Am J Pharmacogenomics 2005; 5 (3): 149-160
1175-2203/05/0003-0149/$34.95/0



# Genetics and Epigenetics in Major Psychiatric Disorders

## Dilemmas, Achievements, Applications, and Future Scope

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## Contents



## Abstract

No specific gene has been identified for any major psychiatric disorder, including schizophrenia, in spite of strong evidence supporting a genetic basis for these complex and devastating disorders. There are several likely reasons for this failure, ranging from poor study design with low statistical power to genetic mechanisms such as polygenic inheritance, epigenetic interactions, and pleiotropy. Most study designs currently in use are inadequate to uncover these mechanisms. However, to date, genetic studies have provided some valuable insight into the causes and potential therapies for psychiatric disorders.

There is a growing body of evidence suggesting that the understanding of the genetic etiology of psychiatric illnesses, including schizophrenia, will be more successful with integrative approaches considering both genetic and epigenetic factors. For example, several genes including those encoding dopamine receptors (*DRD2*, *DRD3*,

and *DRD4*), serotonin receptor 2A (*HTR2A*) and catechol-*O*-methyltransferase (*COMT*) have been implicated in the etiology of schizophrenia and related disorders through meta-analyses and large, multicenter studies. There is also growing evidence for the role of *DRD1*, NMDA receptor genes (*GRIN1*, *GRIN2A*, *GRIN2B*), brain-derived neurotrophic factor (*BDNF*), and dopamine transporter (*SLC6A3*) in both schizophrenia and bipolar disorder. Recent studies have indicated that epigenetic modification of reelin (*RELN*), *BDNF*, and the *DRD2* promoters confer susceptibility to clinical psychiatric conditions.

Pharmacologic therapy of psychiatric disorders will likely be more effective once the molecular pathogenesis is known. For example, the hypoactive alleles of *DRD2* and the hyperactive alleles of *COMT,* which degrade the dopamine in the synaptic cleft, are associated with schizophrenia. It is likely that insufficient dopaminergic transmission in the frontal lobe plays a role in the development of negative symptoms associated with this disorder. Antipsychotic therapies with a partial dopamine $D_2$ receptor agonist effect may be a plausible alternative to current therapies, and would be effective in symptom reduction in psychotic individuals. It is also possible that therapies employing dopamine $D_1/D_2$ receptor agonists or COMT inhibitors will be beneficial for patients with negative symptoms in schizophrenia and bipolar disorder. The complex etiology of schizophrenia, and other psychiatric disorders, warrants the consideration of both genetic and epigenetic systems and the careful design of experiments to illumine the genetic mechanisms conferring liability for these disorders and the benefit of existing and new therapies.

## 1. Issue in Psychiatric Genetics

Epidemiologic data and familial studies strongly suggest a genetic basis for major psychiatric disorders. During the past 2 decades, more than 1000 linkage and associations studies have been conducted in a worldwide search to find the genes underlying psychiatric disorders. Almost all chromosomes harbor genes reported to be linked to psychiatric disorders and at least 150 genes have been implicated in the pathogenesis of schizophrenia and bipolar disorder. However, no specific gene has been definitively identified as having a major or even moderate role in these devastating disorders. Part of this failure could be due to the complexity of mental disorders; the following issues may also contribute to the difficulty of gene discovery for these diseases.

### 1.1 Pleiotropy

Pleiotropy is one issue that is often overlooked and may be very important in the field of psychiatric genetics. Pleiotropy occurs when one gene has several functions in different tissues at the same time or during different developmental periods.[1] Thus, if a pleiotropic gene is dysfunctional there may be several abnormal manifestations in a specific period or in different life stages. It is well known that genes can be activated during critical periods in reaction to environmental or hormonal stimulations through the removal of methylation of their promoters.[2] Accordingly, the activated dysfunctional pleiotropic gene may have various manifestations at the critical period and at other times in which it becomes activated.

Psychiatric disorders with a high prevalence of comorbid psychiatric or somatic disorders are more likely to be influenced by a common gene. Dopamine receptor genes (*DRD2*, for example) and serotonin receptor and transporter genes (e.g. *HTR2A* and *SLC6A4*) may be considered pleiotropic genes, since they have various manifestations in different organs and developmental periods. For example, *DRD2* hypoactivity may result in attention-deficit hyperactivity disorder (ADHD) during childhood, drug abuse in adolescence, and post-traumatic stress disorder (PTSD) and depressive disorders in adulthood.[3] It is also likely that this gene has a role in schizophrenia pathogenesis.[4] Also, since estrogen up-regulates the *DRD2* gene,[5] family members who have a dysfunctional gene may have varying phenotypes depending upon gender. This can confound family genetic studies examining specific genetic associations. For example, the *COMT* Val158 allele (the overactive allele of the Val158Met polymorphism) is associated with schizophrenia in males but not females.[6] Since estrogen down-regulates *COMT* gene expression,[7] the effects of this risk allele for schizophrenia may be attenuated by gender and age-related estrogen down regulation. The same scenario is likely true for the *DRD2* Taq1A1 (hypoactive) allele of the Taq1A restriction fragment length polymorphism (RFLP), which is related to drug abuse in men, but not in women.[3] In this case, the estrogen that up-regulates the *DRD2* gene expression[5] may compensate for hypoactivity.

