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PERSPECTIVES

The Genes for Major Psychosis: Aberrant Sequence or Regulation?

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*A number of recent clinical and molecular observations in major psychosis indicate that epigenetic factors may be operational in the origin of major mental illness. This article further develops the idea that epigenetic factors may play an etiopathogenic role in schizophrenia and bipolar affective disorder. The putative role of epigenetic factors is shown by the epigenetic interpretation of genetic association studies of the genes for serotonin 2A (*HTR2A*) and the dopamine D3 (*DRD3*) receptors in schizophrenia. The idea of epigenetic polymorphism of genetic alleles is introduced, and it is argued that epigenetic variation may explain a number of controversial and unclear findings in allelic and genotypic association studies of* HTR2A *and* DRD3*. In linkage analyses of multiplex families with bipolar affective disorder (BPAD), different loci on chromosome 18 indicated co-segregation of alleles of one parental sex with the disease phenotype, and this finding implies that the epigenetic mechanism of genomic imprinting may be involved. Evidence for genomic imprinting provides the background for epigenetic cloning of BPAD risk factors by searching for differentially modified genes on chromosome 18. Finally, epigenetic studies could be relevant to the better understanding of the molecular action of antipsychotic medications. In addition to this, if epimutations are detected in major psychosis, epigenetic treatment directed at correction of epigenetic status of a specific brain gene may eventually be developed.* ***[Neuropsychopharmacology 23: 1–12, 2000]***

KEY WORDS: *Epigenetics; DNA methylation; Chromatin conformation; Schizophrenia; Bipolar affective disorder; Serotonin 2A receptor gene; Dopamine D3 receptor gene; Chromosome 18; Genetic linkage; Genetic association; Genomic imprinting; Parent-of-origin effect; Epimutation*

Recently, several etiological theories of schizophrenia (SCZ) were re-evaluated from an epigenetic point of view (Petronis et al. 1999). By definition, epigenetics refers to modifications in gene expression that are brought about by heritable, but potentially reversible, changes in DNA methylation and/or chromatin structure (Henikoff and Matzke 1997). In the last several decades, a myriad of epigenetic mechanisms has been uncovered, and a series of important functions of genome regulation have been ascribed to epigenetic DNA modification (Bestor et al. 1994, Riggs and Porter 1996). Epigenetic mechanisms can explain a number of clinical and molecular findings that traditionally have been supporting unrelated and somewhat antagonistic theories of major psychosis (Petronis et al. 1999). In this article, we further develop the epigenetic theory and re-analyze a number of experimental findings in psychiatric genetics that suggest a series of candidate genes and chromosomal loci that may become the targets for epigenetic studies.

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SEROTONIN 2A RECEPTOR GENE (*HTR2A*) AND SCHIZOPHRENIA

Current Status of *HTR2A* Studies in Schizophrenia

Interest in serotonin system genes arose several decades ago when it was detected that serotonin is widely

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distributed throughout the central nervous system and that the hallucinogenic drug LSD demonstrates high affinity for serotonin receptors (reviewed in Lewis et al. 1999). To our knowledge, association of *HTR2A* with SCZ was first detected in a Japanese sample when a higher frequency of C allele of the 102T/C polymorphism was documented (Inayama et al. 1996). Although a number of studies with relatively small sample sizes were negative, a large combined study consisting of over a thousand SCZ patients and ethnically matched controls detected a significant overall association between SCZ and the 102C allele ($p = .003$; OR = 1.3; 95% CI 1.1–1.53; Williams et al. 1996). In addition to the higher rate of allele 102C among SCZ patients, there also existed a statistically significant excess of T-C and C-C genotypes versus T-T genotypes in SCZ ($p = .008$) with a relative risk of 1.7 (95% CI 1.22–2.36). Analytical approaches that are rigorous to population stratification, specifically transmission disequilibrium test, partially confirmed the case-control findings that the 102C allele is associated with SCZ (Spurlock et al. 1998a, but see also, Hawi et al. 1997). A meta-analysis of 15 studies including 1533 patients and 1771 controls, detected a significant excess of 102C allele in patients ($p = .0009$; OR = 1.18; 95% CI 1.07–1.31) with no evidence for heterogeneity between studies (Williams et al. 1997). Although both case-controls studies (Kidd 1993) and meta-analyses (Sharpe 1997) have been criticized, and therefore the results of such studies should be interpreted with caution, at present rejecting the etiological role of the *HTR2A* in SCZ with absolute certainty is impossible (O'Donovan and Owen 1999).

