

WHY SCHIZOPHRENIA GENETICS NEEDS EPIGENETICS: A REVIEW

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SUMMARY

Schizophrenia (SZ) is a highly heritable disorder, with about 80% of the variance attributable to genetic factors. There is accumulating evidence that both common genetic variants with small effects and rare genetic lesions with large effects determine risk of SZ. As recently shown, thousands of common single nucleotide polymorphisms (SNPs), each with small effect, cumulatively could explain about 30% of the underlying genetic risk of SZ. On the other hand, rare and large copy number variants (CNVs) with high but incomplete penetrance, variable in different individual, could explain about additional 30% of SZ cases. Although these rare CNVs frequently develop *de novo*, it is not clear whether they affect risk independently or via interaction with a polygenic liability in the background. Finally, the role of environmental risk factors has been well established in SZ. Environmental factors are rarely sufficient to cause SZ independently, but act in parallel or in synergy with the underlying genetic liability. Epigenetic misregulation of the genome and direct CNS injury are probably the main mechanism to mediate prenatal environmental effects (e.g., viruses, ethanol, or nutritional deficiency) whereas postnatal risk factors (e.g., stress, urbanicity, cannabis use) may also affect risk via use-based potentiation of vulnerable CNS pathways implicated in SZ.

In this review, we outline a general theoretical background of epigenetic mechanisms involved in GxE interactions, and then discuss epigenetic and neurodevelopmental features of SZ based on available information from genetics, epigenetics, epidemiology, neuroscience, and clinical research. We argue that epigenetic model of SZ provides a framework to integrate a variety of diverse empirical data into a powerful etiopathogenetic synthesis. The promising future of this model is the possibility to develop truly specific prevention and treatment strategies for SZ.

Key words: schizophrenia – SZ – epigenetics - gene-environment interactions - review

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INTRODUCTION

Based on converging evidence from a number of research disciplines, it has been generally accepted that both genetic and environmental factors play a significant role in the etiopathogenesis of schizophrenia (SZ). However, the exact nature of these two main etiological factors, their pattern of interaction, and their pathogenic mechanisms are poorly understood, despite extensive neurobiological, clinical, genetic, and epidemiological research.

Recent advances in epigenomics have increased understanding of gene-environment (GxE) interaction by identifying molecular mechanisms that mediate environmental influences on gene expression and activity. These epigenetic findings are of fundamental importance for the conceptualization of complex multifactorial psychiatric disorders such as autism, SZ, etc. For example, epigenetic misregulations in response to a variety of environmental factors have been suggested as a mechanism to explain the increasing risk of SZ in adulthood (Oh & Petronis 2008).

In this paper, we provide a general theoretical background of epigenetic mechanisms involved in GxE interactions. We then review empirical studies indicating a more or less direct involvement of dynamic epigenetic factors in complex neurodevelopment and expression of SZ. We argue that epigenetic model of SZ

provides a framework to integrate a variety of diverse empirical data into a powerful etiopathogenetic synthesis. The promising future of this model is the possibility to develop truly specific prevention and treatment strategies for SZ.

Our analysis and interpretations assume that at least some of the genetic findings associated with SZ are true. This is not a foregone conclusion given the limitations of many of the published studies in psychiatric genetics. Furthermore, we do not attempt reanalysis of existing data, but rather develop arguments based on what appears to be the polymorphous nature of genetic and environmental factors contributing to this complex disorder.

THE RISING FIELD OF EPIGENETICS

Epigenetic programming of the genome

In contrast to the “genocentric” molecular biology of the past, epigenetics focuses on changes in gene function, heritable through mitosis and meiosis, that do not involve changes in DNA sequence.

Genes are epigenetically marked (activated or silenced) during gametogenesis (e.g., gene imprinting that allows only one-parent’s allele expression) and embryogenesis (when epigenetic “instructions” for ontogenetic development are set in place), or later in life, either in response to environmental influences or as

a result of stochastic events (Morgan et al. 2005). Most frequently, gene expression is regulated at the level of transcription, via covalent modifications of gene promoters, or after translation, via covalent modifications of histone tails and resulting chromatin remodeling. Specifically, methylation of 5' cytosine in the DNA sequence on gene promoters disrupts the binding of transcription factors and usually diminishes gene expression, although recently there has been evidence that some methylation of gene promoters can also activate genes (Chahrour et al. 2008, Cohen et al. 2008). Post translational regulation of gene activity is achieved via dynamic modulations of chromatin conformation. Recall that DNA is wrapped around a complex of histone proteins ("nucleosomes") where it is either accessible (loosely packed "euchromatin") or inaccessible (tightly packed "heterochromatin") to transcription factors and RNA polymerases. For example, acetylation of lysine in histone tails loosens the chromatin structure and facilitates gene transcription by creating a negatively charged amid group which repels the negatively charged DNA phosphate group ("euchromatin"). Conversely, deacetylation of lysine creates the positively charged amino group with strong attraction to the negatively charged DNA phosphate, tightening chromatin conformation ("heterochromatin") and resulting in diminished gene expression.

Periodic ordering of genes along the DNA sequence and spatial co-localization with transcription factors optimize functioning of co-regulated genes. In particular, highly transcribed genes, RNA polymerases, and transcription factors gather into discrete spatial foci called transcription factories (Junier et al. 2010). Disruption of this spatial genomic organization by allosteric changes in chromatin conformation results in downregulation of the involved genes (Saha et al. 2006).

In addition to directly affecting gene transcription by chromatin remodeling as described above, chromatin conformation and gene activity are synchronized via bidirectional co-regulation: methylated, inactive DNA recruits enzymes that change chromatin conformation into inactive heterochromatin (Fuks et al. 2003). Conversely, active euchromatin recruits enzymes (e.g., histone acetyl transferase - HAT) that lead to hyperacetylation of histone tails, demethylation of DNA, and gene activation (Szyf et al. 2008, Szyf 2009). Details on mechanisms regulating gene expression via DNA methylation and chromatin remodeling are reviewed in Saha et al. (2006), Klose & Bird (2006), Szyf et al. (2008), Szyf (2009). The long-term pattern of epigenetically modulated genes creates the epigenome (or, as it were, a "programmed" genome).

Epigenetic mechanisms mediate GxE interaction

Epigenetic modulation of gene activity is a well established molecular mechanism to mediate various types of environmental influences. Pioneering work in animals demonstrated that early maternal care modulates gene expression resulting in stable patterns of

glucocorticoid receptor expression in the hippocampus, variable vulnerability to stress, and a number of related behavior features in adult offspring (Weaver et al. 2004, review by Szyf et al. 2008, Sweatt 2009). Likewise, humans with histories of abuse manifest increased methylation and decreased expression of hippocampal glucocorticoid receptors and long-term vulnerability to stress (McGowan et al. 2009). Moreover, poor maternal care coupled with over-protection in childhood ("affectionless control") increase risk of depression, addictions, attention deficit, OCD, anxiety disorders, and antisocial traits in adulthood (review by Champagne 2008 and the references therein). A significant linear negative correlation between cerebrospinal fluid levels of corticotropine releasing factor and reported levels of parental care has been reported (Lee et al. 2006). In contrast, good maternal care correlates with decreased trait anxiety and decreased salivary cortisol in response to stress (Pruessner et al. 2004).

