Methylated Spirits: Epigenetic Hypotheses of Psychiatric Disorders

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NEW TREND IN PSYCHOPHARMACOLOGY

Our spirits may be regulated by the methylation of our genes. Methylation, acetylation, and other biochemical processes are the molecular switches for turning genes on and off. There is evidence now that certain behaviors, feelings, and psychiatric symptoms may be modified by turning various genes on or off. If classical genetics is the sequence of DNA that is inherited, then epigenetics is a parallel process determining whether a given gene (ie, a sequence of DNA coding for transcription) is expressed into its RNA or is silenced. Epigenetics is now entering psychiatry with the hypothesis that normal genes as well as risk genes can both contribute to a mental disorder. That is, it has long been hypothesized that when “abnormal” genes with an altered sequence of DNA are inherited as risk genes for a mental illness, these risk genes will make an abnormal gene product in neurons, contributing to inefficient information processing in various brain circuits and creating risk for developing a symptom of a mental illness. Now comes the role of epigenetic actions in mental illnesses. If normal genes make normal gene products but at the wrong time, either being epigenetically expressed in neurons when they should be silenced or epigenetically silenced in neurons when they should be expressed, particularly under the influence of environmental factors and stress, this, too, can contribute to inefficient information processing in brain circuits, increasing the chance of developing symptoms of a psychiatric disorder. Here we describe the role of epigenetics and methylomics (methylating or demethylating upstream genes and downstream molecules) in various psychiatric disorders, emphasizing schizophrenia, and demonstrate whether your spirits can be truly methylated. In a companion article, we describe how psychiatry is on the threshold of new therapeutics that target epigenetic regulation of brain genes via methylomics and acetylation/de-acetylation.
WHAT IS EPIGENETICS?
Genetics is the DNA code for what a cell can transcribe into specific types of RNA or translate into specific proteins. Epigenetics is a parallel system that determines whether any given gene is actually made into its specific RNA or protein, or if it is instead ignored. If the genome is a lexicon of all protein “words,” then the epigenome is a “story” resulting from arranging the “words” into a coherent tale. The genomic lexicon of all potential proteins is the same in all of the >200 types of cells in the body of a given individual. Thus, the plot of how a liver cell becomes a liver cell or how a neuron becomes a neuron is the selection of which specific genes are expressed or silenced in the two different cell types. For cells that differentiate into neurons, both genetics and epigenetics seem to play a role in determining whether brain circuits connecting these neurons develop into a compelling narrative, such as learning and memory, or regrettably evolve into a tragedy such as drug abuse, stress reactions, or a psychiatric disorder.

WHAT ARE THE MOLECULAR MECHANISMS OF EPIGENETICS?
Epigenetic mechanisms turn genes on and off by modifying the structure of chromatin in the cell nucleus. The character of a cell is fundamentally determined by its chromatin, a substance composed of nucleosomes. Nucleosomes are an octet of proteins called histones around which DNA is wrapped (Figure 1). Epigenetic control over whether a gene is read (ie, expressed) or not read (ie, silenced), is done by modifying the structure of chromatin. Chemical modifications that can do this include not only methylation, but also acetylation, phosphorylation, and others. For example, when DNA or histones are methylated, this compacts the chromatin and acts to close off access of molecular transcription factors to the promoter regions of DNA, with the consequence that the gene in this region is silenced (not expressed), so no RNA or protein is manufactured (Figure 1). Silenced DNA means that the molecular features of that gene are not part of a given cell’s personality.

Histones are methylated by enzymes called histone methyltransferases and this is reversed by enzymes called histone demethylases (Figure 1). Methylation of histones can silence genes whereas demethylation of histones can activate genes. DNA can also be methylated and this, too, silences genes. Demethylation of DNA reverses this. Methylation of DNA is regulated by DNA methyltransferase (DNMT) enzymes and demethylation of DNA by DNA demethylase enzymes (Figure 1). There are many forms of methyltransferase enzymes and they all tag their substrates with methyl groups donated from L-methylfolate via S-adenosyl-methionine (SAMe) (Figure 1).