The nature of the 102T/C polymorphism, however, does not help for further understanding of the controversies of association studies. 102T/C polymorphism does not alter the amino acid sequence in the serotonin 2A receptor which means that the receptors encoded by 102C alleles have exactly the same structure as the ones encoded by 102T alleles. Several other possibilities that would explain the role of the 102T/C polymorphism in SCZ have been suggested. First, it has been hypothesized that mRNA transcribed from the 102T allele may exhibit different secondary structure in comparison to mRNA transcribed from the 102C allele, which may affect the efficiency of translation (Arranz et al. 1995). Direct studies of mRNA of the 102C and 102T alleles have not been performed, however indirect ones did not support the latter hypothesis as no difference in serotonin 2A receptor density in the frontal cortex was detected in individuals carrying 102T versus 102C alleles (Kouzmenko et al. 1997). The second possibility is that the real "culprit" polymorphism or mutation might be in linkage disequilibrium with the 102T/C polymorphism. Several polymorphisms in the exons of *HTR2A* were detected but none of them were associated with SCZ (Erdmann et al. 1996). The search for polymorphisms was later extended to the 5′-region of the gene, which resulted in detection of another polymorphism (−1438)A/G. Although (−1438)A/G is in linkage disequilibrium with 102T/C, the former polymorphism does not exhibit significant differences in the promoter basal activity (Spurlock et al. 1998a), and, therefore, cannot explain the association between *HTR2A* and SCZ. In *HTR2A* expression studies, post-mortem brains showed that amounts of *HTR2A* mRNA were roughly 50% lower in a number of cortical regions in SCZ patients compared to controls (Burnet et al. 1996). In addition, a number of studies (although not all) detected decreased density of serotonin receptors (B_{max}) but no changes in receptor affinity (K_d) in the brains of SCZ patients compared to controls (reviewed in Lewis et al. 1999). Changes in B_{max} but not K_d suggest that serotonergic dysfunction in SCZ is more likely to represent regulatory problems at the *HTR2A* rather than a structural mutation in the protein coding region

Epigenetic Interpretation of *HTR2A* Studies with SCZ

Origin of the "T102C" Polymorphism. From the epigenetic point of view, the 102C (CCG) allele, but not the 102T allele (CTG), is more likely to be the ancestral allele (Clifford and Nunez 1996). This argument is based on the observation that different nucleotides exhibit differential mutability rates. More specifically, CCG→CTG substitution rate is 4.5 times higher than the rate of CTG→CCG, while its homologue CGG mutates to CAG 16 times more often than the reverse CAG→CGG (Krawczak et al. 1998, Table 4, p. 480). The fact that chimpanzee's genotype at this site is C-C (Cargill et al. 1999) finally proves that CCG, but not CTG, is the ancestral allele. The mechanism of C→T mutation may be of epigenetic origin since methylated cytosine (^{met}C) in the CpG dinucleotide is known to be a "hot" spot for DNA mutation (Yang et al. 1996), and it is likely that the C102T polymorphism resulted from the conversion of ^{met}C to uracil and eventually to thymine. The (−1438)A/G polymorphism may be of the same genesis since the complementary strand of A/G is T/C, with T flanked by C and G.

The latter assumption (that 102C allele but not 102T one is the ancestral allele), however, runs into a paradox that the ancestral allele is the risk factor for SCZ, and the frequency of the risk allele in some populations exceeds 50% (He et al. 1999). The fact that more than two-thirds of a population carries the risk allele was used as an argument against *HTR2A* being involved in SCZ (Crow 1996). The analysis of epigenetic status at *HTR2A* may help understanding this and other unclear issues around the role of *HTR2A* in SCZ, and the idea of *HTR2A* epigenetic variation is described below.

Polymorphic Genomic Imprinting of HTR2A. Evidence that epigenetic factors may play a role in regulation of

HTR2A, has been recently provided (Kato, Shimizu, et al. 1996). It was found that *HTR2A* was expressed only in the human fibroblast tissue culture with a maternal allele but not in the cells without a maternal allele (Kato, Shimizu, et al. 1996). Such parent of origin specific monoallelic expression of the gene may represent genomic imprinting at *HTR2A.* By definition, genomic imprinting refers to parent-of-origin dependent epigenetic marking of genes that results in differential expression of such genes (for review see Barlow 1995, Falls et al. 1999, Joyce and Schofield 1998, Ohlsson et al. 1998, Solter 1998). The alleles of imprinted genes appear to be differentially marked during gametogenesis or during the early part of development, and such epigenetic marks are reversible from generation to generation. Consistent with the idea of genomic imprinting, the 5′-region of *HTR2A* was methylated in cells with the maternal gene, and it was not methylated in cells without the maternal gene (Kato, Shimizu, et al. 1996). The fact that the experiment was performed in non-native cells raises the additional question if epigenetic regulation of *HTR2A* in the fibroblast tissue culture is the same as in the native tissue. We recently demonstrated that gene methylation status may change after the transformation of peripheral lymphocytes with the Epstein-Barr virus (Petronis et al. in press). Although generally important, this concern seems to not be critical in the case of *HTR2A* because at least two other studies demonstrated converging evidence for mono-allelic expression and possible genomic imprinting of *HTR2A.* In one of them, it was shown that the mouse homologue, *Htr*2, exhibits genomic imprinting with expression of maternal alleles only in the tissues where this gene is expressed (Kato et al. 1998). The analysis of human brain tissues showed that some individuals exhibit monoallelic expression but in the absence of information about parental origin of such alleles, a stronger conclusion about genomic imprinting could not be drawn (Bunzel et al. 1998). It is important to note that only four out of 18 tested brain tissues exhibited monoallelic *HTR2A* expression, while in the remaining 14 tissues *HTR2A* was expressed biallelically (Bunzel et al. 1998). Such irregularity of imprinting is called polymorphic genomic imprinting, and has already been described for other human genes, such as the genes for insulin-like growth factor II (*IGF2*; Xu et al. 1993) and Wilms tumor (*WT1*; Jinno et al. 1994). The origin of polymorphic genomic imprinting is not clear.