Unfortunately, most genetic studies have focused primarily on binary phenotype definitions and have not considered sub-types or quantitative measures of psychiatric disorders, leading to underestimation of the gene-disease associations and false negative linkage results. Wilcox et al.[8] constructed a set of quantitative traits for schizophrenia. Using the derived trait, they were able to

replicate prior findings and propose new loci that were not linked to the usual binary DSM (*Diagnostic and Statistical Manual of Mental Disorders*) trait. Obsessive compulsive disorder (OCD), eating disorders, impulse control disorders, and some paraphilias may belong to a specific category named 'OCD-related disorders'. The common genetic liability for these comorbid conditions has been suggested in several studies.[9,10] Thus, more elaborate study designs with careful attention to phenotype definitions and close collaboration between geneticists and clinicians are needed to identify the effects of specific genes in psychiatric disorders generally, and schizophrenia specifically.

### 1.2 Epigenetic Interaction

Epigenetic interaction is another overlooked issue in the psychiatric genetic studies. Epigenetics refers to modifications in gene expression that are controlled by heritable but potentially reversible changes in DNA methylation and/or chromatin structure.[11] In fact, a portion of the variability in disease phenotypes that cannot be explained based on traditional genetic data may arise from epigenetic interactions. For example, even monozygotic twins, who share 100% of their genes, can display very different psychiatric phenotypes.[12] To understand this variability, the interaction of the epigenetic risk factors and susceptibility genes should be investigated more thoroughly.

Alteration in the pattern of DNA methylation is an efficient mechanism for genes to adapt functionality in response to a variable environment. This can compensate for the effects of a malfunctional polymorphism. DNA methylation at CpG islands is mediated by the addition of a methyl group to cytosine, in a reaction catalyzed by one of the several DNA methyltransferases in an interactive manner.[13] *S*-adenosyl methionine is the major provider of methyl groups for methylation, while folic acid and vitamin B12/cyanocobalamin are also necessary for recruitment of the demethylated *S*-adenosyl methionine. Environmental insults could also cause altered DNA methylation patterns, leading to corresponding changes in gene expression levels. These insults could be more problematic in individuals with a genetic susceptibility to a specific disease, including psychiatric disorders. For example, if there is already a weak/susceptible allele and the level of illness is not at the threshold, epigenetic insults such as promoter DNA methylation could worsen the background hypo- or hyperactivity, allowing the disease phenotype to be expressed (for review see Abdolmaleky et al.[14]). Therefore, a comprehensive view of the genetic liability for psychiatric disorders will involve the investigation of brain DNA methylation and gene expression analyses as well as functional genetic polymorphisms. This will facilitate the development of an integrated model for the susceptibility to schizophrenia and other psychiatric disorders.

### 1.3 Polygeneity

The polygenic nature of the pathogenesis of psychiatric disorders is another reason genetic studies tend to be inconclusive and not replicable. A conclusion that can be drawn from the history of genetic research in psychiatry indicates that, as is the case with other complex diseases, psychiatric disorders are likely to be multi-factorial and polygenic in origin.[12] Polygenic disorders result from the interaction of multiple common responsible genes. It is usually true that co-inheritance of a few of the genes is necessary to reach the disease threshold and observe the psychiatric phenotype. For example, if we assume the frequency of a risk allele of each of several genes contributing to the liability of a disorder is approximately 20%, at least three risk alleles are necessary to produce the observed population prevalence of schizophrenia or bipolar disorder ($0.2 \times 0.2 \times 0.2 = 0.008$). Therefore, the assumption of a single deleterious gene, made for most association studies (and determining the effect size based upon that assumption), is not accurate, especially with a limited sample size. In these situations, a much larger sample and a homogeneous population is needed to detect the polygenic effects conferring disease liability. To overcome these limitations, multicenter studies and meta-analyses of the existing association studies are valuable to determine population heterogeneity and to obtain a sample large enough to provide statistical power to detect several genes of small effect. Fortunately, there are several meta-analyses as well as multicenter studies in the field of psychiatric genetics in the literature and in progress. Therefore, in this review we focus on meta-analyses and multicenter studies.

### 1.4 Ethics

In spite of above mentioned dilemmas, as well as a long delay due to the abuse of psychiatric genetics during World War II, genetic studies have provided valuable insight into the causes and potential remedies of psychiatric disorders. These disorders are devastating and have substantial public health implications both in terms of personal suffering and public fiscal burden. Most major psychiatric disorders have an early age of onset. This may warrant a focus on the identification of genetic liabilities for the purpose of early intervention and prevention. However, the ethical issues associated with the genetic liabilities for disease in general and psychiatric disorders specifically have not been adequately addressed, making this a difficult proposition.

