agents that interfere with epigenetic modifications and normal development.

DNA methylation is also involved in X-chromosome inactivation. Female cells have two copies of chromosome X, while male cells have a single copy of chromosome X and one chromosome Y. Despite this, the amount of protein produced from genes encoded on chromosome X is equal in both types of cells, because one X chromosome is randomly methylated and inactivated in each female cell [Watson and Goodman, 2002] except in regions that share homology with chromosome Y. This means that females are mosaics, where some cells express the maternally inherited X chromosome and other cells express the paternally inherited X chromosome. This leads to greater variability in the phenotype of females than males and the opportunity for greater variation in chromosome X epigenetic regulation. Males inherit and express only the maternal X chromosome and the approximately 200 genes on chromosome Y.

HYPERMUTABILITY OF CpG SEQUENCES

The rate of mutation at 5-mCpG is 20–40 times higher than for other dinucleotides [Cooper and Youssoufian, 1988]. This occurs because spontaneous deamination events convert 5-mC to thymine, and convert cytosine to uracil. Uracil is removed from DNA while the T-G mismatched base-pair is mutagenic [Hendrich et al., 1999b].

Deamination accounts for the 5–20-fold lower than expected occurrence of CpGs and the higher than expected frequency of TpG dinucleotides in eukaryotic genomes [Singal and Ginder, 1999]. This means that new traits may be evolved more rapidly by mutations at 5-mC residues especially present in inactive genes or silent foreign DNA elements integrated into the genome (e.g., endogenous retroviruses). Methylated and inactive foreign DNA is prone to C-to-T mutations and the creation of new genes from reactivation sequences [Monk, 1995].

DNA METHYLATION AND MENTAL DISORDERS

Alcohol abuse during pregnancy is well known for disturbing fetal brain development. In animal studies, alcohol administered during gestation reduced the production of brain-derived neurotrophic factor (BDNF) mRNA, the number of BDNF-synthesizing neurons, and glial fibrillary acidic protein (GFAP) mRNA in progeny. These changes are associated with hypermethylation of the respective genes [Valles et al., 1997; Maier et al., 1999]. Paternal alcohol consumption is also associated with reduced methyltransferase mRNA expression, and presumably DNA methylation, in spermatozoa [Bielawski et al., 2002].

In rats, DNA methylation increases with age [Rath and Kanungo, 1989]. Generally, increased DNA methylation is associated with aging in some, but not all, tissues [Bird, 2002]. DNA methylation may be abnormal in neurodegenerative disorders. For example, the levels of IGF2 and c-Fos expression, both important in the central nervous system, decrease coincidently with the degree and pattern of methylation in aged rat brain [Kitraki et al., 1993]. Moreover, cytosine hypomethylation was detected in atherosclerotic lesions and in APOE knock-out mice [Hiltunen et al., 2002].

A decrease in 5-mC in the promoter of the amyloid precursor protein (APP) gene was detected in elder individuals. This change may "contribute to amyloid beta protein deposition in aged brain" [Tohgi et al., 1999a]. Hypomethylation of this gene in an Alzheimer's disease patient has been reported as well [West et al., 1995]. In human brain, DNA methylation at specific loci may increase [Tohgi et al., 1999b] or decrease [Tohgi et al., 1999b] with age. Hence, DNA methylation varies with age in both time as well as place, and aberrant DNA

methylation in old age may lead to neurodegenerative disorders [Tohgi et al., 1999b] or cancer [Chen and Wu, 2002]. Interestingly, folic acid, which is known to facilitate methylation, is reported to be effective for prevention of some cancers and dementia [Young and Ghadirian, 1989].

Rett's syndrome and Angelman's syndrome, commonly co-morbid with epilepsy and mental disorders, are linked to DNA methylation function [Costello and Plass, 2001]. In fact, Rett's syndrome is caused by a mutation in MECP2, a methyl CpG binding transcriptional repressor protein. This mutation influences the interplay of the MECP2 protein with methylated DNA and consequently changes the repression of transcription [Kaludov and Wolffe, 2000].