There is accumulating evidence that dynamic chromatin conformation provides the link between external environment and gene expression and activity (Champagne 2005, 2008, Szyf et al. 2008, Sweatt 2009). This holds not only for chemical or biological environmental pathogens (Tremolizzo et al. 2002, Weaver et al. 2004, 2005), but also for psychosocial exposures (Nithianantharajah & Hannan 2006, Miller & Sweatt 2007). Epigenetic marks established by early environmental conditions tend to be stable (Champagne 2005), but are reversible, even in adulthood, through sustained effects of changing environments (Weaver et al. 2004, Nithianantharajah & Hannan 2006). This is called "environment x environment" interaction (ExE).

Genomic susceptibility to environmental influences continues over lifetime, even in mature, differentiated somatic cells. For example, 3 year old MZ twins are roughly concordant for the degree of DNA methylation and histone acetylation in peripheral blood lymphocytes and other non-neural tissue, but at the age of 50 years they have amassed a fourfold difference (Fraga et al. 2005). In the Fraga et al. (2005) study, twins raised apart manifested greater discordance in DNA methylation than twins raised together, indicating that exposure to discordant environments, rather than stochastic effects, created the difference (Champaign & Curley 2009, Connor & Akbarian 2008). On the positive side, lifelong genetic susceptibility to environmental influences provides avenues for prevention and treatment strategies for medical and psychiatric disorders. On the negative side, epigenetic mechanisms open the door for environmental pathogens to reach the cell nucleus and alter the genome, either by potentiating existing or by creating new genetic liabilities.

Epigenome and genome: complementary regulation of phenotypic features

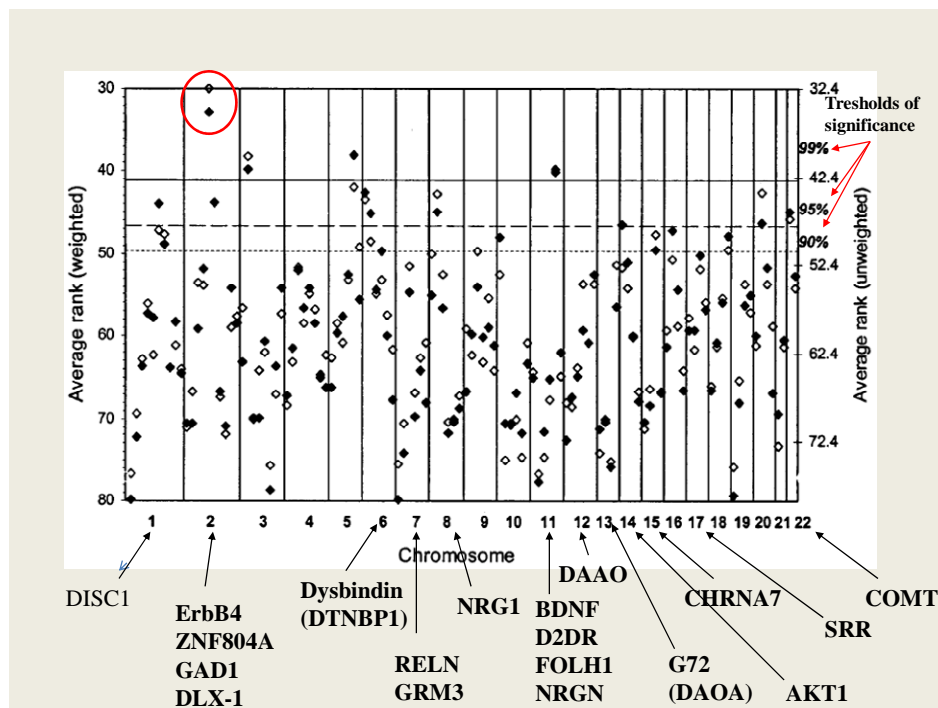
In contrast to but complementary with genetic changes, epigenetic mechanisms are fast (they mediate acute regulation of gene activity in response to environment), affect only selected tissues and cell types

(genetic changes affect all cell types), and provide a mechanism for non-genomic, “Lamarckian” inheritance (information about environment can be transmitted to generations of offspring via incomplete removal of epigenetic markings in the germ line (Waterland & Jirtle 2003). As Hochberg et al. (2011) summarized, epigenetic mechanisms provide plasticity in developmental programming and rapid adaptation to environmental influences that has evolved in order to maximize chances of survival and reproduction under changing environments.

GENETIC RISK ARCHITECTURE OF SCHIZOPHRENIA

Genetic liability to SZ may possibly involve hundreds (Mill et al. 2008) even thousands of genes (Int Sch Consortium 2009). In a meta-analysis of linkage results from 20 studies of SZ vs. non SZ genome scans we and other colleagues (Lewis et al. 2003), studied the risk of certain polymorphisms associated with SZ. The study was similar to genome wide association studies (GWAS), but we looked at linkage to certain chromosome regions with markers rather than at a particular allele.

Only one significant region on the long arm of chromosome 2 was consistently found across the 20 studies ($p < 0.000417$), but there were a number of nominally significant regions ($p < 0.05$) that emerged out of many tests conducted using the Monte Carlo method. P AvgRnk (the probability of observing, by chance, each bin’s average rank) was observed in 12 consecutive bins on nine chromosomes (5q, 3p, 11q, 6p, 1q, 22q, 8p, 20q, 4p). Likewise P Ord (the probability of observing, by chance, a bin with the same place (1st, 2nd) in order of average ranks in each permutation) was observed in 19 consecutive bins on six chromosomes (16q, 18q, 10p, 15q, 6q, 17q). The emphasis here is on consecutive bins involving large contiguous segments of the genome, meaning that it was not just random or chance association in one bin. The main conclusion of this large-scale study was that “...some or all of these regions contain loci that increase susceptibility to schizophrenia in diverse populations” (Lewis et al. 2003, p. 34). This study practically marked the end of search for a single gene conferring risk of SZ. Moreover, the identified susceptibility regions in SZ corresponded to unstable segments of the genome with high rates of copy number variants (CNV) repeatedly reported to be involved in SZ (Sebat et al. 2009).



Legend:

DISC1 - Disrupted-in-Schizophrenia 1; ErbB-4- encodes tyrosine-protein kinase ErbB-4 receptor for Neuregulin1; ZNF804A – zinc finger protein 804A; GAD1 – encodes glutamic acid decarboxylase GAD 67; DLX 1- encodes Homeobox protein DLX-1; DTNBP1- dysbindin; RELN – encodes reelin; GRM3 – encodes metabotropic glutamate receptor 3; NRG1 – neuregulin; BDNF – encodes for Brain Derived Neurotrophic Factor; D2DR- encodes for D2 dopamine receptor; FOLH1 – encodes for Glutamate carboxypeptidase II (GCPII); NRGN – encodes for neurogranin involved in protein kinase C signaling pathway; DAAO – encodes for D Amino Acid Oxidase involved in D Serine metabolism; G72 (DAOA) – encodes for D Amino Acid Oxidase Activator; CHRNA7 – encodes nicotinic receptor alpha 7; AKT 1 – encodes RAC-alpha serine/threonine-protein kinase involved in neuronal survival; SRR – serine racemase involved in Serine metabolism – glutamatergic coactivator; COMT – encodes COMT.

Figure 1. Scatterplot of average ranks for each bin weighted (◆) and unweighted (◇) for sample size (the higher the bin position on Figure, the more significant the bin)

Figure 1 shows the scatter plot of average ranks for each bin weighted (◆) and unweighted (◇) for sample size (the higher the bin position, the more significant the bin). As shown in Figure 1, with the exception of chromosome 2, other bins (markers) were not significant. A selection of candidate SZ genes and their corresponding chromosomal locations are illustrated at the bottom of Figure 1. Note: these genes have not been confirmed by Lewis et al. (2003) and this is solely intended to illustrate genome-wide abnormalities in SZ.