Polymorphic genomic imprinting of *HTR2A* can be a significant confounding factor in genetic association studies. It is critically important to know how many individuals express both *HTR2A* alleles and how many individuals express only one *HTR2A* allele in the groups of controls and affected subjects (see Figure 1). None of the numerous association studies of *HTR2A* in SCZ accounted for the polymorphic genomic imprinting, which questions the validity of such studies.

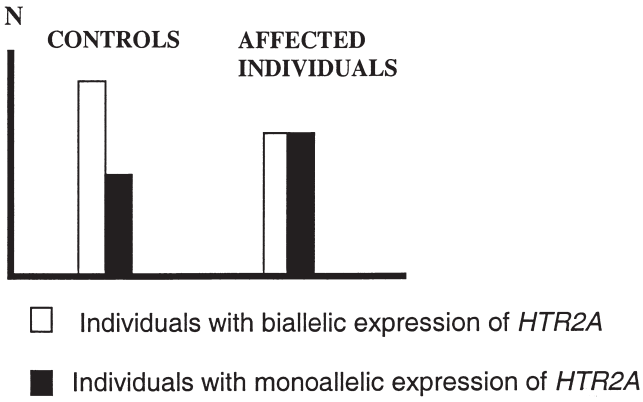

Figure 1. Monoallelic and biallelic expression of *HTR2A.* The proportions of monoallelically and biallelically expressed *HTR2A* may be different in controls and affected individuals.

Other Types of Epigenetic Polymorphism at the HTR2A. In addition to genomic imprinting, the *expressing HTR2A* alleles may be subjected to other types of epigenetic regulation. In our recent studies of the dopamine D2 receptor gene (*DRD2*) we showed that each DNA sample exhibited a unique pattern of DNA methylation at the 5′-region of *DRD2* with a high degree of somatic variability of ^{met}C (Popendikyte et al. 1999). There were no two DNA samples with identical patterns of *DRD2* methylation, which is not surprising given the metastable nature of epigenetic signals. In a similar way to genetic polymorphism, which refers to DNA sequence differences across individuals, variation of epigenetic patterns can be called *epigenetic polymorphism.* The idea of epigenetic polymorphism suggests that in addition to "yes/no" type regulation by genomic imprinting, a "many shades of grey"-type epigenetic regulation may be present in *HTR2A.* A combination of the two types of regulation, polymorphic imprinting and epigenetic polymorphism, may account for differential degree of *HTR2A* expression across individuals.

The epigenetic polymorphism may help explain the role of *HTR2A* 102C/T polymorphism in SCZ in the following way. A sample of 102C alleles, although they possess identical DNA sequence, may be different from the epigenetic point of view. Some of the 102C alleles may be heavily methylated in their regulatory regions and, therefore, expressed to a relatively low degree, while other 102C alleles may be hypomethylated, which results in high expression of the gene. The same idea is applicable to 102T alleles. Such epigenetic variants of genetic alleles can be called *epialleles.* 102C and 102T epialleles that are beyond some critical "threshold" level of epigenetic modification may be predisposing to SCZ. Evidence for genetic association is detected because on average one type of alleles (e.g., 102C) is subjected to a stronger epigenetic suppression of gene expression than the other alleles (e.g., 102T). In this scenario, a genetic

polymorphism does not have any biological effects, and such genetic variation is nothing but a "reporter" of the epigenetic status of an allele. The idea of a genetic reporter of epigenetic status was recently developed to explain the association between *Taq*I"A" polymorphism of *DRD2* and D2 receptor density in the brain (Petronis 1999), where it was suggested that *DRD2 Taq*I"A" polymorphism resulted from the mutation of a methylated C (^{met}C), which is a "hot" spot for DNA mutation (Yang et al. 1996) and which led to the rarer *Taq*I"A" allele, TTGA. It was hypothesized that various *DRD2* alleles (haplotypes) are subjected to various patterns of DNA methylation around the *Taq*I"A" polymorphic site. In this case, the di-allelic *Taq*I"A" site serves only as a reporter of the epigenetic status of the region surrounding the *Taq*I "A" site. The role of a reporter may eventually degenerate because each meiosis evokes a major rearrangment of epigenetic status in the gametes (Holliday 1996; Shemer and Razin 996), and the epigenetic status of *DRD2* may undergo significant changes. At some point, polymorphic DNA sites may fail to represent the epigenetic modification of a specific *DRD2* allele (Petronis 1999). For the same reason, absence of *DRD2* linkage to SCZ and controversial results in *DRD2* genetic association studies do not exclude the putative role of epigenetic regulation in the etiopathogenesis of the disease.

The same argument is applicable to the C102T polymorphism for *HTR2A.* As discussed above, the 102T allele may have originated from deamination of ^{met}C to uracil and eventually to thymine. The discriminatory power of the C102T polymorphism of the epigenetic variation of genes may vary across populations and samples. The higher the epigenetic difference between 102C and 102T alleles, the stronger evidence for association with SCZ. Samples with a negligible difference in epigenetic status across 102C and 102T alleles will exhibit no association with SCZ. The relationship between genetic and epigenetic association is graphically represented in Figure 2. The idea of epigenetic dysregulation of *HTR2A* in SCZ is consistent with the decreased amounts of *HTR2A* mRNA (Burnet et al. 1996) and serotonin receptor type 2 density (B_{max}) (Lewis et al. 1999) in post-mortem brains of SCZ patients compared to controls.