Methylation of DNA can eventually lead to de-acetylation of histones as well, by activating enzymes called histone de-acetylases (HDACs). Deacetylation of histones also has a silencing action on gene expression (Figure 1). Methylation and de-acetylation compress chromatin, as though a molecular gate has been closed, and transcription factors that activate genes can not get access to their promoter regions. Thus, the genes are silenced and not transcribed into RNA or translated into proteins (Figure 1). On the other hand, demethylation and acetylation do just the opposite; they

FIGURE 1. Gene activation and silencing

Molecular gates are opened by acetylation and/or demethylation of histones, allowing transcription factors access to genes, thus activating them. Molecular gates are closed by deacetylation and/or methylation provided by the methyl donor SAMe derived from L-methylfolate. This prevents access of transcription factors to genes, thus silencing them.

Ac=acetyl; Me=methyl; DNMT=DNA methyltransferase; TF=transcription factor; SAMe=S-adenosyl-methionine; L-MF=L-methylfolate.

decompress chromatin as though a molecular gate has been opened, and thus transcription factors can get to the promoter regions of genes and activate them (Figure 1). Activated genes then become part of the molecular personality of a given cell.

MAINTAINING VERSUS CHANGING THE STATUS QUO
Some enzymes try to maintain the status quo of a cell, such as the enzyme DNMT1, which maintains the methylation of specific areas of DNA and keeps various genes quiet for a lifetime. For example, this process keeps a liver cell always a liver cell, including when that cell divides into another one. This process also keeps a neuron a neuron, by always silencing a different set of genes. Presumably methylation is maintained at genes that one cell does not need, even though another cell type might.

It used to be thought that once a cell differentiated, the epigenetic pattern of gene activation and gene silencing remained stable for the lifetime of that cell. Now, however, it is hypothesized that there are various circumstances in which epigenetics may change in mature, differentiated cells. For example, in certain cancers, there may be de novo and unwanted activation of diabolical genes to produce cancerous villains as the storyline of certain cell types. Cancer therapeutics is vigorously exploring how to selectively silence such evil genes by using heroic epigenetic mechanisms to vanquish cancerous villains, resolve the cellular crisis, and have only the good cells live happily ever after.

Similarly in neurobiology, the initial epigenetic pattern of a neuron is indeed set during neurodevelopment to give each neuron its own lifelong “personality.” However, it now appears that the storyline of some neurons is that they respond to their narrative experiences throughout life with a changing character arc, thus causing de novo alterations in their epigenome. Depending upon what happens to a neuron, such as experiencing child abuse, adult stress, dietary deficiencies, or productive new encounters, it now seems that previously silenced genes can become activated and/or previously active genes can become silenced. When this happens, both favorable and unfavorable developments can occur in the character of neurons. Favorable epigenetic mechanisms may be triggered in order for one to learn (eg, spatial memory formation) or to experience the therapeutic actions of psychopharmacologic agents. On the other hand, unfavorable epigenetic mechanisms may be triggered in order for one to become addicted to drugs of abuse or to experience various forms of “abnormal learning,” such as when one develops fear conditioning, an anxiety disorder, or a chronic pain condition.

How these epigenetic mechanisms arrive at the scene of the crime remains a compelling neurobiological and psychiatric mystery. Nevertheless, a legion of scientific detectives is working these cases and is beginning to show how epigenetic mechanisms are mediators of psychiatric disorders. There is also the possibility that epigenetic mechanisms can be harnessed to treat addictions, extinguish fear, prevent the development of chronic pain states, and maybe even prevent disease progression of psychiatric disorders such as schizophrenia by identifying high risk individuals before the “plot thickens” and the disorder is irreversibly established.

One of the mechanisms for changing the status quo of epigenomic patterns in a mature cell is via de novo DNA methylation by a type of DNMT enzyme known as DNMT2 or DNMT3. These enzymes target neuronal genes for silencing that were previously active in a mature neuron. Of course, deacetylation of histones near previously active genes would do the same thing, namely silence them, and this is mediated by enzymes called HDACs. In reverse, demethylation or acetylation of genes both activate genes that were previously silent. The real question is how does a neuron know which genes among its thousands to silence or activate in response to the environment, including stress, drugs, and diet? How might this go wrong when a psychiatric disorder develops? This part of the story remains a twisted mystery but some very interesting detective work has already been done by various investigators who hope to understand how some neuronal stories evolve into psychiatric tragedies. These investigations may set the stage for rewriting the narrative of various psychiatric disorders by therapeutically altering the epigenetics of key neuronal characters so that the story has a happy ending. That possibility is described in a companion article.
METHYLOMICS AND THE SCIENCE OF METHYLATED SPIRITS IN PSYCHIATRY