Interestingly, in several linkage studies, the suspected chromosomal locations for susceptibility to schizophrenia and mood disorders are compatible with the loci of genes that contribute to DNA methylation. For example, chromosomes 19p [Badenhop et al., 2002], 10p [Faraone et al., 1998; Nurnberger and Foroud, 2000; Maziade et al., 2001], 20q [Williams et al., 1999], 21q [Nurnberger and Foroud, 2000; Berrettini, 2001], 18q [Van Broeckhoven and Verheyen, 1999; Nurnberger and Foroud, 2000; Berrettini, 2001], and 3q [Maziade et al., 2001] are the loci for production of DNMT1, DNMT2, DNMT3B, DNMT3L, MBD1, MBD2, and MBD4, respectively. Furthermore the nitric oxide synthase gene that has a role in DNA methylation is located in 12q24, which is also related to schizophrenia [Shinkai et al., 2002]. Psychiatric morbidity is common in fragile-X syndrome as well, where hypermethylation of expanded CGG trinucleotides is associated with *FMR1* gene silencing [Tassone et al., 2000].

DNA hypomethylation is correlated with lupus erythematosis and other autoimmune diseases [Attwood et al., 2002]. Some demethylating drugs (e.g., hydralazine and procainamide) induce lupus erythematosis. Interestingly, these diseases have a high co-morbidity with major psychiatric disorders [Sadock and Sadock, 2000]. Similarly, steroid hormones, useful in the treatment of some autoimmune diseases, have strong effects on DNA methylation and may induce psychotic or mood disorders in high doses [Sadock and Sadock, 2000].

In schizophrenia and bipolar disorder, the mRNA of reelin, a protein necessary for neuronal migration, axonal branching, and synaptogenesis, and cell signaling in adult brain, is reduced by 50% in post-mortem brain [Fatemi et al., 2000; Costa et al., 2001]. Reelin gene expression is correlated with the methylation state of its promoter. Hence, hypoactivity of the reelin gene in these disorders may be related to the hypermethylation of the gene's promoter [Chen et al., 2002].

Several studies in the early 1970s indicated that L-methionine, a precursor of *S*-adenosyl methionine, which provides methyl groups for DNA methylation, exacerbates symptoms in most schizophrenic and manic patients [c.f., Chen et al., 2002]. L-methionine treatment decreases reelin mRNA levels in animal studies; hence, exacerbation may be mediated through reelin promoter methylation. In contrast, valproate, a compound useful in schizophrenia and bipolar disorder, is a DNA demethylating agent [Alonso-Aperte et al., 1999; Chen et al., 2002; Manev and Uz, 2002].

Based on our recent meta-analysis, the C allele of the T102C polymorphism of the *HTR2A* gene is associated with schizophrenia in patients of European, but not Far Eastern, decent [Mostafavi Abdolmaleky et al., in press]. In normal controls and schizophrenic patients, the C allele is hypoactive, but in schizophrenic patients the C allele has less activity than controls [Polesskaya and Sokolov, 2002]. The receptor protein structure is identical in both alleles but anti-psychotic treatments increase the expression of the C allele in schizophrenic patients [Polesskaya and Sokolov, 2002], suggesting gene regulation might be abnormal in this polymorphism. The critical cytosine in the C allele occurs in a CpG dinucleotide, a potential methylation target [Petronis, 2000] whose methylation in those of European decent may lead to hypoactivity of the C allele and a greater schizophrenia frequency among individuals with the homozygous C/C genotype. However, methylated C hyper-mutability, through deamination, could lead to its change to T [Petronis, 2000] and a greater frequency of the T allele in the Far East population [Mostafavi Abdolmaleky et al., in press]. Since methylation of specific CpG sites has been reported to be associated with a change in gene expression [Ngo et al., 1996; Attwood et al., 2002], studying the possibility of whether this particular CpG site in the HTR2A gene could also have such an effect should be under consideration.

In another recent meta-analysis, a C311S DRD2 polymorphism was associated with schizophrenia [Glatt et al., in press], indicating that DRD2 is involved in the disorder. The *DRD2* gene has several CpG dinucleotide methylation targets [Popendikyte et al., 1999]. Hence, DNA methylation could be important for the regulation of expression of this gene as well [Petronis, 2000]. The DRD2 A1 allele is a regulatory region polymorphism associated with a nearly 30% decrease in dopamine receptor D2 expression and linked to Tourette's syndrome, addiction, and depression [Blum et al., 1996]. In addition, the severity of Tourette's syndrome [Tasman et al., 2003] and the frequency of schizophrenia [Stabenau and Pollin, 1993] are greater in twins with a lower birth weight. A lower birth weight, indicative of under-nutrition, may alter methylation of genes such as the *DRD2* gene, and produce psychopathology. In the human brain, the ratio of 5-mC:C at the DRD2 locus is significantly higher in the right vs. left striatum [Popendikyte et al., 1999]. Perhaps, DNA methylation or other epigenetic factors control brain hemisphere laterality, which could be under epigenetic and developmental influences.