## 2. Genetic Studies in the Field of Psychiatry

### 2.1 Brain Dopaminergic System

Dopaminergic neurons are those that release dopamine, which is involved in several brain activities including attention, executive memory, desire, hedonic activities, natural rewards, and biological activities such as cell signaling.[12,15] Most of these effects are mediated by dopamine $D_1$- and $D_2$-like receptors that act on other cell-signaling pathways. As we will discuss later, $D_1$- and $D_2$-like receptor polymorphisms are involved in psycho-pathogenesis. The functional consequences of polymorphisms in many genes involved in dopamine production are not well known. However, the recognized polymorphisms in the genes encoding the catabolizing enzyme cathechol-*O*-methyl transferase (*COMT*), and the dopamine transporter (*SLC6A3*), involved with reuptake of dopamine from the synaptic cleft, may both have roles in psychiatric disorders.

#### 2.1.1 Cathechol-O-Methyl Transferase

The *COMT* gene is located in the 22q11.21 chromosomal band. Several linkage studies have linked this region to schizophrenia and bipolar disorder.[16,17] *COMT* encodes two isoforms of the COMT enzyme, a membrane-bound form (MB-COMT) and a soluble form (S-COMT), which result from transcription from different promoters. The MB-COMT form is involved in dopamine catabolism in the human brain.[18] COMT is involved in attention, executive cognition, and working memory performance.[19] COMT overactivity is related to social withdrawal[12] and neuroticism.[20]

The *COMT* transcript encoding MB-COMT has a functional polymorphism at codon 158 (Val158Met). Homozygosity for the *COMT* Val158 allele leads to a 3- to 4-fold increase in enzymatic activity, compared with homozygosity for *COMT* Met158.[21] Genetic studies regarding the association of this polymorphism with schizophrenia have been inconclusive and somewhat contradictory; however, a recent study with a very large sample (720 patients and 2970 controls) reported the association of Val158 homozygosity (the over-active allele) with schizophrenia in men.[6] Since estrogen down-regulates the COMT production,[7] this sexual difference might explain the gender difference in the findings. Furthermore, recently a family-based meta-analysis confirmed the association of the Val158 allele with schizophrenia.[22] Another meta-analysis reported evidence for the association of Met158 allele with OCD.[23] However, a large, multicenter study reported the association of the Val158 allele with early onset major depressive disorder.[24]

Gene expression analysis applying *in situ* hybridization histochemistry (FISH) in the dorsolateral prefrontal cortex of schizophrenia patients indicated that *COMT* gene expression was reduced in superficial cortical layers, but increased in deep layers.[25] Another study analyzing *COMT* expression in schizophrenia and mood disorders indicated no difference in the levels of transcripts between cases and controls.[26] Recently, we found a highly significant level of hypomethylation of the MB-*COMT* promoter in postmortem brains of patients with schizophrenia and bipolar disorder compared with control subjects, particularly in the left frontal lobe. In addition, the degree of DNA methylation was inversely correlated with transcript quantities as determined by quantitative real-time PCR, using primers that exclusively amplify the MB-*COMT* isoform (Abdolmaleky HM et al., unpublished data). The primers of the previous studies were not specific for the MB-COMT isoform, which is the predominant form involved in the degradation of synaptic dopamine in the human brain.

These results suggest that hyperactivity of the *COMT* gene may confer liability for schizophrenia and mood disorders through rapid degradation of dopamine in the synaptic cleft, leading to a frontal lobe hypodopaminergic state and the reported frontal hypoactivity in schizophrenic patients.[27] The known schizophrenia-associated problems in attention, desire, hedonic and social activity, cognitive processes, and working memory could be related to dopamine dysfunction of the frontal lobe. Therefore, new antipsychotic drugs with partial agonistic effects on the $D_2$ receptor, or dopamine auto receptors agonist drugs,[28] may be more useful in improving attention, cognitive processing, and negative symptoms in patients with schizophrenia.

#### 2.1.2 Dopamine Transporter

The dopamine transporter (DAT) is involved in dopamine reuptake from the synaptic cleft, while COMT has catabolic effect over dopamine. Several reports indicate that dysfunctional polymorphisms in the gene encoding DAT (*SLC6A3*) are related to bipolar disorder (e.g. Greenwood et al.[29]); however, there are many negative reports as well (e.g. Georgieva et al.[30]). A recent study reported a highly significant ($p = 0.0003$) association of an *SLC6A3* promoter polymorphism in schizophrenic patients.[31] Concurrent dysfunction of the *SLC6A3* gene may exaggerate the effects of the above-mentioned *COMT* over-activity. Thus, investigating the interaction of *SLC6A3* and *COMT* functional polymorphisms will likely improve our understanding of gene-gene interactions in psycho-pathogenesis and provide an integrative model of the genetic liability for psychiatric disorders.