DOPAMINE D3 RECEPTOR GENE (*DRD3*) AND SCHIZOPHRENIA

DRD3 has been one of the most popular genes in genetic association studies of SCZ. The *Bal*I (or *Msc*I) polymorphism represents a serine (Ser) to glycine (Gly) substitution in the codon 9 of the first exon of *DRD3.* Since the primary finding of increased homozygosity of *Bal*I alleles among SCZ patients (Crocq et al. 1992), this polymorphism has been analyzed in more than 30 independent studies. Although individual studies with relatively small sample sizes led to quite controversial conclusions, a large European study using more than 300 SCZ cases and controls, replicated the excess of *DRD3* homozygotes of both alleles in SCZ patients ($p = .003$; Spurlock et al. 1998b). Several meta-analyses have been performed, and these have supported the risk effect of *DRD3.* One of them detected excess of Ser/Ser carriers among SCZ patients in the both African and Caucasian groups ($p < .05$; Dubertret et al. 1998). Another meta-analysis which investigated a sample of 5351 individuals, detected a significant excess of both forms of homozygotes in the group of SCZ patients ($p = .0009$; Williams et al. 1998). A transmission disequilibrium test showed a significant excess of both homozygotes in SCZ patients ($p = .004$; Williams et al. 1998). The above findings suggest that either homozygosity of *DRD3 Bal*I

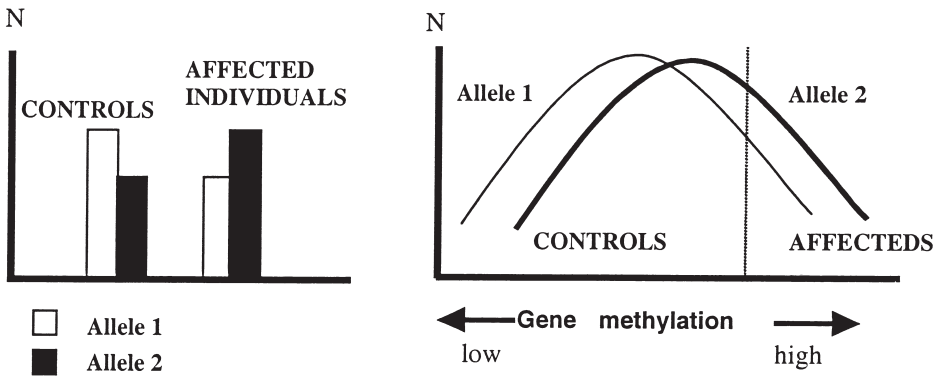

Figure 2. Genetic and epigenetic association with a disease. **(A)** Distribution of alleles 1 and 2 in the groups of affected individuals and controls. Allele 2 is associated with a disease. **(B)** Distribution of alleles 1 and 2 according to their epigenetic status. Both alleles exhibit a normal distribution of epialleles, although the mean degree of DNA methylation of allele 2 (thick line) is higher in comparison to allele 1 (thin line). Punctuated vertical line represents a hypothetical threshold at which epialleles begin to noticeably increase the risk of a disease. Epialleles of both alleles predispose to a disease, however the proportion of epialleles 2 is greater than that of epialleles 1.

is a predisposing condition to SCZ, or heterozygosity for alleles for this *DRD3* polymorphism is a protective factor. This effect is called overdominance (also "heterosis" or "hybrid vigor"). At least three samples studied in two centers showed a sex effect, that is, significant excess of homozygotes was observed in males ($p = 0.00021$) but not in females (Asherson et al. 1996, Griffon et al. 1996).

In *DRD3* expression studies, cortical regions of postmortem brains from patients affected with SCZ revealed significantly lower levels of D3 mRNA than in controls (Meador-Woodruff et al. 1997). Interestingly, the expression of a D3 splicing variant named D3nf (a truncated D3 variant that differs from the D3-receptor only in the carboxyl terminus) appeared to be unaffected in SCZ brains (Schmauss et al. 1993, Schmauss 1996). It was suggested that disproportion between D3nf and regular D3 proteins may affect efficiency of the formation of heteroligomeric D3 complexes (Nimchinsky et al. 1997) which potentially disturb dopaminergic function in the brain of SCZ patients.

Epigenetic Interpretation of the *DRD3* Findings in SCZ

The easiest finding to explain from the epigenetic point of view is the presence of *DRD3* association in the group of SCZ males, but absence of such association in the SCZ females. Differential effects of hormones, including sex hormones, have a significant impact on gene expression, and this is achieved by changing chromatin conformation (Jantzen et al. 1987, Truss et al. 1992) and/or local pattern of gene methylation (Saluz et al. 1986). Some genes exhibit even a clear-cut sexually dimorphic DNA methylation pattern (Yokomori et al. 1995).