Methyomics is how the body uses methyl groups for various metabolic functions, from maintaining DNA methylation to the synthesis of vital cellular components to the inactivation of various biological substances. The body cannot make methyl groups, but gets them from dietary sources, especially by converting folate to L-methylfolate, methionine, and ultimately to SAMe which is the universal methyl donor (Figure 2). Methyl groups are important in the synthesis of nucleic acid bases, which are linked not only to the synthesis of DNA and RNA, but also to the synthesis of biopterin (Figure 3), the critical cofactor required by the enzymes that synthesize the monoamine neurotransmitters dopamine (DA), norepinephrine (NE), and serotonin (5-HT) (Figure 4). Methyl groups are also involved in the synthesis of melatonin and epinephrine, and in the inactivation of DA and NE, the latter by the methyltransferase enzyme known as catechol-o-methyl transferase (COMT) (Figure 5). Thus, methyomics is a critical regulatory process for genetics, epigenetics, and neurotransmitters, and is in a key position to influence our spirits, not only those that occur during normal brain development, but those that represent symptoms of psychiatric disorders.

UPSTREAM VERSUS DOWNSTREAM METHYLOMICS IN PSYCHIATRY

Information flow starts at the genome and can be considered “upstream.” Genetic instructions then flow “downstream” into various gene products, small molecules, and cellular functions. Epigenetic molecular mechanisms act upstream and are thought to negotiate how the environment interfaces with genes. For example, epigenetic mechanisms are recruited to drive experience-dependent modifications in cognition and behavior. When these upstream mechanisms go awry in the brain, they are thought to be capable of producing psychiatric symptoms and mental illnesses. Downstream consequences of epigenetic mechanisms include the ability to form new synapses with input of new information, to make enzymes and receptors capable of regulating neurotransmitter levels, and to regulate the availability of methyl groups themselves for use in both upstream and downstream methyomics. The efficient operation of these downstream functions is also necessary in order to prevent breakdown of neuronal functioning, and potentially, to prevent psychiatric disorders.

Upstream histone and DNA methylation provide important clues about gene expression in the human brain during normal development and in certain disease states. For example,
mutations within genes encoding for various histone methyltransferases are linked to mental retardation (eg, the H3K9 specific histone methyltransferase EHMT1) and to autism (eg, H3K4 specific histone memethylase JARID1C/SMCX). Mutations in the gene for a methylated DNA binding protein (MeCP2) that normally silences genes are linked to the behavioral abnormalities of Rett syndrome. Histone hyper-trimethylation has been described in Huntington’s Disease.

SCHIZOPHRENIA IS A VAST CONSPIRACY
For a long time, studies of identical twins have hinted that epigenetics are in play in schizophrenia. An explanation is needed for why only half of co-twins of schizophrenics also have this illness even though both have inherited all the same genes. A leading hypothesis today is that genes interact epigenetically with the environment, essentially conspiring to cause schizophrenia when some genes are expressed in the affected twin, and yet not to cause schizophrenia in the unaffected twin because some critical risk genes remain silent.2

Another hint that such epigenetic mechanisms are involved in schizophrenia is the observation that methionine administration can exacerbate schizophrenia. This used to be attributed to downstream methylogics, namely the hypothetical formation of transmethylated neurotransmitters that were hallucinogenic,16 but this was never substantiated. Perhaps instead this methyl donor upsets the delicate balance of upstream methylogics, conspiring with epigenetic mechanisms to cause unwanted epigenetic changes and thus exacerbation of schizophrenia. The mechanism of methionine-induced actions in schizophrenia, however, still remains undetermined.

Today, the most active theories about schizophrenia have to do with neurodevelopmental hypotheses due to a confluence of multiple genetic, epigenetic, and environmental factors, each named as potential co-conspirators in schizophrenia.9 Alterations in slowly developing changes in the normal neurodevelopment of the brain are hypothetically mediated by alterations in the upstream epigenetic regulation of gene expression over time2-4 and these are linked to the cause of schizophrenia and other psychiatric illnesses.2-4 For example, the H3K4 specific histone methyl transferase MLL1 is essential for hippocampal synaptic plasticity and might be involved in cortical dysfunction in some cases of schizophrenia.14 Upstream epigenetic alterations in DNA methylation of some genes or gene promoters, such as those for COMT,17,18 for various types of glutamate receptors,9 for glutamic acid decarboxylase (GAD) the enzyme that synthesizes γ-amino butyric acid (GABA),14,19-22 and for the critical synaptic structural protein reelin, have been described in some,20-26 but not

**FIGURE 4.** Biopterin cofactor for monoamine neurotransmitter synthesis

![Biopterin cofactor for monoamine neurotransmitter synthesis](image)

L-MF=L-methylfolate; BH=biopterin; DA=dopamine; NE=norepinephrine; 5-HT=serotonin.