#### 2.1.3 Dopamine $D_1$ receptor

The dopamine $D_1$ receptor (DRD1) is involved in the modulation of synaptic plasticity in the prefrontal cortex[32] and striatum.[33,34] DRD1 also regulates expression of several genes (e.g. brain-derived neurotropic factor gene [*BDNF*]) through cyc-

lic adenosine monophosphate (cAMP) and cAMP response element modulator (CREM) by acting on the cAMP response elements (CREs).[35]

The *DRD1* gene has several polymorphisms that are not currently known to be associated with schizophrenia or mood disorders. However, haplotype analysis indicates a significant association between *DRD1* and bipolar disorder.[36] Moreover, patients receiving the $D_1/D_2$ receptor agonist drug pramipexole have shown improvement in the depressive phase of bipolar disorder.[37,38]

#### 2.1.4 Dopamine $D_2$ Receptor

The dopamine $D_2$ receptor (DRD2) has been a central subject of study in major psychiatric disorders for several decades. Many studies have shown an up-regulation of *DRD2* gene expression in schizophrenia.[39] However, there are several studies that have reported its down-regulation in schizophrenia, drug dependency, depressive disorders, and PTSD.[3] The *DRD2* gene is located on the long arm of chromosome 11, a region that has been linked to schizophrenia and mood disorders.[40] Although genetic association studies on the role of the *DRD2* in schizophrenia showed contradicting results, a recent meta-analysis reported a highly significant association of the *DRD2* Cys311Ser polymorphism with schizophrenia.[41] This meta-analysis suggests that this *DRD2* hypoactive allele (Cys311) is, at least in part, involved in schizophrenia pathogenesis.

There is also some evidence indicating that *DRD2* promoter DNA methylation could be under epigenetic modulation. The *DRD2* gene has several potential CpG dinucleotides which could serve as methylation targets.[42]

Recently, an investigation of the patterns of epigenetic DNA modifications in the 5′-regulatory region of the *DRD2* gene of twins with schizophrenia showed that the affected twin from a discordant pair was epigenetically 'closer' to the affected twin of another pair than to his unaffected monozygotic co-twin.[43] Furthermore, other experiments indicate that antipsychotic drugs modulate *DRD2* expression through chromatin modification.[44] Although this effect was transient, it implies that *DRD2* could be under epigenetic influences. This may increase the probability of schizophrenia, especially in individuals with the hypoactive allele.

#### 2.1.5 Dopamine $D_2$-like receptors

The dopamine $D_3$ and $D_4$ receptors (DRD3 and DRD4; the $D_2$-like receptors) have also been the focus of genetic studies. According to two meta-analyses, the *DRD3* Ser9Gly variant confers susceptibility to schizophrenia.[45,46] In another meta-analysis, the promoter polymorphism of *DRD4* was associated with schizophrenia.[47] However, exon 1 and exon 3 *DRD4* polymorphisms were not associated with schizophrenia.[48] One report indicates that *DRD4* exon 3 variants are associated with delusional symptomatology in major psychoses.[49] A polymorphism in *DRD4* was also reported to be associated with ADHD in several studies, including two meta-analyses.[50,51]

### 2.2 Brain-Derived Neurotrophic Factor

The brain-derived neurotrophic factor (BDNF) is a member of the neurotrophin family involved in neuronal development and cell survival.[52] Human studies indicate that BDNF has a role in affective disorders.[53] Family-based association studies have indicated that the *BDNF* gene is a risk locus for bipolar disorder[54,55] and the Val66 allele of the *BDNF* Val66Met polymorphism is associated with OCD[56] and neuroticism.[57] Studies with animal models of depression indicated that maternal deprivation is correlated with reduced *BDNF* expression. This finding suggests a role for BDNF in the biology of mood disorders,[58] schizophrenia,[59,60] and anxiety disorders.

The antidepressant activity of BDNF was also shown in animal model studies.[61] Most of the antidepressant drugs act through increasing monoamines in the synaptic cleft and production of the BDNF protein.[53,62] Also, olanzapine, an atypical antipsychotic drug, inhibits stress-induced BDNF reduction in animal studies.[63] New reports indicate that olanzapine, which induces the expression of the *BDNF* gene, may be more appropriate for psychotic patients because BDNF is hypoexpressed in patients with schizophrenia.[64]

The production of BDNF is correlated with the degree of methylation of its gene's promoter secondary to neuronal activation through dopamine $D_1$-like receptors. $D_1$-like receptors, through a cascade of events (mediated by cAMP/protein kinase A [PKA] and CREM), activate a CRE site on the *BDNF* promoter, and concurrently the DNA becomes de-methylated resulting in increased gene expression. Neuronal inactivation leads to *BDNF* promoter methylation.[65] COMT overactivity may reduce dopamine and the availability of other monoamines in the synaptic cleft leading to BDNF under-stimulation and methylation of its promoter. An investigational focus on this issue could provide new treatment modalities, such as COMT inhibitor drugs, which through an increase of dopamine in the synaptic cleft may stimulate BDNF expression reducing the risk of depressive or psychotic disorders.