The role of epigenetic factors in other *DRD3* findings in SCZ is not straightforward. Despite numerous phenotypic observations of overdominance, molecular mechanisms of this phenomenon are not clear, and experimental studies on this issue are sparse. A classical example of overdominance is that related to the heterozygous condition of the gene encoding the B chain of hemoglobin (*HBB*). DNA mutations that lead to replacement of amino acid sequence in *HBB*, significantly reduce the ability of Hb protein to bind oxygen, and homozygosity for *HBB* mutation leads to a recessive disease, sickle cell anemia. Sickle cell trait, or heterozygosity for the recessive mutation in *HBB*, is evolutionary advantageous because a wild type allele provides an organism with a normal supply of oxygen while the presence of a mutant allele significantly reduces the risk for malaria. The example of sickle cell trait, however, is hardly applicable to the *DRD3* findings in SCZ. Mutant and wild-type *HBB* homozygotes are susceptible to two very different diseases, while in the case of *DRD3* it is the same disease. At the molecular level, mutant hemoglobin exhibits a very different affinity for oxygen in comparison to the wild hemoglobin, while the receptors deriving from Ser and Gly alleles of *DRD3* exhibit only marginal, if any, difference in their affinity for dopamine (Lundstrom and Turpin 1996). In addition, there is no evident difference in the expression of mutant *HBB* alleles in comparison to wild-type alleles, at least at the protein level, while *DRD3* expression seems to be disturbed in SCZ. All these differences allow for thinking that overdominance of D3 receptor in SCZ is mechanistically different from the one in the sickle cell trait. Can it be epigenetic?

In the literature, there are at least two examples that relate overdominance to epigenetic regulation of gene expression. Expression of *Pl′-mah*, an epiallele of a maize transcriptional regulator gene for antocyanin synthesis, may vary depending on the second *pl* allele (Hollick and Chandler 1998). Expression of *Pl′-mah* is relatively low and is stable when this epiallele is homozygous or heterozygous with another epiallele, *Pl-Rh*, but such expression increases significantly when it appears in combination with other *pl* alleles or when *Pl′-mah* is hemizygous (Hollick and Chandler 1998). Such a significantly higher expression of *Pl′-mah* in a combination with *pl* alleles cannot be explained by a simple additive effect of *Pl′-mah* and *pl* alleles, and is likely to represent a case of overdominance. The increase in *Pl′-mah* expression is thought to be related to the absence of allelic interaction between *Pl′-mah* and *pl* alleles while epialleles *Pl′-mah* and *Pl-Rh* are likely to "communicate" with each other, which sustains relatively low level of *Pl′-mah* expression.

In addition to plants, evidence for epigenetic factors in overdominance derives from the genetic studies of *callipyge* sheep that exhibit the phenotype of muscular hypertrophy (Cockett et al. 1996). Molecular studies have shown that only heterozygous sheep (*CLPG/cplg*) that inherited the *callipyge* mutation, but not homozygous carriers of the mutant alleles (*CLPG/CLPG*), expressed the phenotype of muscular hypertrophy (Cockett et al. 1996). The idea that *CLPG* overdominance might be of epigenetic origin is based on the fact that the *cplg* locus exhibits strong evidence for genomic imprinting since only matings with *CPLG* rams, but not *CPLG* ewes, produced offspring with muscular hypertrophy. Although it would be naïve to directly extrapolate from plant and animal findings to *DRD3* studies in SCZ, given the lack of alternative explanations, epigenetic analyses may be one of the possible approaches.

The epigenetic mechanism of *DRD3* overdominance becomes even more complicated if reduced expression of *DRD3* is a result of abnormal splicing of D3 mRNA. In this case, the putative role of epigenetic factors derives from the observation that sites of gene transcription and mRNA splicing co-exist in the nuclear compartments (Moen et al. 1995, Zirbel et al. 1993), which favors the idea that the two processes are temporally and spatially linked in the cell nucleus. It has also been

suggested that nuclear compartmentalization is an epigenetic mechanism (Riggs and Porter 1996), and, therefore, the epigenetic factors may play a role in the further processing of mRNAs. At least two experimental studies suggested the role of DNA template-based (*cis*) factors but not cytoplasmatic (*trans*) factors in alternative splicing (Adami and Babiss 1991, Hayward et al. 1998). The first study demonstrated that different spliced versions of the E1B gene may originate from late and early adenoviral genomes which co-existed in the same cell. Neither genome was able to impose its regulated splicing pattern on the other, indicating that the cue for the switch in viral gene splicing is not directly dependent on global changes in *trans*-acting splicing factors. This suggests a model where the signal for changes in RNA processing for the *E1B* is linked to the state of the DNA template or its localization within nuclear subcompartments. Another example comes from human *GNAS1*, the gene that encodes maternally, paternally, and biallelically derived proteins. It was shown that mRNA of the paternally derived large G protein, XL, is differentially spliced in comparison to the mRNA transcripts of the neuroendocrine secretory protein NESP55, which is expressed exclusively from the maternal allele. In addition, consistently with the idea of epigenetic influence on differential splicing, a series of genes were shown to exhibit tissue-specific and developmental stage-specific expression o particular alternative mRNA molecules (Lopez 1998). Both tissue specificity and developmental changes are known to be epigenetic functions, and it can be hypothesized that concomitant alterations in the gene splicing patterns may also be related to epigenetic modification of the genome.