**FIGURE 5.** Inactivation of dopamine and norepinephrine by COMT

![Inactivation of dopamine and norepinephrine by COMT](image)

COMT=Catechol-O-Methyl Transferase; DA=dopamine; Me=methyl; SAMe=S-adenosyl-methionine; L-MF=L-methylfolate.

all, 27-29 studies of cerebral cortex of patients with psychosis. Whether the abnormalities in methyl marks on DNA that are postulated to be present in psychiatric disorders such as schizophrenia are due to inherited factors, acquired factors, or both, is not yet known but both are suspected.

Modern theories of major mental illnesses do not suggest that genetics is the cause of mental illnesses so much as it is a co-conspirator with epigenetics interfacing with the environment. 3,4,9,30 There appear to be no major mental illnesses due to a single gene mutation, but there do appear to be many mental illnesses linked to the inheritance of multiple simultaneous risk genes. 5 Even this is not enough for most mental illnesses to become manifest as it seems that stress from the environment, probably because it triggers aberrant epigenetic mechanisms, is also required before the brain decompensates and develops a mental disorder. 4,5,30 Thus, mental illness may really be a conspiracy among many genetic, epigenetic, and environmental co-conspirators.

GLUTAMATE AND GABA AS CO-CONSPIRATORS IN SCHIZOPHRENIA

Although there is no “gene for schizophrenia” there are multiple candidates for risk genes, many of which may need to be simultaneously inherited in order for schizophrenia to occur. Several of these genes are thought to be those that express proteins whose functions converge at glutamate synapses. 5,10-26 This observation has led to the hypothesis that the glutamate synapse with N-methyl-d-aspartate (NMDA) glutamate receptors that regulate DA circuits is the scene of the crime for schizophrenia. 9 One notion is that there is abnormal connectivity between GABAergic interneurons and glutamatergic pyramidal neurons, or between various glutamatergic pyramidal neurons in prefrontal cortex (PFC) in schizophrenia due to the diabolical actions of GABA and glutamate co-conspirators at glutamate synapses. 9

Speaking epigenetically, glutamate receptor genes (for metabotropic GRM 1-7 and ionotropic NMDA, kainite glutamate receptors) undergo dynamic, region, and cell specific changes in expression during the course of normal brain development, accompanied by complementary alterations in methylation (H3K4 di- and trimethylation) at the sites of the corresponding promoters. 14,31 There is also normally progressive upregulation of GABAergic mRNAs during development of human PFC reflected by parallel increases in J3K4 methylation at sites of these promoters, 14,32 suggesting H3K4 di- and trimethylation defining actively expressed genes in the normally developing human brain. 14

Hypothetically, schizophrenia causes inherited and environment- or experience-triggered, disease-related changes in gene expression for glutamate and GABA systems, potentially explained by alterations of H3K4 trimethylation or other histone modifications. 14 A deficit in H3K4me3 marks at the promoter of the GABA synthesis gene GAD (GAD1/GAD67) has been described in the postmortem schizophrenic brain, along with a deficit in GAD1 transcript and increased levels of the repressive marker H3K27me3. 14 Altered methylation of DNA of a more global nature has also been described in schizophrenia. 33,34 Most theories of schizophrenia that involve glutamate and GABA suggest that multiple simultaneous co-conspirators, acting either epigenetically or genetically, must plot together in the conspiracy in order for schizophrenia to occur. 9