Traditional genetic designs, such as linkage studies, have notoriously failed to replicate candidate genes for SZ. The reason? Genetic risk in SZ involves multiple and variable genes with variable pattern of involvement, all interacting nonlinearly with other genes (epistasis) and variable environmental factors (GxE and ExE interaction). As a result, the same (different) genotype may underlie different (same) phenotypes in different individuals. Hence, individual genetic effects are easily lost in statistical averages of combined family pedigrees and erroneous assumptions of linearity, all common in genetic studies of the past.

Genetic causes of SZ: Common variants with small effect or rare variants with large effects?

Single nucleotide polymorphisms (SNPs) - a single base pair mutation at a specific locus are the most common types of genetic variation - occur in more than 1% in population and more than 1 million per individual. GWAS test association between common SNPs and SZ phenotypes genome wide. As Bray et al. (2010) noted, results to date have indicated that no common genetic variant confers in itself more than a very small increase in risk for SZ in general populations (all Odds Ratios < 2). However, recent report by the International Schizophrenia Consortium (2009a, 2009b) demonstrated that thousands of common alleles each with small effect cumulatively could explain about 30% of the underlying genetic risk of SZ.

In contrast, Copy Number Variants (CNVs) are rare (less than 1%) but large genetic lesions that involve both deletions and duplications spanning at least 1 Kb and usually encompassing several genes. Recently, the International Schizophrenia Consortium (2008) showed increased frequency of multiple rare CNVs (each spanning more than 100 Kb) in SZ. Walsh et al. (2008) also found multiple rare CNVs in SZ, affecting genes involved in neurodevelopment, specifically in neuregulin and glutamatergic pathways. An increase in de novo CNVs have been shown in sporadic cases of SZ (Xu et al 2008). As summarized by Bray et al. (2010), individual CNVs do increase the risk for SZ compared to smaller genetic variants (Odds Ratios range is between 4 and 30), but CNVs are also observed in controls, and increase risk for other mental disorders (e.g., autism). Although these rare genetic variants may explain a number of sporadic and some familial cases of SZ, they cannot fully characterize genetic risk for SZ. We agree with Bray et al. (2010, p.3) that “phenotypic

consequences of even large genetic lesions ... will depend upon additional genetic (and possibly also environmental) factors”.

Adding to the genetic heterogeneity of SZ, de novo genetic factors, called immediate early genes, may be induced by unfolding neuropathological processes. For example, hypofunction of NMDA receptors expressed on GABA interneurons induces a number of immediate early genes (review by Farber 2003). While not specific for SZ, they may have independent etiopathogenetic effects and could provide clues to “understanding the more enduring intracellular and nuclear events that occur in response to the disinhibition syndrome induced by NMDA hypofunction” (Farber 2003, p.122).

In summary, it appears that SZ can be caused both by a large number of common variants with small effect and by rare structural genetic variants with large effects in different individuals. In the extreme, each clinical case of SZ could be genetically specific, albeit probably not unique, a “moving target” reflecting an interactive combination of a wide but ultimately limited spectrum of pathological genetic variants.

Mixed model of genetic risk architecture in SZ

SZ is a highly heritable disorder, i.e., up to 80% of phenotypic variance in liability for SZ is attributable to genetic factors (Sullivan et al. 2003). Lifetime risk of SZ increases with quantitative genetic additions: if one parent has SZ, the risk for each child is between 10%-15%, if both parents have SZ, the risk increases to 35%-46% (Gottesman 1991). On the other side of these percentages, about 90% of SZ persons have no SZ parents and up to about 60 % have no 1st or 2nd degree relative with SZ. In other words, most cases of SZ appear sporadic, despite evidence of high heritability.

In some cases, rare and large genetic lesions, such as CNVs, are sufficient to produce SZ (The International Schizophrenia Consortium 2008, Walsh et al. 2008, Xu et al. 2008). These rare mutations have been observed within many different chromosomal regions, encompass a number of different genes, and usually have high but incomplete penetrance, thereby explaining the frequent impression of sporadic occurrence of SZ (Mitchell & Porteous 2011). As shown by Xu et al. (2011), in about half of SZ cases studied, identified CNVs were de novo and not inherited. Whether these rare lesions increase risk of SZ independently or through interaction with the polygenic liability in the background is unknown, but certainly worth studying. In summary, many different chromosomal regions with a rare CNV can cause SZ, but usually do so one at the time (Mitchell & Porteous 2011). This scenario is consistent with the heterogeneity model of genetic risk in SZ. Environmental effects are not necessary, but may contribute to the severity of phenotypic expression. In addition, as the rare variant is not always sufficient to cause SZ, it may need epistatic assistance from its “genetic background”, consisting of other normal and/or polymorphic genes, consistent with the “mixed model” (Mitchell & Porteous 2011).

In other cases, environmental factors synergistically increase risk of SZ, but only if superimposed on familial history of psychosis, not as independent risk factors (Maki et al. 2010). Here, a low penetrance polygenic liability, consisting of variable rare and/or common structural genetic lesions, is potentiated through GxE interactions. The emphasis here is on synergistic potentiation between genes and environment because, in this scenario, both factors are necessary but neither is sufficient to cause the illness. Such synergism could be mediated by epigenetic misregulation of the inherited liable genome (e.g., by increasing its penetrance), or by increasing susceptibility to SZ via specific effects (e.g., use-based augmentation of vulnerable biological pathways) or via non-specific effects (e.g., direct brain injury) or perhaps by all of the mechanisms in some proportion.

As we describe in detail elsewhere (Svrakic et al., in preparation), this scenario accounts for abnormal, large effect epigenetic states that are superimposed on a multifactorial background in which each factor has small effects on liability to illness. Such a mixed model can account for GxE interaction and/or shared family environmental effects in addition to having properties similar to cases in which a single structural variant of large effect is superimposed on a multifactorial background as described above.

The technical meaning of a "mixed" model is that there are multiple factors that are large enough to affect the liability distribution, which is no longer a simple normal bell-shaped curve, but one that has "bumps"

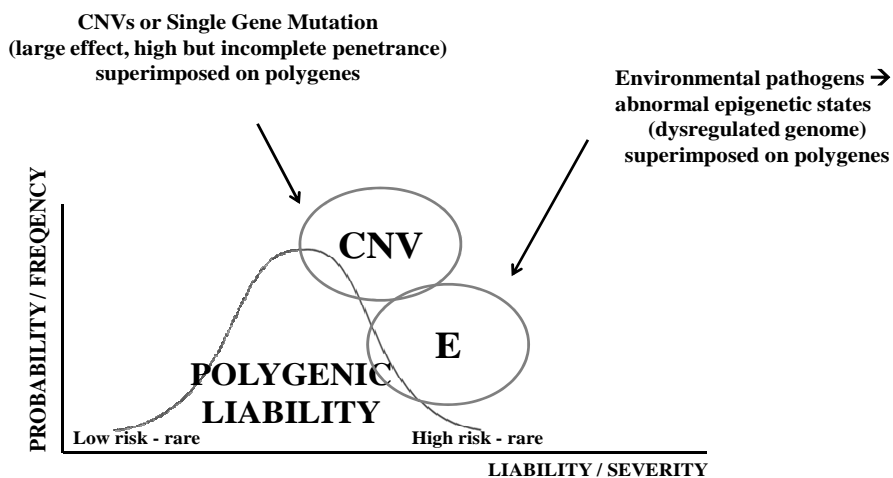
indicating admixture of multiple distributions contributing to liability, like a large single gene or a large CNV plus the many small effects of polygenes that have a smooth bell-shaped curve (Figure 2).