CHROMOSOME 18 AND BIPOLAR AFFECTIVE DISORDER

Chromosome 18 in bipolar affective disease (BPAD) is interesting from at least two points of view. This chromosome exhibits evidence for linkage in a proportion of bipolar disease families, and some linked loci on chromosome 18 demonstrate parent-of-origin effects. Excess sharing of the paternal but not maternal alleles was observed at D18S41, a marker on chromosome 18q21 in bipolar and recurrent unipolar depression ($p = .0004$; Stine et al. 1995). When only paternal pedigrees (i.e., when those in which the father or one of the father's sibs is affected), the same marker, D18S41, showed even greater degree of allele sharing (81%; $p = .00002$; LOD = 3.51; 2 = 0.0; Stine et al. 1995). Interestingly, D18S464, a marker on 18p11, showed evidence for linkage to exclusively maternal alleles ($p = .02$ and $p = .004$ for bipolar and recurrent unipolar depression, and for bipolar disease, respectively; Stine et al. 1995). As expected in complex traits, effort to replicate the above finding led to quite controversial results (Van Broeckhoven and Verheyen 1998, 1999). Several studies, however, could not reject the hypothesis that parental-sex specific genetic factors on chromosome 18 markers contribute to etiology of BPAD. In a new family collection the Johns Hopkins University group detected excess sharing of paternal alleles among BPAD patients at the markers D18S38 (68%; $p = .054$) and D18S541 (78%; $p = .015$; McMahon et al. 1997) which are ~7 cM and ~18 cM distal to D18S41 on the male-specific genetic map (see http://cedar.genetics.soton.ac.uk/public_html), respectively. Another study detected that mixed paternal-maternal families showed evidence for linkage to chromosome 18 centromeric markers to BPAD, in the region D18S37–D18S56 (affected sib pair method, p values from 10^{-5} to 0.03 for different markers), but no evidence for linkage was detected in pure maternal families (Gershon et al. 1996). This finding has recently been confirmed using multipoint ASPEX analysis (Detera-Wadleigh et al. 1999).

Parent-of-origin effect has been investigated in several combined studies of linkage data on chromosome 18 in BPAD from several centers in North America (including the above) and in Europe (Rice 1997). Linkage results varied widely depending on selection of datasets, statistical approaches used for meta-analysis, linkage parameters, and phenotypic definition of the disease (Collins and Go 1997, Donald et al. 1997, Greenwood and Bull 1997, Lin and Bale 1997), with the overall conclusion that parent-of-origin effect cannot be excluded for chromosome 18p and 18q markers in BPAD.

Interestingly, parent-of-origin effect for BPAD has been suggested in clinical studies. The risk for BPAD was significantly higher for maternal relatives ($p = .006$), and for the offspring of affected mothers ($p = .017$), while effected fathers repeatedly failed to transmit the disease to their daughters or sons (McMahon et al. 1995). The maternal effect was replicated in some studies (Gershon et al. 1996), while in others the size of maternal effect depended on the definition (boundaries) of the disease phenotype (Kato, Winokur, et al. 1996) or was not seen at all (Grigoroiu-Serbanescu et al. 1998). A cautionary note is that some of the above BPAD samples were ascertained for linkage analysis, and population-based studies are necessary for verification of the risk for BPAD depending on the sex of affected parent. On the other hand, females are more commonly affected with BPAD than males; therefore, lower genetic loading should be required for females to reach the threshold of clinical disease, which should result in lower disease risk to maternal but not paternal offspring; the opposite observation suggests that genuine genomic imprinting is operating in BPAD.

Clinical findings of parent-of-origin effect have direct implications for linkage studies in BPAD because

these indicate that at least some chromosomal loci may exhibit differential linkage depending on their parental origin. No contradiction exists between the evidence for maternal effect in BPAD families and paternal effect in linkage analysis to chromosome 18 markers in BPAD. The paternal effects on chromosome 18 may be outweighed by more common maternal genetic risk factors on other chromosomes, which results in the overall evidence for preferential maternal transmission of BPAD. The above studies suggest that linkage analyses have to be performed in a parental sex-specific way, that is, excess of allele sharing has to be sought for maternal and paternal alleles separately.

Parent-of-origin specific linkage analyses have at least two advantages in comparison to the ones that ignore parental origin of alleles. First, random co-segregation with disease phenotype is unlikely to be limited to the same-sex parents. Given that evidence for linkage in complex diseases is usually weak, and that it is always difficult to differentiate between a true predisposing locus and a false positive, parent-sex specific linkage may be of significant heuristic value. "Lukewarm" linkage that derives from a parent of one sex may be of greater importance than the same evidence of linkage that originates from both parents. Similarly, the idea of parental effect was creatively applied to a controversial phenomenon of genetic anticipation (increased severity and earlier age of a disease onset in subsequent generations). Several years before the discovery of unstable DNA, it was suggested that if anticipation occurs as a consequence of ascertainment bias, then it would be equally apparent in father/offspring and mother/offspring pairs, while anticipation confined to one sex of parent indicates some specific genetic or epigenetic event (Ridley et al. 1988). The same reasoning can be applied to linkage studies in complex traits. Note well, however, that not every evidence for paternal or maternal linkage can be immediately treated as genomic imprinting. Differential rates of meiotic recombination in male and female meioses may lead to artefactual differential linkage. To avoid this bias, higher density of markers in the region of putative linkage and multipoint linkage analysis is indicated.