L-METHYLFOLATE AS A CO-CONSPIRATOR IN SCHIZOPHRENIA

Another interesting link of schizophrenia with upstream methylomics is that to the enzyme methylene tetrahydrofolate reductase (MTHFR), the enzyme that synthesizes the methyl donor L-methyl folate 11-13 (Figure 2). MTHFR supplies methyl groups used by upstream and downstream methylomic reactions by synthesizing L-methylfolate from folate precursors derived from the diet 11-13 (Figure 2). Severe MTHFR deficiency, although rare, is associated with psychosis and developmental delay. 35 A more common inherited form of MTHFR, known as the C677T variant and also called the T allele, shows a less profound reduction in enzyme activity but nevertheless causes elevation in homocysteine levels, an indirect indication of L-methylfolate deficiency 26,37 (Figure 6). The T allele of MTHFR may thus be associated with a marginal functional availability of methyl donor groups. Interestingly, homocysteine levels have been reported to be high and folate levels low in patients with schizophrenia. Folate levels correlate with the severity of negative symptoms of schizophrenia. 38,40 Administration of folate or L-methylfolate raise L-methylfolate levels and
reduce homocysteine levels. In schizophrenic patients, folate and L-methylfolate have also been reported to improve positive, negative, and cognitive symptoms.

Inheriting the T form of MTHFR increases the risk for schizophrenia and especially the risk for executive dysfunction in schizophrenia. Since inheriting the T allele of this enzyme means significantly reduced enzyme activity of MTHFR as well as reduced availability of methyl donors, this allele could compromise both upstream DNA methylation and downstream metabolic reactions dependent upon methylation. It thereby increases the risk for schizophrenia or cognitive dysfunction in schizophrenia.

Another link of methyl donor deficiency to schizophrenia comes from observations that variants of two additional enzymes regulating synthesis and metabolism of L-methylfolate are both associated with increased risk for schizophrenia.

**COMT REGULATION OF DA SIGNALING**

The enzyme COMT inactivates the catecholamines DA and NE by a transmethylation reaction that involves the transfer to DA or NE of a methyl group derived directly from SAMe (Figure 5), which is itself synthesized from folate and L-methylfolate via MTHFR (Figure 6). Although COMT is active at all DA and NE synapses, it is much more important for regulating DA levels in brain areas such as PFC that lack a competing inactivation process, namely the DA reuptake pump, or dopamine transporter (DAT) which has only low levels in PFC.

Thus, when COMT enzyme activity is high in PFC, DA levels are low; and vice versa, when COMT enzyme activity is low, DA levels are high in PFC. Since DA profoundly affects the information processing of pyramidal neurons in PFC, it also profoundly influences cognitive functioning. DA does this by its actions specifically in dorsolateral PFC, not only in normal controls, but also in patients who have cognitive dysfunction associated with a number of disorders including schizophrenia, depression, and attention-deficit/hyperactivity disorder. Thus, the activity of COMT is a key regulator of cognition because it is a key regulator of DA in PFC.

This is in contrast to other brain areas, such as basal ganglia or nucleus accumbens, that have high densities of DAT and where DA levels are therefore regulated far less profoundly by COMT. This is important to understand in order to interpret the importance of COMT regulation in schizophrenia, since the DA hypothesis of schizophrenia suggests that DA levels are overly active in limbic areas such as nucleus accumbens yet hypoactive in cortical areas such as PFC. Thus, changes in COMT activity would be expected to change DA levels much more in PFC than in...
nucleus accumbens and by extension, to regulate cognitive symptoms (thought to be regulated in PFC) much more than positive symptoms (thought to be regulated in nucleus accumbens).

**WHEN METHYLOMICS AND DA ACT AS CO-CONSPIRATORS IN SCHIZOPHRENIA: THE DOUBLE WHAMMY**

The activity of COMT is regulated both by genetics and by epigenetics. Epigenetically, upstream methylation of the COMT promoter decreases the amount of COMT enzyme that is made, and thus reduces COMT activity and raises PFC DA. Interestingly, the amount of methylation that this promoter has is strongly inherited. This may affect the efficiency of information processing even in normals. In schizophrenia, deficits are reported in the methylation of the COMT promoter, perhaps caused by or compounded in patients who have the T form of MTHFR, who might therefore have inefficiency in their ability to methylate. We begin to see the shape of a conspiracy between methylomics and DA here. Reduced COMT promoter methylation causes increased amounts of COMT to be expressed, and thus increased COMT enzyme activity, lower PFC DA, and reduced efficiency of information processing. Thus, decreased availability of methyl donors due to inheriting decreased MTHFR activity from the T allele could lead epigenetically to increased amount of COMT enzyme being made, and thus reduced DA signaling in PFC.