"Fetal hypoxia is associated with greater structural brain abnormality amongst schizophrenic patients and their non-schizophrenic siblings than controls at low genetic risk for schizophrenia," and these effects are more prominent in siblings with low birth weight [Cannon et al., 2002]. DNA methylation increases in response to transient ischemic incidents that endanger the survival of neurons in the central nervous system, while inhibition of DNA methylation prevents brain damage [Endres et al., 2000, 2001]. This suggests that

the methylation process could be abnormal in at-risk individuals.

Nitric oxide, among other activities, activates DNA methyltransferases [Hmadcha et al., 1999]. Also, the interaction of nitric oxide with glutamate [Stewart et al., 2002] during immobilization stress [De Cristobal et al., 2002] or focal ischemia [Cuzzocrea et al., 2000] produces neurodegenerative effects in animal studies. Hyperproduction of nitric oxide and the nitric oxide synthase polymorphism are both linked to schizophrenia [Shinkai et al., 2002]; therefore, nitric oxide could be a candidate mediator for inducing aberrant methylation in schizophrenic patients.

Moreover, pathologic folate metabolism [Herran et al., 1999] and dopamine receptor D4 (DRD4) abnormalities have been suggested to be causative in schizophrenia. DRD4 modulates cellular methylation processes [Serretti et al., 2001]. Recently, the D4 receptor was shown to have a specific intra-neuronal cytoplasmic distribution [Wedzony et al., 2000], so it may modulate DNA methylation as well. Since individuals with schizophrenia have a fourfold lower level of phospholipid methylation [Sharma et al., 1999], D4 receptor abnormalities may influence risk for schizophrenia through their influence on DNA methylation patterns.

Hence, DNA hypomethylation "perturbs the function and survival of central nervous system neurons in postnatal" stages [Fan et al., 2001], and any abnormal DNA methylation change may produce brain damage. Further, a balance of DNA methylation levels is important for neuronal survival [Fan et al., 2001]. As it was mentioned before, dietary levels of methyl-donor precursors such as folic acid can affect DNA methylation patterns. This also hints at the importance of an "optimal methylation diet" [Van Den Veyver, 2002] in brain diseases.

In some patients with severe depression, intracellular folic acid deficiencies, in the absence of anemia, are associated with central nervous system deficiencies in monoamine metabolites; e.g., 5-HIAA, HVA, and MHPG [Bottiglieri et al., 2000]. Alcoholism, which leads to folic acid deficiency, is linked to depressive disorders and dementia [Sadock and Sadock, 2000]. Interestingly, folic acid is useful in treating refractory major depressive disorder and dementia [Young and Ghadirian, 1989; Bottiglieri et al., 2000]. Similarly, methionine, useful in depression and dementia, exacerbates manic symptoms [Mischoulon and Fava, 2002; Pancheri et al., 2002]. This suggests that DNA methylation may be involved in mood disorders as well.

CONCLUSIONS

There is a dynamic interaction between DNA methylation and the environment throughout the lifespan. DNA methylation permits genome plasticity and short-term adaptation to a variable environment in each generation. In contrast, mutations that involve base changes in germ-line cells allow long-term adaptation. Hence, evolution occurs not only through selection of the "fittest" mutations, but also through the selection of short-term adaptive mechanisms involving DNA methylation and other epigenetic phenomena. Epigenetic processes increase the complexity of genomic responses by allowing short-term fine-tuning of the genome, and provide a mechanism for preserving information on environmental exposures. There is increasing evidence that complex diseases, such as mental and developmental disorders, may be mediated through aberrant epigenetic effects.

The considerations raised in this article suggest directions for future research in:

1. Studies of gene methylation, as these may provide a useful method for the study of gene–environment interaction, since most psychiatric disorders are multi-factorial and DNA methylation is responsible for fine-tuning of gene expression in early life.
2. The study of effects of environmental factors such as stress, place and season of birth, nutrition, etc., on the methylation of genes expressed in the central nervous system.
3. The study of methylation patterns of genes involved in neurodegenerative disorders, because of the ability of DNA methylation changes to produce apoptosis in the central nervous system, which also mimics the known pathologies of major psychiatric disorders.
4. The study of DNA methylation in monozygotic twins, concordant and discordant for any trait, as these may be helpful in understanding the role of DNA methylation in psychiatric disorders, especially in those genes having CpG islands in their promoter area (e.g., DRD2, HTR2A, RELN, NRF, APOL, and COMT).

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