Secondly, parental-sex specific linkage suggests that genomic imprinting may be operating in the etiology of a disease. Genomic imprinting implies that the gene(s) that predispose to a disease, carry different epigenetic marks depending on their parental origin. Genomic imprinting at disease gene(s) opens new opportunities in cloning such gene(s). Several laboratory methods can be applied to direct cloning of differentially imprinted genomic loci, and such methods will be briefly described in the next section. In complex traits, experimental approaches alternative to traditional positional cloning may be of significant importance because linkage analyses often are not able to narrowly define the region of linkage. At present chromosome 18 regions linked to BPAD are clearly too wide (Van Broeckhoven and Verheyen 1999) for the application of positional cloning and expectations of a rapid cloning of disease genes are unrealistic.

Epigenetic studies of chromosome 18 in BPAD may also help understanding the yet unknown disease mechanisms. In a study by Stine et al. (1995), a marker on the short arm of chromosome 18, D18S464, exhibited excess sharing of maternal alleles ($p = .004$ and $p = .02$ for phenotype of bipolar disease and bipolar disease plus recurrent unipolar depression, respectively), but surprisingly this excess sharing was detected in the paternal pedigrees. A similar phenomenon was detected in Nöthen et al. (1999), where evidence for linkage on chromosome 18p was the strongest for a recessive model in the paternal pedigrees (e.g., recessive narrow model, LOD = 1.67, 1.91, and 1.65 for markers D18S37, S453, and S40, respectively). Markers D18S37 and D18S40 are 17cM and 3cM away from D18S464 in the female-specific genetic map, respectively, which suggests that evidence for linkage may be to the same genetic locus in both studies. In both studies evidence for linkage originated predominantly from the non-affected parent (i.e., the mother). To our knowledge, no experimentally proven mechanism for such a pattern of disease transmission is yet available. It is worthwhile mentioning, however, the example of a susceptibility locus for type 1 diabetes, *IDDM2. IDDM2* is represented by allelic variation of a minisatellite polymorphism at the regulatory region of insulin gene (*INS*). Class I alleles of *INS* predispose to type 1 diabetes. A particular class I allele exists, however, that does not predispose to the disease when paternally inherited, which is compatible with polymorphic genomic imprinting (Bennett et al. 1997). The paternal effect is observed only when the father's untransmitted allele belongs to class III alleles (Bennett et al. 1997). This putative allelic interaction is similar to an epigenetic phenomenon of paramutation described in plants. It could be the case that in BPAD genetic risk factors on chromosome 18p are regulated by complex interaction btween transmitted and untransmitted alleles in addition to parental- and sex- specific DNA modifications. The above examples of type 1 diabetes and BPAD once again remind that complex disease may be caused by far more complicated mechanisms than the ones identified in more simple genetic diseases such as sickle cell anemia or cystic fibrosis.

EPIGENETICS ASPECTS OF ANTIPSYCHOTIC TREATMENT

In addition to relevance to major psychiatric disease, epigenetic status of specific genes may be one of the tar-

gets of antipsychotic neuroleptic treatment as well as one of the mechanisms of the action of neuroleptic medications. The idea that medication may affect DNA methylation is not new, and has been discussed over years in other fields of medicine (Szyf 1996). The epigenetic hypothesis may address one of the most unclear issues in psychopharmacology of why a two to three-week delay is necessary for achieving clinical antipsychotic effect (Bender et al. 1998, Johnstone et al. 1978) when neuroleptics block specific receptors within hours after they get into an organism (Hornykiewicz 1982). Epigenetic changes in the cell are relatively slow and may take weeks or even months to generate such a change in epigenetic regulation (Camus et al. 1990, Varela-Moreiras et al. 1995), which makes it consistent with the delayed antipsychotic effect of neuroleptics. The increasing evidence that epigenetic factors regulate not only the amount of mRNA molecules of a specific gene but also the nuclear location and the time of expression during a specific period in the cell cycle (Riggs and Porter 1996) may lead to uncovering the specific mechanisms of neuroleptic-epigenotype interactions.

Epigenetic characterisation of the genes that encode receptors that are the targets of antipsychotic medications, may also contribute to better differentiation between potential responders and non-responders to a specific drug. There is evidence that the above described 102C/T polymorphism at the *HTR2A* is a predictor of the clinical response to clozapine (Arranz et al. 1998) and typical neuroleptics (Joober et al. 1999). Response to neuroleptics may be partially determined by the differential expression of *HTR2A* epialleles, which results in differential density of serotonin 2A receptor in the brain tissues and effectively differential number of target receptors for the antipsychotic drug.