Downstream, the conspiracy deepens. Decreased availability of methyl donors for use in metabolic reactions due to inheriting this same decreased MTHFR activity via the T allele could lead to reduced synthesis of the bipterin cofactor for DA synthesis via tyrosine hydroxylase (Figures 3 and 4). This reduction of DA would conspire to compound the already reduced DA signaling caused by reduced methylation of the COMT promoter and increased activity of COMT described above. Even though reduced availability of methyl donors might simultaneously decrease the downstream inactivation of DA by COMT, which itself uses methyl donors, this is unlikely to change DA levels since other inactivating mechanisms such as the norepinephrine transporter (NET) and diffusion would take up any slack.

Bottom line: inheriting reduced MTHFR activity via the T allele could conspire with DA to result in a double whammy of reduced DA availability by both an upstream mechanism that increases DA inactivation by increasing COMT activity and by a downstream mechanism that reduces the synthesis of DA via reducing the availability of the bipterin cofactor for tyrosine hydroxylase. Since decreased DA signaling in PFC is linked to executive dysfunction such as problem solving difficulties from deficient working memory in many disorders, including schizophrenia, this may underlie the observations of reduced executive functioning in schizophrenia patients who inherit the T allele of MTHFR.

**COMT AS A CO-CONSPIRATOR IN SCHIZOPHRENIA**

An additional regulator of COMT activity is not just epigenetic methylation and genetic control of methylation via MTHFR, but also which type of COMT that is inherited, valine or methionine (val or met). The val form of COMT is more stable and has 2–4 times higher enzyme activity. Thus, it has lower PFC DA levels compared to the met form of COMT, which is more labile, has lower enzyme activity, and is less able to inactivate DA in PFC, thus raising DA levels and increasing DA signaling in PFC. The val form of COMT, presumably because it is associated with lower DA levels in PFC, has been consistently shown to be associated with less efficient activation of PFC brain circuits during tests of memory performance. However, this does not appear to be specific for schizophrenia and even occurs in normals. Some investigators also suggest that inheriting the val form of COMT may increase either the risk of schizophrenia or at least the risk of cognitive dysfunction in schizophrenia.

**METHYLOMICS AND THE ENVIRONMENT**

There are many situations in which the environment can trigger aberrant epigenetic reactions, leading to the activation or silencing of normal genes, but the wrong genes at the wrong time. For example, both good and bad experiences can drive the production of epigenetic methyl “marks” even in adults. That is, epigenetic changes in gene transcription seem to underlie long term memories, good and bad. Thus, experimental animals have epigenetic mechanisms linked not only to normal
hippocampus dependent spatial memory formation but also to associative fear conditioning, a model of anxiety disorders, and to extinction of learned fear, a model of psychotherapeutic recovery from anxiety disorders. The environment can also influence epigenetics indirectly if it leads to folate, L-methylfolate, and methyl donor deficiencies. In addition to an environment in which there is poor nutritional intake of folate, several other environmental facts can reduce the availability of methyl donors, including smoking, alcohol, many anticonvulsant drugs, and many other medications. Also, if the “environment” of the brain includes the rest of a person’s body, various illnesses can reduce the availability of folate and methyl donors, such as pregnancy, gastrointestinal and absorption disorders, and eating disorders. Finally, if the environment during prenatal development includes the uterus, methyl donor deficiencies of the mother could affect embryonic development, methylation, and epigenetics of the unborn child. It could be that the MTHFR genotype of the mother is just as important as that of the patient in determining the risk of schizophrenia, but this is rarely determined.

**Upstream**

The environment can drive epigenetics by altering methyl marks due to regulating the availability of downstream methyl donors used in upstream epigenetic mechanisms. For a cell to preserve its stable characteristics, its upstream maintenance of DNA methylation is vital. The use of methyl groups for maintenance DNA methylation is so important, that it takes precedence over the use of methyl groups downstream for other purposes. In fact, the enzyme DNMT1 seems to act as a sensor for the DNA methylation capacity of a cell. If the downstream availability of methyl groups for upstream DNA methylation goes down, there is a compensatory increase in DNMT1 activity, perhaps due to lack of methylation of its own promoter at its own gene. This increased DNMT1 activity is due to the synthesis of more copies of DNMT1, which theoretically can then more successfully compete for available methyl groups in order to preserve maintenance DNA methylation at the most critical sites in DNA.