### 2.3 Reelin

Reelin is an extra-cellular matrix protein, mainly produced by gamma-aminobutyric acid (GABA)-ergic inter-neurons involved in neuronal migration, axonal branching, and synaptogenesis throughout brain development and later life.[66,67] These activities

are mediated through binding to lipoprotein receptors and activation of a tyrosine kinase cascade leading to DAB1 phosphorylation. Reelin also activates a second messenger cascade, influencing gene expression that leads to long lasting structural changes.[68,69] The reelin gene (*RELN*) is located on chromosome 7, having a long CpG-rich promoter.[70] Reelin promoter *in vitro* methylation reduces the gene expression, which could be recovered by treatment with 5-aza-deoxycytidine, confirming that its expression is regulated by promoter methylation.[71]

L-methionine (a known methyl donor) decreases *RELN* mRNA levels in animal studies and is associated with an increase in the number of methylated cytosines in the CpG islands of the *RELN* promoter region.[72] In schizophrenia and bipolar disorder, *RELN* mRNA is severely reduced in postmortem brain studies.[66,67,73,74] L-methionine can exacerbate symptoms in most schizophrenic and bipolar patients, suggesting that hypo-activity of *RELN* may be due to hyper-methylation of the gene's promoter. Interestingly, valproate, which is useful in schizophrenia and bipolar disorder, is known as a demethylating agent through inhibition of histone deacetylase[71,72,75] and can prevent this methionine-induced hypermethylation in animal studies.[72]

A recent study reported an over-expression of DNA methyl transferase (DNMT)-1 (an enzyme that primarily acts to maintain DNA methylation) in the GABAergic interneurons of patients with schizophrenia using the RNA *in situ* hybridization technique.[76]

We recently reported increased DNA methylation of the CpG islands localized to the *RELN* promoter regulatory regions, including a CRE and several SP1 transcription factor binding sites and concurrent *RELN* hypo-expression, in postmortem brain samples from patients who had been diagnosed with schizophrenia.[77]

Thus, the benefits of sodium valproate in schizophrenia and bipolar disorder may be due to its inhibition of the histone deacetylases which results in the removal of the methyl groups from the methylated cytosine of the DNA.[72,75] Further attention to this class of drugs may provide additional insight. Interestingly, butyrate, a milk product known to inhibit histone deacetylases,[78] or tea polyphenol (−)-epigallocatechin-3-gallate, which inhibits DNMT, could also influence the methylation status of some genes.[79]

### 2.4 Serotoninergic System

Secondary to environmental stimulation, serotonin (5-HT) activates genes to produce several proteins involved in synapse formation, a process mediated by cAMP and CRE-binding protein (CREB). Those synapses already activated by serotonin can utilize these proteins for growth and long-lasting structural changes.[80] There are several types of serotonin receptors in the brain.

The serotonin type 2 receptor (HTR2A), which is the main target of antidepressants and atypical antipsychotic drugs, has been the focus of many studies in the field of psychiatric genetics. A meta-analysis of whole-genome linkage scans confirmed a linkage between schizophrenia and markers on the long arm of chromosome 13.[81] The *HTR2A* gene is located in this region,[82] and has many known polymorphisms in the population. The 102T/C single nucleotide polymorphism has been the subject of most studies. In this polymorphism, the base in the nucleotide at position 102 may be thymine (T) or cytosine (C), with possible genotypes TT, TC, or CC. However, this mutation does not result in an amino acid change. There is a significant association between TT genotype and platelet HTR2A density.[83] In human postmortem studies, the expression of HTR2A receptors in the temporal cortex is about 20% less for the CC compared with the TT genotypes. This difference is greater for schizophrenic patients than healthy controls with the same alleles. Moreover, *HTR2A* mRNA level is inversely correlated with the duration of neuroleptic-free intervals indicating that neuroleptics may increase HTR2A expression.[84] In electro-physiologic studies, schizophrenic patients with the TT and CC genotypes show a differential response to treatment with clozapine.[85] Following treatment, patients homozygous for the 102C allele had higher N100 amplitudes than patients with other genotypes.[86] This may be an important biomarker for the potential efficacy of treatment with this agent.

The *HTR2A* 102T/C polymorphism is located in exon 1 with possible promoter activity that may regulate gene expression. The C in position 102 is followed by G, making this mutation is a candidate for methylation.[87] C-methylation can prevent gene expression;[2,88,89] in some locations even single nucleotide methylation can change gene expression.[90] Therefore, the 102C allele could be under epigenetic modification influencing gene expression. Moreover, new data indicates that silent mutations may influence mRNA stability and gene expression level,[91] supporting the idea that the *HTR2A* 102C allele could reduce HTR2A expression.

There have been numerous association studies of the *HTR2A* 102T/C polymorphism with aspects of schizophrenia, including a formal diagnosis,[82] earlier onset with poorer outcome,[92] drug response,[85] and susceptibility to tardive dyskinesia.[93,94] The association between the C allele and schizophrenia was confirmed by a meta-analysis.[82] However, there is an ongoing debate because negative findings persist in the literature.

We recently performed a meta-analysis of case-control and family-based association studies.[95] In the 6 years between the

original work and our analysis, the number of available studies doubled (to 35).