A series of studies have been dedicated to serotonin transporter gene (*SLC6A4* or 5-*HTT*). DNA sequence variation was detected in the upstream regulatory sequence of *SLC6A4*, and the alleles of this genetic polymorphism was shown to have an effect on gene transcription (Lesch et al. 1996). Lymphoblastoid cells with at least one copy of the short allele ('s') of *SLC6A4* demonstrated a significantly lower degree of *SLC6A4* expression, serotonin uptake potential and binding in comparison to the homozygotes for the long ('l') allele (Lesch et al. 1996). A recent pharmacogenetic study suggested the putative role of DNA sequence variation in the promoter of *SLC6A4* in response to treatment of major depression with fluvoxamine, a selective serotonin re-uptake inhibitor (Smeraldi et al. 1998). The homozygotes 'l/l' as well as heterozygotes 's/l' were better responders to fluvoxamine in comparison to the 's/s' homozygotes. Although the results are intriguing, it is not clear why the 'l/l' and 's/l' individuals showed similar response to fluvoxamine, while the earlier functional studies demonstrated that genotype 's/l' is much more similar to 's/s' in comparison to 'l/l' (Lesch et al. 1996). These discrepancies suggest that there could be tissue specific factors that lead to functional differences between *in vitro* lymphoblastoid cell lines and *in vivo* CNS tissues, with a good chance that such factors are epigenetic. The findings that the degree of expression may be involved in the drug response as well as predisposition to bipolar and unipolar disease (Furlong et al. 1998) warrants a detailed analysis of epigenetic regulation of the *SLC6A4.* It is expected that the *SLC6A4* will exhibit a number of variants of epigenetic regulation that may act relatively independently from the DNA sequence-based factors.

FINAL NOTES

Ten years of intensive molecular genetic searches for DNA mutations that would cause or predispose to major psychosis, unfortunately, have not been very productive. Experimental data of genetic linkage and association studies accumulated over this decade are either controversial or negative. Research strategies that worked relatively well in other complex diseases, such as breast cancer and Alzheimer's disease, turned out to be significantly less efficient in major psychosis. To paraphrase the well-known commandment (Paterson 1998), it looks like "all diseases are complex but some of them are more complex than others". For the "more complex" diseases the epigenetic paradigm could bring new theoretical and experimental opportunities. The criteria for selection of target genes or chromosomal loci for epigenetic studies are straightforward, which include parent-of-origin effect, sex effects, evidence for association with polymorphisms that have no evident role in functioning of a gene, and other epigenetic phenomena.

The above hypotheses can be tested using a series of epigenetic strategies. DNA methylation status in serotonin and dopamine receptor genes can be investigated using pairs of methylation sensitive and insensitive restriction enzyme, such as *Msp*I and *Hpa*II, *Cfr9*I and *Sma*I (see http://www.fermentas.com), as well as the more informative approach that utilizes bisulphite-induced modification of genomic DNA (Frommer et al. 1992). If the target gene is unknown, but evidence exists that epigenetic factors are operating (e.g., chromosome 18 in BPAD), screening for epigenetic differences can be performed using at least two techniques. Restriction landmark genomic scanning (RLGS) is a method that employs two-dimensional electrophoresis and allows for simultaneous visualization of a large number of DNA loci (Hayashizaki et al. 1993) including differentially methylated DNA fragments (Kawai et al. 1993). A modification of representational difference analysis can also be applied for cloning of DNA sequences that are subjected to differential DNA methylation. This method is called methylation sensitive representational difference analysis (MS-RDA) (Toyota et al. 1999, Ushijima et al. 1997).

Another important component of regulation of gene activity is chromatin structure. It has been known for a long time that in addition to DNA methylation, a high degree of gene expression correlates with histone hyperacetylation, while a low degree of such expression is linked to hypoacetylation of histones (Fletcher and Hansen 1996). The proportion of acetylated histones to deacetylated ones in each specific chromosomal region can be detected using anti-acetylated histone antibodies and quantitative polymerase chain reaction (e.g., Coffee et al. 1999). Interestingly, over the last several years relationship between DNA methylation and histone acetylation, two fundamental epigenetic mechanisms, has been established, which was detected that a methylcytosine-binding protein (MeCP2) that binds to methylated DNA, via other proteins attracts histone deacetylases (HDAC1 and HDAC2; Jones et al. 1998, Nan et al. 1998). For a number of genes, DNA methylation and histone acetylation may act synergistically.

Identification of patients to whom epimutations, but not DNA mutations, play an etiopathogenic role would be of a significant importance. Exclusion of the "epigenetic" subgroup of SCZ or BPAD patients from samples would reduce the degree of heterogeneity of major psychosis, and, therefore, increase the power of the more traditional genetic linkage and association studies in the rest of the sample. Additionally, evidence for any epigenetic changes at the candidate genes to major psychosis may lead to new DNA modification-based therapies. Recently, a compound protein consisting of DNA methyltransferase and zinc-finger protein was constructed (Xu and Bestor 1997). The mechanism of action of such a protein consists of the recognition of the target DNA sequence by the zinc-finger protein that is specific for the target sequence and subsequent methylation of the surrounding cytosines by DNA methyltransferase. This design may exhibit a number of advantages in comparison to the more traditional therapeutic approaches, including higher specificity as well as lower toxicity with fewer side effects. If DNA methylation defects are detected in SCZ or BPAD, a specific fusion protein recognising the sites of epimutations and restoring the normal epigenetic patterns can be generated.

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