On the one hand, this “up regulation” of DNMT1 activity may also lead to unwanted hypermethylation of some other components of DNA, causing unwanted silencing of some normal genes. On the other hand, shunting methyl groups towards use by DNMT1 for maintenance DNA methylation may simultaneously starve the cell of the other important uses of methyl groups, including for de novo DNA methylation, histone methylation, nucleic acid synthesis, and neurotransmitter synthesis. This would theoretically lead to unwanted hypomethylation of other components of DNA that could cause abnormal activation of certain genes.

**Downstream**

Another consequence of methyl donor deficiency, whether due to genetic factors such as MTHFR or to environmental factors such as those that reduce folate levels, is downstream due to the resultant decrease in L-methylfolate levels and increase in the levels of the SAMe metabolite homocysteine (Figure 6). If severe, this condition is sometimes called hyperhomocysteinemia and is caused when SAMe is not replenished due to L-methylfolate deficiency. In fact, elevated homocysteine levels can be a sensitive indicator of folate and L-methylfolate deficiency, and are normalized by administering either folate or L-methylfolate. Elevated levels of homocysteine are also associated with hypomethylation of certain components of DNA and histones, and theoretically could lead to activating some important genes, thus linking reduction in methyl donor availability with upstream epigenetic methyllomics. This is thought to occur in some conditions such as uremia, but it is not known whether the same thing happens in psychiatric disorders associated with elevated homocysteine levels such as schizophrenia, although it is suspected. Folate deficiency is well known to cause problems in DNA repair and to activate promoters in cancer cells, and presumably would likely have the same consequences in neurons.

**THE TRIPLE WHAMMY: WHEN METHYLOMICS CONSPIRE**

Methyllomic mechanisms are candidate co-conspirators that are also hypothetically involved in schizophrenia, potentially participating in both upstream and downstream mechanisms. As discussed, some risk genes for aberrant methyllomics may be inherited as potential co-conspirators along with glutamate
and GABA risk genes; furthermore, the environment could trigger aberrant epigenetic mechanisms due to methyl donor deficiency caused by metabolic, dietary, concomitant drugs, concomitant illnesses, and other environmental stressors. This could add to the stress that the environment places on glutamate, GABA, and inherited epigenetic risk genes.

The interaction of the risk genes for COMT and MTHFR is an example of how methylomic risk factors can conspire to make cognitive functioning worse in schizophrenia.\textsuperscript{43,47,48} Discussed above are the specific risk genes, the val allele of COMT and the T allele of MTHFR. If a person has the T allele of MTHFR, this on its own renders a double whammy to DA signaling: the T allele has the potential to reduce DA signaling both because it can increase DA inactivation by increasing COMT activity via reduced methylation of the COMT promoter, and because it can reduce DA synthesis by reducing tyrosine hydroxylase activity via decreasing the levels of biopterin. Now, if this same person inherited the gene for the highly active val form of COMT, this val COMT enzyme could conspire with the T form of the MTHFR to deliver a third blow to DA signaling: the val form of COMT causes high enzyme activity in each copy of the enzyme, whereas the T form of MTHFR could reduce methylation of the COMT promoter and cause synthesis of more copies of the enzyme. Both conspire to drive down DA levels due to excessive inactivation of DA by COMT.\textsuperscript{43,48} Furthermore, the T form of MTHFR could further conspire to reduce DA by reducing its synthesis in the first place by making methyl donors unavailable for optimal synthesis of biopterin downstream, thus reducing the DA synthetic enzyme tyrosine hydroxylase’s activity.\textsuperscript{47,48} A triple whammy!

Indeed, it has been shown that the val form of COMT interacts with the T form of MTHFR in schizophrenia to compromise executive function on cognitive tests and to disrupt functional activation of prefrontal circuits on brain imaging.\textsuperscript{47,48} Maybe COMT val forms of the enzyme are not robust enough in themselves to increase the risk for schizophrenia but when they conspire with the T form of MTHFR, they can amplify the risk of that gene. One can image that inheriting several abnormal genes regulating glutamate or GABA might have a similar interaction, but this has not yet been proven.

\textbf{CONCLUSION}

Both inherited risk genes as well as abnormalities in epigenetic regulation of normal genes have been implicated in the pathophysiology of many psychiatric disorders. Methylation, the regulation of gene expression and silencing by methylating DNA and histones, is a key epigenetic mechanism. A conspiracy of multiple risk genes with a stressful environment that triggers epigenetic changes in the expression of normal genes is a current leading hypothesis for schizophrenia and most major mental illnesses. \textbf{CNS}

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