Our results were similar to the previous meta-analysis[82] (odds ratio [OR] = 1.18). We found a significant association between the C allele of the *HTR2A* 102T/C polymorphism and schizophrenia (OR = 1.1). The OR was slightly higher for the European sample (OR = 1.2). We also found significant allelic heterogeneity between European and East Asian populations. In the East Asian population, the 102C allele was not associated with schizophrenia. This heterogeneity, along with the documented differences in allele frequency between the two ethnic groups, suggests that data from European and East Asian samples should not be pooled when evaluating the evidence for involvement of this gene in schizophrenia risk.

We did not find significant evidence for an association of the 102C allele with schizophrenia in five family-based association studies. However, the pooled OR from 473 parent-offspring trios was 1.3. Thus, the pattern of results was similar to those from case-control studies. This evidence may help us estimate the magnitude of this association in distinct population subgroups. Polymorphic imprinting may influence the magnitude of the effect in genetic studies. Bunzel et al.,[96] showed that approximately 20% of individuals have mono-allelic expression of *HTR2A* in the brain, confounding the magnitude of association of a specific allele.

The HTR2A receptor has modulatory effects on dopaminergic neurons.[97] Part of its role in the pathogenesis of schizophrenia may be through actions on the dopaminergic system. The dopaminergic system is under the influence of several other neurotransmitters. Thus, estimating the effect of the HTR2A 102C allele would be confounded by the influences of these other genes and environmental factors.

### 2.5 Glutaminergic System

N-methyl-*D*-aspartate (NMDA) receptor antagonists produce a schizophrenia-like syndrome. NMDA receptors include an ionotropic NMDA-1 (GRIN1) subunit and one of four GRIN2 subunits (A, B, C, or D). The NMDA receptor 2B (GRIN2B) subunit is one of the brain-specific proteins in the postsynaptic density at glutamatergic synapses. The carboxyl-terminal domain of the GRIN2B subunit is involved in the intracellular signal transduction. The NMDA receptor participates in the regulation of dopamine, norepinephrine (noradrenaline), acetylcholine, and GABA.[98] Supportive evidence for involvement of NMDA in schizophrenia is provided from pharmacological studies and from animal models showing schizophrenia-like symptoms (decreased social activity and increased stereotypy) in mice that have a deficiency in expression of NMDA receptors GRIN1 and GRIN2.[99] The NMDA receptor has several polymorphisms in the coding and non-coding regions that, in most instances, do not change the protein structure.[100] Some of these silent polymorphisms are not associated with schizophrenia,[101,102] although other polymorphisms have been associated with the disorder.[103,104]

The promoter region of the *GRIN2A* gene exhibits a variable length GT repeat polymorphism, and a longer GT repeat has been correlated with reduced gene expression may be a risk factor for schizophrenia and bipolar disorder.[105,106] There is also some evidence that the G alleles of *GRIN1* 1001G/C and 6608G/C polymorphisms may have a role in bipolar disorder.[107] Overall, genetic studies provide evidence for the involvement of the NMDA receptor in the pathogenesis of major psychiatric disorders. These findings suggest that research focusing on the genes involved in glutaminergic transmission may be successful in deciphering the genetic liability for major psychiatric illnesses, including schizophrenia. However, the complexity of glutamine-related genes has made this task difficult.

Evidence indicates that atypical antipsychotic drugs such as clozapine, through increasing activity of the NMDA receptor, may facilitate glutamatergic transmission.[108] As there is a close interaction with the dopaminergic system,[109] any malfunction of the glutamatergic pathway affects dopaminergic pathways as well. Clozapine increases dopamine release in the frontal lobe thereby improving cognition in schizophrenic patients.[110] The prevalence of smoking in schizophrenic and bipolar patients is significantly higher than in the general population.[111] One extant hypothesis is that nicotine (as a dopamine agonist) is abused to overcome cognitive and depressive symptoms. As clozapine affects the NMDA receptor, concurrent use of nicotine with classical antipsychotic drugs also has some positive effects on the NMDA receptor activity.[112] In light of these effects, physicians and researchers should consider more appropriate drugs to overcome these symptoms and avoid self-medication by patients using otherwise harmful drugs.

### 2.6 Other Genes

In a small number of studies, other genes were also reported to be linked to schizophrenia, including those encoding nitric oxide synthase 1 (*NOS1*),[113] the apolipoprotein L genes *APOL1*, *APOL2*, and *APOL4*,[114] neuregulin 1 (*NRG1*),[115] tumor protein 53 (*TP53*),[116] the DAOA (D-amino acid oxidase activator; *G72*), D-amino-acid oxidase (*DAO*), proline dehydroxylase 2 (*PRODH2*), dystrobrevin binding protein 1 (*DTNBP1*), neuronal nicotinic cholinergic receptor alpha polypeptide 7 (*CHRNA7*), and the protein kinase oncogene *AKT1*, as reviewed comprehensively elsewhere.[117] The function of most of these genes is not well

understood in the brain, and the therapeutic application of these findings is unclear at present. Among these genes, there is most supporting evidence for the involvement of *NRG1* (located in the short arm of chromosome 8) in the pathogenesis of schizophrenia in a minority of patients. The *NRG1* risk haplotype is present in 15.4% of patients with schizophrenia, compared with 7.5% of the general population.[115] This gene is implicated in glutamate receptor expression and therefore justifies the current extensive investigation in its role in psychiatric disorders worldwide. However, *NRG1* is a very complex gene with several isoforms. More than 1000 SNPs have been identified to date.[115] However, no specific functional polymorphism has been linked to schizophrenia. This is probably due to the complexity of this gene.