EPIGENETIC MISREGULATION IN SZ: REVIEW OF EVIDENCE

Epigenetic misregulation of the genome could result from two sources: epimutations or environmental effects, or both. Primary epimutations (i.e., errors in DNA methylation programming) occur during gametogenesis and embryogenesis, when epigenetic marks are set in place to specify temporally- and tissue-specific steps in development (Kato 2008). Fidelity of the transmission of DNA methylation patterns is lower than that of the DNA sequence (Ushijima et al. 2003). Hence, de novo epimutations are quite frequent, in fact one or two orders of magnitude greater than somatic DNA mutation (Horsthemke 2006). This implicates their significant contribution to human disease such as cancer (Dobrovic & Kristensen 2009) and possibly SZ.

Primary epimutations and epigenetic misregulations by environmental pathogens always coexist in some proportion (epimutations are occurring anyway, with or without genetic liability for SZ, but their actual contribution to the overall SZ risk is unknown). Whatever the origin, epigenetic markings of DNA are heritable, i.e., they are transmitted through mitosis in somatic cells during morphogenesis and growth (Champagne 2005, 2008). This provides a mechanism by which epigenetic

Risk Architecture of Schizophrenia: different scenarios (models) account for genetic and environmental factors



“Bumps” in normal risk distribution illustrate admixture of multiple distributions caused by genetic and environmental factors contributing to liability to SZ

Figure 2. Risk Architecture of Schizophrenia

effects of early environmental pathogens propagate through development (Dolinoy et al. 2007) as molecular precursors of evolving structural and functional abnormalities in SZ.

Pidsley & Mill (2011) review literature suggesting and/or demonstrating GxE interaction by virtue of epigenetic mechanisms in SZ. Here, we report selected data from a spectrum of disciplines, ranging from genetic epidemiology, molecular biology, to molecular genetics.

Excessive methylation worsens SZ symptoms

- High methionine diet leads to a profound exacerbation of SZ symptoms (e.g., Pollin et al. 1961, Brune & Himwich 1962);
- Psychiatric medications (valproate, clozapine) reduce DNA methylation and improve SZ symptoms (Dong et al. 2008);
- High levels of homocysteine (demethylated methionine), rising during the exacerbation phase and decreasing in remission, are found in the plasma of SZ patients (Petronijevic et al. 2008);
- Increased levels of methyl group donor - SAM were found in prefrontal cortex of SZ patients (Guidotti et al. 2007).

Methylation patterns in MZ twins discordant for SZ

- MZ twins discordant for SZ do not differ in their underlying structural genomic abnormalities (Ono et al. 2010), implicating causative epigenomic or developmental processes.
- MZ twins discordant for SZ manifest significant differences in the methylation pattern of the DRD2 gene (one of the candidate SZ genes) (Petronis et al. 2003). Strikingly, the affected twin was epigenetically more similar to the non-related affected individual with SZ than to his own unaffected MZ co-twin.

Epigenetic misregulation of γ -aminobutyric acid (GABA) genes in SZ

- Significant reduction of GABAergic proteins (GAD₆₇ and reelin) concurrent with increased DNA methyltransferase1(DNMT1) in the same cortical, hippocampal, and striatal GABA interneurons thought to be involved in SZ (e.g., Guidotti et al. 2000, Grayson et al. 2005, Veldic et al. 2005).
- Hypermethylation of GABA gene promoters has been shown to mediate this downregulation (Huang et al. 2007, Ruzicka et al. 2007) and the effect is reversed by histone deacetylase inhibitors (Tremolizzo et al. 2002).

SZ is protective against cancer - a prototypic GxE illness with epigenetic DNA misregulation

- While a number of factors may contribute to this peculiar dissociation, including an overall shortened lifespan, a possible explanation is that epigenetic modulations of genes shared by SZ and cancer may have opposite effects on the phenotype (e.g., MET proto oncogene is associated with risk of SZ and is

also involved in normal tissue differentiation, tumor growth, and metastases) (Sharma et al. 2010, review by Burdick et al. 2010)

Massive methylation of neurodevelopmental genes in SZ

- Mill et al (2008) studied overall methylation patterns (“methylome”) in postmortem frontal cortex of SZ and Bipolar subjects and found evidence for disease-associated aberrant DNA methylation in about 100 loci, including genes regulating glutamatergic and GABAergic systems, genes for stress response, and genes for neurodevelopment (Mill et al. 2008). Also, a lower degree of modularity was found in SZ than in controls, potentially revealing a systemic epigenetic dysfunction rather than isolated epigenetic missteps.
- In absolute amounts, DNA hypomethylation and hypermethylation were about equally represented in SZ with DNA hypermethylation being probably the primary epigenetic mechanisms in SZ (Mill et al. 2008).

Histone modifications in SZ

- Epigenetic misregulation in SZ may also involve changes in chromatin conformation and other regulatory mechanisms of gene expression, such as histone modifications resulting in downregulation of several metabolic genes (Akbarian et al. 2005).

Epigenetic misregulation of the genome: possible targets

Epigenetic mechanisms can misregulate the genome in different ways to increase its pathogenicity:

Inherited polymorphic genes. Using analogy with cancer, this would correspond to the proposed “two hit” scenario in carcinogenesis in which a genetic defect (e.g., recessive mutation) would not result in illness unless accompanied by some other genetic or environmental variable (e.g., somatic mutation or epigenetic silencing of the normal allele, respectively) leading to the expression of the mutation. For example, a functional SNP, causing increased proline oxidase activity within the PRODH gene, is positively associated with SZ, whereas two functional SNPs, which decrease this activity, are negatively associated with SZ (Kempf et al. 2008). Epigenetic, “second-hit” silencing of protective allelic variants is likely to shift the net effect towards increased risk of SZ and vice versa.

Normal genes. This refers to epigenetic misregulations of normal genes involved in neurodevelopment of SZ. Examples are numerous and include DISC1, Neuregulin1-ErbB4, COMT, BDNF, and DTNP1 (dysbindin), among others (<http://www.schizophreniaforum.org>). Epigenetic misregulation of these neurodevelopmental genes could result in a number of genetic abnormalities, such as aberrant monoallelic expression of a gene, inadequate dosing of a gene, and mistiming of genomic activity, among others.

Protective genes. This refers to genes with positive effects on cognition, motivation, emotion, and neurodevelopment. For example, the COMT Met/Met variant is associated with reduced risk of SZ, has a favorable effect on working memory (Egan et al. 2001) and protects against psychosis in cannabis users (Caspi et al. 2005). If Met/Met homozygosity is misregulated (e.g., underexpressed) by epigenetic mechanisms, this is likely to increase both the risk of SZ and the severity of SZ symptoms.

Pathological activation of genes. As Mill et al. (2008) showed, both hypo- and hypermethylation characterize the pathological genome in SZ. Although not as efficient as hypermethylation in modifying gene activity in neural tissue, hypomethylation is associated with some gene activation (Etchevery et al. 2010, Mill et al. 2008). Incidentally, pathological continuation of gene activity (instead of normal age-related down-regulation) has been shown for a number of neurodevelopmental and transmitter related genes in SZ (Torkamani et al. 2010). The mechanism underlying this continuing genomic activity is not clear (homeostatic vs. epigenetic), and could conceivably include hypomethylation of gene promoters.

Conditionally pathological genes. This refers to normal genes expressed in glia and other somatic cells, regulating intercellular communication and immune response (e.g., genes encoding for cytokines). Infection-related activation of these genes during sensitive in utero developmental periods leads to aberrant neurodevelopment, as we described earlier. This is consistent with numerous genetic studies implicating immune system involvement in SZ (e.g., The International Schizophrenia Consortium 2009b).