## 3. Gene-Gene and Gene-Environment Interactions: an Integrative Model

Although several lines of evidence indicate that *COMT*, *RELN*, *BDNF*, *HTR2A*, *GRIN1*, *GRIN2A*, *GRIN2B*, *SLC6A3*, *DRD1*, and *DRD2*-like receptor genes are involved in major psychiatric disorders, genetic polymorphism studies have shown only weak association. New evidence strongly suggests a potential role for DNA methylation in psychiatric disorders. Generally, the promoter regions of these genes contain stretches of CpG islands and their methylation status appears to be involved in the regulation of gene expression. It is now widely believed that each tissue type may consist of its own characteristic methylome represented by regulatory CpG island methylation patterns. These methylomes are susceptible to epigenetic modifications in response to environmental insults resulting in inappropriate gene expression. Thus, a gene's methylome can be viewed as a template for gene/environment interactions, and may be involved with the fine tuning of adaptive genes. Fragile sites are another backbone for such interactions that may disrupt the gene function.

Changes in methylation may be more problematic in individuals with a genetic susceptibility to specific diseases. Biological, chemical, and physical factors (such as nutrition, minerals, and weather) participate in gene-environment interactions. Cultural and educational backgrounds and response to stress, which subsequently influence lifestyle, also participate in gene-environment interactions. These interactions may influence gene expression and thereby the risk of psychopathology. As illustrated in figure 1, the language of environmental factors, after translation to chemical language, is understood by the regulatory region of genes, influencing the fine-tuning of gene expression through DNA methylation. DNA methylation can be thought of as a mechanism for memorizing such environmental modulations.[2,14]

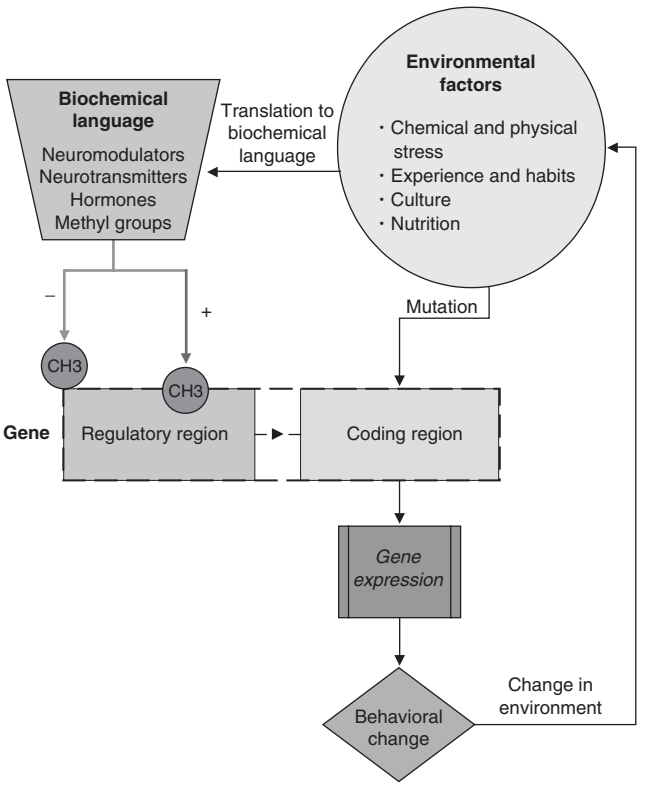


**Fig. 1.** Integrative model for gene-environment interactions.

We believe that the investigation of the interaction of epigenetic risk factors and genetic susceptibility will contribute to a comprehensive picture of the genetic etiology of disease and result in an integrated model. It will be important to determine both the genetic polymorphisms and epigenetic alterations and describe the methods by which they interact.

## 4. Clinical Implications of Genetic Studies

Recent studies indicate that in the prefrontal cortex, a $D_1$ receptor-mediated dopaminergic mechanism is responsible for working memory function[118] and synaptic plasticity.[32] $D_1$-like receptors, through a cascade of events mediated by cAMP/PKA and CREM, activate a CRE at the promoter of several genes including *BDNF*. Concurrently, the DNA becomes de-methylated, resulting in gene expression. Neuronal inactivation leads to *BDNF* promoter methylation.[65] The promoter region of the *RELN* gene harbors a CRE that could be a target for regulation by dopamine through the $D_1$-like receptors leading to activation of the gene expression.

COMT over-activity may reduce dopamine and the availability of other monoamines in the synaptic cleft, leading to DRD1 underactivity, and, subsequently, to *BDNF* methylation. COMT overactivity may be especially important in the prefrontal cortex where

the degradation of dopamine is more reliant on COMT because of the relative scarcity of DATs in this region.[22] However, concurrent genetically or epigenetically determined DAT hyperactivity may result in rapid dopamine reuptake, leading to more dopamine deficiency in the synaptic cleft. This will lead to under-stimulation of the $D_1$ and $D_2$ receptors. Subsequent DNA methylation of the CRE binding sites of the *BDNF* and *RELN* gene promoters could lead to reduced expression and development of negative symptoms in schizophrenia or depression. The HTR2A receptor, which has modulatory effects on dopaminergic neurons[97] as well as serotonin and its transporter, may also participate in this pathway.

There are a few findings from studies that challenge this conclusion. Dopamine agonists can induce psychosis in a small number of patients.[12] One likely explanation is that dopaminergic drugs act on several types of dopamine receptors taht may be involved in psychotic episodes. Research devoted to the clinical effects of other receptor-specific dopaminergic drugs that target the frontal lobe hypodopaminergic pathways is warranted. Another potential factor is the differential down-regulation of dopamine receptors following dopamine excess induced by dopamine agonists. This down-regulation may be more pronounced for the $D_1$/$D_2$ receptors in the frontal lobe, leading to hypo-activity of the downstream targets. In mesolimbic pathways, this down-regulation may not occur, instead dopamine agonists may increase dopaminergic transmission and the emergence of positive symptoms. Research over the past decade has confirmed this model in which a differential role for the cortical and sub-cortical dopaminergic pathway is also considered.[12] Interestingly, the *COMT* Val158 allele is associated with increased tyrosine hydroxylase (*TH*) gene expression in mesencephalic dopamine neurons in the human brain.[119] This implies that cortical dopamine deficiency may induce sub-cortical dopamine production.

In general, antidepressants and atypical antipsychotic drugs can attenuate most of the above-mentioned neurochemical imbalances such as induction of *BDNF* expression and enhancement of dopamine release in the frontal cortex. Antipsychotic drugs can also act on NMDA receptors and blockade HTR2A and DRD2 receptors. However, the mechanisms of action of mood stabilizers are not well understood; valproate may remove gene promoter methylation. Since a substantial portion of psychiatric patients do not show improvement with these drugs, new therapeutic measures are still needed.

Dopamine receptor agonists have provided significant benefit for patients with unipolar and bipolar depression in several open[120] and randomized blind clinical trials.[37,38] In fact, if a dopamine deficiency is the underlying cause, prescription of DRD1 agonists should be beneficial. However, further double-blind clinical trials are needed to confirm this hypothesis.

COMT inhibitors have still to be tested in clinical trials to establish their utility in treating schizophrenia, bipolar disorder, and major depression. In Parkinson disease, the COMT inhibitor entacapone was reported to improve patients' symptoms and quality of life dramatically, with minor adverse effects.[121] In animal studies, these drugs could prevent stress-induced anhedonic state,[122] improve prefrontal cortex performance, and potentate clozapine-induced extracellular dopamine release.[123]

In human studies,[124,125] the COMT inhibitor tolcapone has shown beneficial effects in major depressive disorders. Recent data indicates that mirtazapine, an antagonist of $\alpha$2-adrenoceptors with noradrenergic property, was more effective than selective serotonin reuptake inhibitors (SSRIs) in depressed patients with the *COMT* Val158 allele.[126] Schizophrenic patients with the Val158 allele also show less improvement in working memory performance and prefrontal physiology even with atypical antipsychotics such as olanzapine.[127] Nevertheless, amphetamine enhances the efficiency of prefrontal cortex functions in working memory tasks in subjects with the *COMT* Val/Val genotype.[128] These finding suggest that negative symptoms of schizophrenia as well as depressive symptoms of unipolar and bipolar disorders will be a potential target for COMT inhibitor drugs, particularly in patients with the Val/Val genotype. In patients with little or no risk for psychotic features, this treatment strategy may also relieve anhedonia and improve working memory and attention. However, when the risk of a psychotic episode is high, concurrent usage of DRD2 partial agonists can prevent the sub-cortical dopaminergic outrage and the psychotic episode. Thus, new anti-psychotic drugs with partial agonistic effects on the dopaminergic system (e.g. aripiprazole) may be more useful to patients with both negative and positive symptoms. These drugs are believed to stimulate hypoactive dopaminergic pathways and, at a same time, can block DRD2 receptors in the hyperactive dopaminergic pathways resulting in diminished positive symptoms.[129,130]

The development of the science of pharmacogenomics will have profound implications for the accurate diagnosis, prevention, and development of new treatment modalities in psychiatry. The nascent science of methylomics will likely come to be considered a complement to genomics in investigating the pathogenesis of complex mental disorders.

## Acknowledgments

We thank Dr Stephen J. Glatt for his critical review of this manuscript.

This work was supported by grants from NIMH (R25MH60485) and NIH (ES10377 and CA101773).

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