Metastable epialleles. These are defined as loci that can be epigenetically modified in a variable and reversible manner, such that a range of phenotypic outcomes (healthy and sick) can occur from genetically identical cells. A classic example is the mouse A^{vy} - viable yellow agouti epiallele (Duhl et al. 1994, Morgan et al. 1999). Metastable epialleles are sensitive to environmental factors especially during prenatal development (Duhl et al. 1994). Only a few genes with metastable epialleles have been identified so far (review by Jirtle & Skinner 2007) but epigenetic modulation of these variants may be of particular interest for psychiatry.

All of the above. All of the above alternatives may occur in SZ, given the context of non specific, regional misregulation of the genome by early environmental factors.

The main interacting components of the epigenetic model of SZ are summarized graphically in Figure 1.

ENVIRONMENTAL RISK FACTORS IN SCHIZOPHRENIA

Environmental factors are integral in SZ pathogenesis, much like in other complex GxE disorders, such as cancer. Reviews by Rutten & Mill (2009), Brown & Derkits (2010), van Os et al. (2008) and van Os et al. (2010) summarize environmental pathogens in SZ with evidence for GxE interaction.

Prenatal “first hit” factors

Prenatal factors include viral infections (particularly influenza), toxoplasmosis, and genitourinary infections, especially during the 1st and 2nd trimesters of pregnancy and in synergy with genetic liability to psychosis (review by Brown & Derkits 2010, Clarke et al. 2009). As most viruses do not cross the placenta, the pathological mechanism to the fetus was postulated to be indirect, probably related to maternal antiviral responses - such as proinflammatory cytokines (Ellman et al. 2010). Indeed, fetal exposure to interleukin-8 increases risk of SZ in offspring (Brown et al. 2004) and leads to structural neuroanatomic alterations in CNS regions corresponding to those implicated in SZ (Ellman et al. 2010). In addition, severe maternal stress during the 1st trimester of pregnancy (Khashan et al. 2008), maternal depression (Maki et al. 2009) are also associated with increased risk of SZ. Interestingly, the latter was associated with a four-fold increased risk in the offspring, again in the context of a family history of SZ, not as an independent factor.

Other prenatal factors associated with SZ include hypoxia secondary to obstetric complications (Nicodemus et al. 2008), and protein and other nutritional deficiencies, particularly deficiencies in dietary nutrients required for the formation of S-adenosylmethionine (SAM), such as folate, choline, vitamin B12 (Rutten & Mill 2009). Recall that SAM provides methyl groups for methylation processes, implicating epigenetic mechanisms in SZ.

At present, three different mechanisms are shown to mediate prenatal infection effects, most likely with some overlap:

Epigenetic downregulation of placental/fetal genes. In a recent study, Bobetsis et al. (2010) identified 74 placental/fetal genes epigenetically misregulated by bacterial infection during murine pregnancy. Most of the genes involved in fetal development were downregulated, and included, among others, two genes involved in neurodevelopment, the synaptotagmin X (SYT10), and the neuropeptide galanin (GAL) and its receptor (GALR3). SYT10 regulates the secretion of neurotransmitters and signaling between neurons, whereas mutations in the GAL gene underlie broad CNS impairments, for example lower numbers of sensory neurons and reduced capability for nerve regeneration.

Pathological activation of microglia. It is not certain whether all maternal inflammatory cytokines cross the placenta, and some (e.g., IL-6) do so early but not late in gestation (pointing to the importance of the timing of exposure). In vitro studies demonstrate activation of microglia by pro-inflammatory cytokines. Microglia is abundant in the fetal brain and, when activated, produce chemokines and cytokines that can be toxic during neurodevelopment. Molecular mechanisms underlying microglial effects have not been completely specified, but involve increased protein production and thus implicate epigenetic mechanisms. With bacterial infections, endotoxin lipopolysaccharide does cross placenta and induces cytokines in the fetal brain, as determined by increased levels of the corresponding mRNA (Jonakait 2007) directly implicating underlying epigenetic mechanisms.

Compromised fetal-placental-maternal link. Pro-inflammatory cytokines play an important role in maintaining the complex environment of the fetal-placental link and, at increased levels, can damage the fetus. In humans, IL-6 facilitates maternal host-versus-graft reaction and correlates with spontaneous abortion. In a less drastic outcome, maternal rejection of the fetus may only partially compromise the integrity of the fetal-placental link, and increase SZ risk either via distress-related neurotoxic damage (likely involving nutritional or oxygenation problems) or by distress-induced epigenetic misregulation of the genome.

Epigenetic misregulation and prenatal “first hit” risk-factors

Depending on the timing of environmental pathogens, different neurobiological targets are affected, different pathogenic mechanisms are involved, and different phenotypic outcomes may be generated. Prenatal in utero factors primarily affect the genome and/or the developing brain tissue – and we call them “first hit” risk factors. These are mostly biological and/or chemical pathogens (viruses, toxins, hypoxia, etc.), but also indirectly reflect social environment via maternal stress (Khashan et al. 2008).

Epigenetic misregulations of the liable genome are probably the main mechanism to mediate prenatal or early postnatal environmental effects. Epigenome is especially sensitive to disruption prenatally, during rapid cell replication and precise posting of epigenetic markings to drive development (Dolinoy et al. 2007). In addition, prenatal factors frequently cause direct brain injury (e.g., ethanol, lead poisoning, etc). The ensuing aberrant neurodevelopment creates early aberrant neural structures and functions (“prodromal CNS”) with increased sensitivity to environmental influences. This prodromal brain in fact becomes an independent risk factor for SZ, an emerging substrate for interactions with the environment which may or may not lead to the phenotypic expression of SZ in individual cases (Svrakic et al., in preparation).

Postnatal “second hit” risk-factors

After birth, aberrant early CNS, together with initial genetic liability operating in its background, are exposed to a variety of environmental influences including those documented to increase risk of SZ (Rutten & Mill 2009, van Os et al. 2010). We call these “second hit” risk factors and they extend from early postnatal period, through childhood, to late adolescence or even later. These postnatal or “second hit” factors include social pathogens (e.g., urbanicity, international migration), psychological pathogens (e.g., stress), and chemical pathogens (e.g., cannabis use) among others (reviews by Van Os et al. 2004, Rutten & Mill 2009). There is suggestive evidence for the causal effect of postnatal environmental effects (van Os et al. 2010). For example, incidence of SZ varies across urban vs rural areas, between minority groups, and is associated with high attributable risk (van Os et al. 2004).

Precise mechanisms underlying postnatal environmental factors are largely unknown but are unlikely to be homogenous. Epigenetic modulation of the genome is certainly a possibility, given the lifelong genomic susceptibility to such modifications. In fact, dynamic regulation of DNA methylation changes continues in differentiated cortical neurons (Ravindran et al. 2006, Siegmund et al. 2007), influenced by a variety of social factors (Rampon et al. 2000), alcohol (Ravindran & Ticku 2004), and methamphetamines (Numachi et al. 2004, 2007).

In addition to epigenetic misregulations of the genome (as described above), environmental factors may affect neurodevelopment via use-dependent potentiation of biological pathways implicated in SZ. For example, adolescent cannabis use increases risk of SZ during sensitive periods of corticogenesis. Specifically, carriers of the COMT Val allele (this variant is associated with rapid dopamine metabolism, low cortical and high midbrain dopamine) were more likely to develop psychosis if they used cannabis, compared to subjects carrying the COMT Met allele (Caspi et al. 2002) or adult-onset cannabis users carrying the COMT Val polymorphism (Caspi et al. 2005). Cannabis causes a significant decrease in cortical dopamine (Stokes et al. 2010) and increase in midbrain dopamine (Voruganti et al. 2001), both implicated pathological mechanisms in SZ. Although recent studies have failed to replicate this finding, cannabis use provides an example where the preexisting heritable dopamine dysfunction is amplified by environmental pathogens leading to increased risk of psychosis. This example highlights our mixed model of genetic risk architecture, in which abnormal epigenetic states potentiate preexisting polygenic vulnerabilities where each individual factor has small effect on risk.

Acute psychosocial stress, another established postnatal factor in SZ, has been postulated to increase SZ risk via GxE interaction (Rutten & Mill 2009), perhaps involving cytokines (You 2011). In addition, stress has also been shown to increase striatal dopamine

(Pruessner et al 2004), a well known factor in positive SZ symptoms especially in the context of an existing predisposition (Lisman et al. 2010). Migration and associated lower SES may create the so called “social defeat” stress or “unstable social hierarchy” stress, both shown to involve CNS nuclei and cortical circuits implicated in SZ (Zink et al. 2008, Meyer-Lindenberg 2010).

Recent studies provide evidence that use-dependent functional augmentation of biological pathways implicated in SZ could become long term and “hardwired”. Use-based refinement of synaptic connections is reported in the finalization of functional cortical architecture and networks (Singer 1995). Although genetic information primarily drives corticogenesis (Kaschube et al. 2002) these two mechanisms, genetic-based and use-based, co-exist in some proportion towards the very end of brain development. In other words, in some cases, environmental pathogens appear to increase the risk via augmentation of biological pathways implicated in SZ during the fine tuning of cortical architecture and function, a process that extends from early puberty to late adolescence and early adulthood.

COMPLEX NONLINEAR DEVELOPMENT OF SZ

Complex genetic liability for SZ represents the necessary condition (“*conditio sine qua non*”) for

subsequent neuropathological processes to unfold and also a substrate for epigenetic modulations by environmental pathogens. In some cases, genetic factors alone (e.g., CNVs, private point mutations) can carry the illness into the phenotype. In other cases, epigenetic factors are superimposed on the background involving multiple genetic abnormalities, each with small effect size. As both genetic and environmental factors vary in individual cases, clinical expression and evolution of SZ are also variable. Indeed, the impact of environmental factors may vary depending on several factors:

Timing - same factor, occurring prenatally or postnatally, may have different and variable effects on CNS at different stages of development (discussed below). In addition, timing of in utero environmental pathogens seems important as well, as, different gestational periods may correspond to time windows leading to specific disturbances in fetal brain development and different adult psychopathology (Meyer et al. 2007);

Type - different factors (viruses, hypoxia, cannabis, stress, or urban surroundings etc.), may potentiate risk of SZ via different processes (van Os et al. 2010);

Severity - mild pathogenic effects frequently generate milder outcomes, and vice versa (Farber 2003);

Chronicity of exposure - chronic exposure is usually more pathogenic, as shown for cannabis (Van Os et al. 2002);

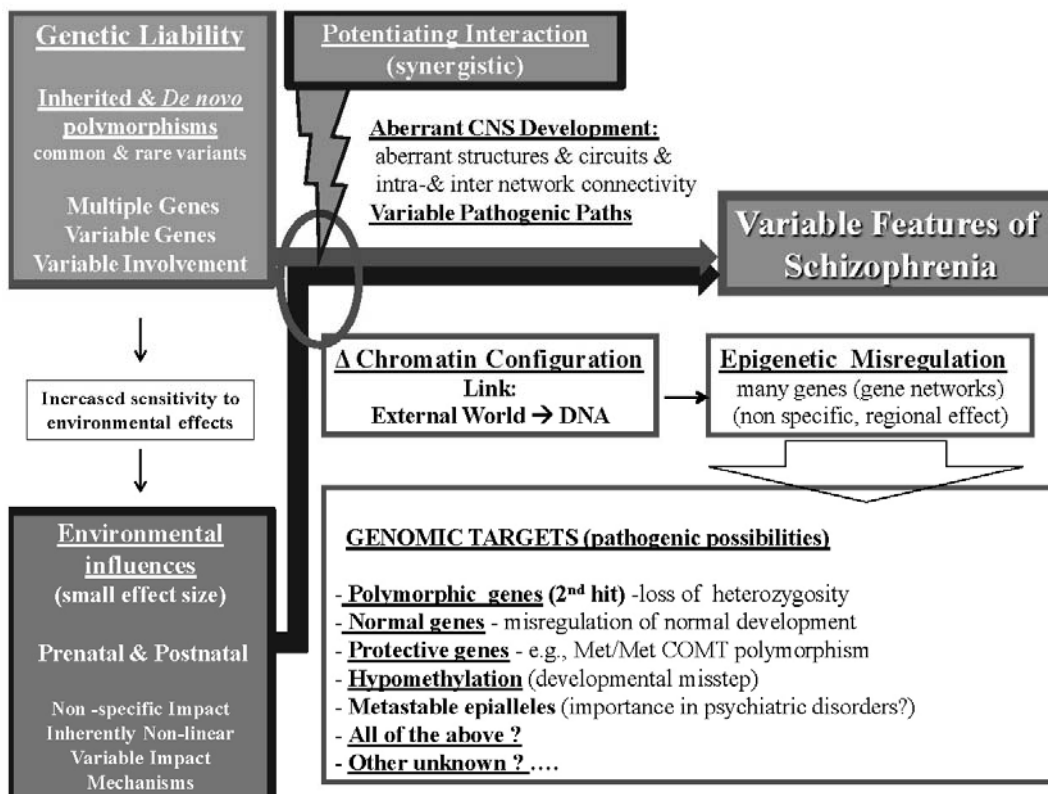


Figure 3. Epigenetic Model of Schizophrenia – simplified schematic presentation

Co-existence of protective factors, such as corrective effects of caring environments (Tienari et al. 2004), or protective genetic variants such as COMT Met/Met alleles (Egan et al. 2001, Meyer-Lindenberg & Weinberger 2006) or protective MET proto oncogene haplotypes (Burdick et al. 2010).

Sensitivity of epigenetic processes active at the time of exposure (e.g., basic neurodevelopment in utero, fine-grain corticogenesis in adolescence).

During the whole process of SZ pathodevelopment, de novo epistatic interactions (protective or pathological), de novo mutations and/or epimutations, immediate early genes, and a number of other internal or external influences, including chance effects, may unfold. All this is likely to further contribute to the variability of pathological mechanisms and phenotypic expressions of SZ. This creates a nightmare scenario for researchers with numerous, optional, and variable interdependent factors interacting in parallel, additively, or in synergy. Hence, we propose that progress in the epigenomics of SZ is likely to require formulation of its development as a misregulated complex adaptive system with nonlinear dynamics (Svrakic et al., in preparation). Figure 3 depicts some of the main components of the epigenetic model of SZ.

NEUROBIOLOGY OF SZ DOES NOT MATCH ITS CLINICAL CLASSIFICATION

Each of the three main groups of SZ symptoms (positive, negative, and cognitive) can develop via a number of different neurobiological pathways. These are all well described in the literature, either at a system level (Lisman et al. 2008, 2010) or as individual findings (review by Keshavan et al. 2008). Different biological pathways leading to SZ symptoms can, in part or in toto, occur simultaneously, as independent or as interdependent processes. For example, reduced telencephalic expression of GABAergic proteins (such as GAD₆₇ and reelin) accounts for a number of structural and functional abnormalities postulated to underlie negative, cognitive, and positive symptoms (review by Lisman et al. 2008). Similarly, down-regulation of NMDA receptors expressed on GABA interneurons produces disinhibition of cortical and hippocampal pyramidal cells which is associated with cognitive and negative symptoms (Farber 2003). In turn, such disinhibition of hippocampal pyramidal neurons can account for increased dopamine in the striatum (Floresco et al. 2001) believed to underlie positive symptoms of psychosis even in normal subjects (Angrist 1994). In animal models of SZ, early postnatal ablation of 40-50% NMDA receptors in mice cortex and hippocampus leads to delayed SZ-like neurobiological abnormalities, such as GAD₆₇ and GABA deficit, loss of synchrony in firing of pyramidal neurons, and a number of behavioral correlates of “mouse SZ” (Belforte et al. 2010). Finally, each of the above biological pathways

may be caused by a primary hyperdopaminergic condition, due to a CNS-wide presynaptic expression of D2 dopamine receptors controlling other CNS networks (Farber 2003). In other words, each of these different biological pathways can independently or in succession or in combination whatever the case may be drive SZ symptoms. However, in research and everyday practice, diagnosis of SZ is based on the predominance of negative and cognitive symptoms for the Residual and Disorganized subtype, predominance of positive symptoms (delusions and hallucinations) for the Paranoid subtype, or on a mixture of all symptoms for the Undifferentiated subtype. Clearly, these clinical diagnoses (subtypes) involve symptoms with many possible underlying biological mechanism which makes them grossly misleading for research and treatment.

The nonlinearity of the ethiopathogenetic process in SZ implies that the final phenotypic outcome cannot be reduced to or predicted by initial conditions. In other words, the non linear pathogenesis of SZ provides the stage for either equifinality – where more than one underlying etiological factor converge to create one clinical subtype, or multifinality - where one etiopathogenic factor and/or aberrant pathway (e.g., GABA deficit) underlies different phenotypic outcomes (e.g., Paranoid, Undifferentiated, and Disorganized SZ). Both equifinality and multifinality are characteristic of multifactorial, complex biological systems such as personality (Svrakic et al. 1996, Cloninger et al. 1997) and other psychiatric disorders such as SZ (Svrakic et al., submitted). In such complex biological systems, phenotypic differences arise when multiple and variable genetic factors interact nonlinearly among themselves and with multiple and variable environmental factors, all with variable timing, duration, and severity. Non linearity of the process also indicates that genetic liability for SZ does not ipso facto mean phenotypic expression of the illness, but rather implies a graded presentation that includes a spectrum from mild to most severe cases (Kety et al. 1994). As we discuss elsewhere (Svrakic et al., in preparation), there is compelling evidence that SZ may be the most severe outcome of a familial polygenic liability for aberrant CNS architecture and function, also called “SZ spectrum” disorders.

SZ AS A NEURODEVELOPMENTAL BRAIN DISORDER

An increasing number of authors conceptualize SZ as a neurodevelopmental disorder, as opposed to a static brain lesion or a failure of brain maturation in its final phases of development (review by Rapoport et al. 2005, Keshavan et al. 2008, Insel 2010). The neurodevelopmental nature of SZ is supported by converging evidence from a number of different perspectives. These range from comparative longitudinal neuroimaging studies of cortical maturation, via task related neurophysiological studies of synchronization of neural oscillations, via population-based studies of prodromal symptoms

before manifest illness, to animal studies demonstrating that delayed manifestations of early CNS lesions are indeed possible (Niwa et al. 2010, Belforte et al. 2010, Insel 2010, review by Rapoport et al. 2005).

It takes time for the initial SZ liability to materialize into aberrant CNS structures, connectivity, and function. Prepubertal humans rarely develop psychosis after exposure to NMDAR antagonists, such as PCP or ketamine (Reich & Silvey 1989). As Farber (2003, p.125) pointed out, NMDAR-hypo state that was created prenatally can remain quiescent throughout childhood until maturational changes in brain circuitry make the brain function more vulnerable to the underlying biological defects which creates the stage for SZ symptoms to begin to appear. In other words, developing CNS is, as it were, “not ready” for SZ because its full functionality is not yet established. Rather, it takes an aberrantly developed CNS to expose and amplify defects in higher cognitive functions, attention, memory, emotions, perception, or unity of conscious perception.

During its typical neurodevelopmental course, the syndrome of SZ unfolds over time in succession, prodromal symptoms first, psychosis last. In fact, four valid phases of SZ – beginning with risk, via prodrome, psychosis, and chronic disability have been established (Yung et al. 2008, Insel 2010 and the references therein). It has been recently shown that progression from prodromal symptoms to manifest SZ in ultra-high risk subjects can be significantly reduced with neuroprotective compounds, such as long chain omega 3 fatty acids (Amminger et al 2010). If replicated, this finding could represent an important advance in preventing late stages of SZ. Additionally, this implicates that prodrome in SZ is not simply a “stage” of the illness, as there is no linear progression to SZ even in ultra-high risk subjects. Rather prodrome is better conceptualized as an emerging, independent risk factor in SZ, creating an interactive triad (genes x brain x environment) susceptible to pathological and protective influences.

As Insel (2010) suggested, neurodevelopmental model may change our concept of SZ, so that first manifestations of psychotic symptoms would be seen not as the onset but as the late stage of this illness, which is likely to have lasting and important implications for research and especially treatment and prevention.

SCHIZOPHRENIA AS A MODULAR BRAIN DISORDER

Human brain is organized into functional networks (also called intrinsic connectivity networks) which mediate perceptual, emotional, motivational, and cognitive information (Fox et al. 2005). Recent scientific and technological advances have molded our understanding of SZ as a modular disorder which involves CNS as a whole, with no distinct, circumscribed defect (i.e., there

is “no disease center”). Pathophysiology of SZ includes impairments in connectivity among distributed and local neuronal assemblies. Using fMRI BOLD method, Zhou et al. (2007) showed that SZ patients manifest a pathologically increased connectivity within the default (no task) network and thus have difficulties switching between default network and task-networks (Zhou et al. 2007). This tendency to be “mentally stuck” in the default mode may account for a number of cognitive, perceptual, emotional, and attention symptoms in SZ. Based on available data from a number of research perspectives (diffusion tensor imaging, fMRI BOLD, electrophysiology, neuropsychology...) SZ reflects a distributed impairment in many cortical and subcortical areas and most likely involves all neurotransmitter systems (GABA, dopamine, glutamate, acetylcholine... and other).

Electrophysiological studies provide further evidence for impaired connectivity in SZ. Neural oscillations (i.e., rhythmic, repetitive neural activity at frequency bands ranging from 1-200 Hz) are a fundamental mechanism for enabling coordinated activity during normal brain functioning (Singer 1999). This oscillatory activity (“oscillating brain”) mediates communication within and between cortical areas and orchestrates collective neural behavior in higher cognitive functions, attention, memory, and integrity of consciousness (Uhlhaas et al. 2008, 2006, Uhlhaas & Singer 2010). Phase of oscillations encodes stimulus properties, while phase synchronization provides a mechanism for integration of collective neural responses (Uhlhaas et al. 2008). Well-synchronized oscillatory activity in cortical theta band (4-7Hz), beta band (13-30Hz) and gamma-band (30-300Hz) emerge during transition from adolescence to adulthood, preceded by a significant reduction of beta- and gamma-bands during late adolescence (transient destabilization before emergence of mature cortical networks) (Uhlhaas et al. 2008). SZ patients consistently show task related abnormalities in phase synchrony in the beta-band (20-25 Hz) as well as delayed onset of phase synchronization of the gamma-band (Uhlhaas et al. 2006, 2008, Uhlhaas & Singer 2010). These findings illustrate not only the widespread connectivity problem in SZ but also its modular nature. Moreover, these findings point to late adolescence as the critical development period during which temporal patterning of brain activity is expected to reach adult levels. A failure of this process could fully expose and even accentuate underlying brain abnormalities in SZ, usually first manifested during late adolescence.

PAST MISCONCEPTIONS AND FUTURE DIRECTIONS

Modern GWAS, designed to pinpoint genetic factors in SZ by examining up to 1 percent of normal and pathological genomes, have failed to identify more than just a small fraction of genetic involvement in SZ. In a

much more optimistic tone, a number of replications of larger genetic structural variants, such as CNVs, in sporadic cases of SZ have finally beginning to emerge. As already noted, there is accumulating evidence for shared genetic liability among major mental disorders, psychotic and non psychotic (Guilmatre et al. 2009, Bornovalova et al. 2010, Kendler et al. 2011). This general inherited liability for CNS dysfunction does not always develop into SZ, or for that matter into any diagnosable psychopathology, blurring the boundaries in comparisons of normal and pathological genotypes. We know about significant genetic contribution to the variability in SZ primarily from population genetics, not from molecular biology, because the former method is sensitive to the overall genetic effect but not to its detailed molecular structure.

There is a general trend in genetic studies to move away from single genes and proteins. The new standard is genome wide studies which involve a variety of genetic and epigenetic features, ranging from common and rare genetic polymorphisms (GWAS studies), via coexpressed gene networks (a map of all active genes at that time in the CNS) or co-expression of gene products (“proteoms”), to studies of genome wide epigenetic patterns, such as the binding patterns of transcription factors (“transcriptomes”) or genome-wide methylation patterns (“methylomes”). Compared to genetic studies of the past, studies using microarray-based genotyping technology are much better suited to detect coregulation and modularity among large number of genes in complex disorders such as SZ. Several data sets that unify the representation of genes and gene products across species (such as Gene Ontology Enrichment Analysis or Ingenuity Systems Pathways Analysis) are now used to cross reference neurophysiological functions encoded by gene networks, making it possible to better understand pathophysiological mechanisms involved in SZ. Torkamani et al. (2010) study is a good example that these new research designs and strategies can provide refreshingly new insights into genetic mechanisms involved in SZ, including longitudinal patterns of gene expression and activity. In particular, Torkamani et al. (2010) found a pathological continuation of gene activity (instead of normal age-related downregulation of neurodevelopmental genes) for a number of neurodevelopmental and transmitter related genes in SZ. The mechanism underlying this continuing genomic activity is not clear (homeostatic vs. epigenetic), and could conceivably include hypomethylation of gene promoters.

Having in mind that gene expression is governed not only by allelic specificity, but also by its epigenetic status, non coding RNAs, interactions with gene products co-involved in the particular process, etc., proper study designs should involve simultaneous analyses of all these effects. Thus, simultaneous genomic, methylomic, transcriptomic, and neuroproteomic data should be a complete package that can reveal all sequential phases of molecular processes involved in the pathogenesis of SZ and related mental disorders.

“Enriched” samples, i.e., studies of high risk populations in high risk environments are expected to amplify underlying etiological factors and thereby demonstrate more robust effects. Such studies are optimally done using unbiased approach to data analysis, another new trend in psychiatric research, which means that analyses are not driven or influenced by a particular biological or phenotypic hypothesis. Instead, biological and phenotypic data are analyzed separately and patterns of their interaction established at the end.

“Enviromics” is a new initiative to match specific environments, environmental processes, or environmental conditions with their specific effects (pathogenic, protective, and beneficial) on different levels of biological organization (e.g., growth, reproduction, and survival), including mental health. Basically, the idea is to match specific environmental factors with their complementary gene networks and protein systems. As a parallel to the Human Genome Project, Anthony (1995, 2001) suggested “Psychiatric Envirome Project” or mass-screening of environmental risk factors for psychiatric epidemiology that seeks the total collection of environments that either affect the occurrence or the course of mental disorders - independently, in combination with, or in addition to the influence of genes and their proteins. A massive task, but certainly with potential benefits if (when) completed.

Animal models have been useful in figuring out crude, basic mechanisms underlying neurodevelopment, but not as much in deciphering uniquely human pathology involved in SZ, such as prefrontal neuroanatomy and the executive function deficits (Insel 2010). Instead, as Insel (2010) suggests, “model animals” may be a much more productive strategy. Here, an animal is “implanted” with a hypothesized biological factor (e.g., a CNV), perhaps exposed to a variety of high-risk environments, and monitored for developmental structural and functional consequences.

One of the most challenging tasks in the future will be to solve the problem of tissue and cell specificity of epigenetic CNS changes. Indirect (peripheral) assessment of CNS epigenetic changes has been attempted using peripheral mononuclear blood cells with some promising results (Gavin et al. 2009). Obviously, if epigenetic misregulation during early developmental stages is related to neurodevelopment, it might be detectable in blood cells (Kato 2008). The concern is that these peripheral correlates provide only a rough approximation of CNS changes below the level of detail needed to decipher the complex etiological puzzle SZ. At present, postmortem CNS tissue is used most frequently, with all the problems inherent in this method (review by Pidsley & Mill 2011). Brain biopsy is not done for this purpose for obvious ethical reasons. One possibility is to study CNS tissue obtained from SZ patients with comorbid temporal lobe epilepsy who are surgically treated for epilepsy, as this would provide tissue from temporal cortical areas of interest in SZ.

Such cases are not very common, but even if they were, the problem could be chronic antiepileptic and antipsychotic medication (e.g., valproate, clozapine) as well as repeated seizures, as all have significant epigenetic effects. Finally, recent research using fibroblasts which are converted into Induced Pluripotent Stem Cells and then grown into neurons and glia, has enabled prediction of phenotypes based on the irregularities observed in vitro developed neural tissue.

With few exceptions, the above advances in biological research are not matched by improved definitions of clinical phenotypes, which are, at present, the weakest link in psychiatric research. The next step is more reliable classifications of phenotypes, most likely based on endophenotypes or intermediate phenotypes. Both are closer to the underlying genetic or neurophysiological mechanisms respectively (Munafò et al. 2008) and thus likely to provide a more valid classification of phenotypes. There are numerous candidate intermediate phenotypes and endophenotypes in the literature, ranging from neurophysiology (e.g., prepulse inhibition, mismatch negativity, abnormal phase synchronicity in beta- and gamma- oscillations, etc), molecular biology (e.g., COMT polymorphism, etc), to neuropsychological tests (e.g., working memory tests, etc). For critical discussion and review we refer the reader to Meyer-Lindenberg & Weinberger (2006), Meyer-Lindenberg (2010), Keshavan et al. (2008) and Stober et al. (2009).

CONCLUSION

SZ is a complex multifactorial disorder with highly variable course and clinical expression. The complexity of SZ is indicated by the dynamic interplay among many risk and protective factors that influence its evolution and expression. Environmental factors are integral in SZ pathogenesis. One of the recognized molecular mechanisms to mediate various types of environmental influences is epigenetic modulation of gene activity. We propose that progress in the epigenomics of SZ is likely to require formulation of its development as a misregulated complex adaptive system with nonlinear dynamics (Svrakic et al., in preparation).

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