Review

Biological Psychiatry

Schizophrenia as a Disorder of Molecular Pathways

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ABSTRACT

Over the last decade, transcriptome studies of postmortem tissue from subjects with schizophrenia revealed that synaptic, mitochondrial, immune system, gamma-aminobutyric acidergic, and oligodendrocytic changes are all integral parts of the disease process. The combined genetic and transcriptomic studies argue that the molecular underpinnings of the disease are even more varied than the symptomatic diversity of schizophrenia. Ultimately, to decipher the pathophysiology of human disorders in general, we will need to understand the function of hundreds of genes and regulatory elements in our genome and the consequences of their overexpression and reduced expression in a developmental context. Furthermore, integration of knowledge from various data sources remains a monumental challenge that has to be systematically addressed in the upcoming decades. In the end, our success in interpreting the molecular changes in schizophrenia will depend on our ability to understand the biology using innovative ideas and cannot depend on the hope of developing novel, more powerful technologies.

Keywords: Convergence, Gene Expression, Gene Network, Genetics, Postmortem, Schizophrenia

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Gene expression changes in the postmortem brains of subjects with schizophrenia have been studied for many decades. These studies initially included low throughput methodologies, encompassing northern blotting and in situ hybridization and were expanded more recently to quantitative polymerase chain reaction, DNA microarrays, and RNA sequencing (RNA-seq) (1). Over the last half century, a tremendous amount of data have been generated, yet our understanding of gene expression changes in schizophrenia is still limited. Reasons for this incomplete knowledge are complex and include both disease-related factors and technical limitation. Schizophrenia is a spectrum disorder rather than a single diagnosis (2) and the etiology of the disease is complex, encompassing both environmental and genetic factors (3). Substance abuse is quite common in the patient population (4), and various comorbidities and lifestyle differences also have a strong effect on the findings. The picture is further complicated by limited availability of postmortem material, postmortem interval, medication history, circumstances of death, and the disease progression between disease onset and the brain harvest (5). In addition, considering the potential cohort biases and very small sample sizes, differences in experimental methodology, and the diverse data analytical methods used, it is perhaps not surprising that transcriptome findings often do not replicate across the investigated cohorts (6).

Still, while most of the single-gene expression changes in schizophrenia have poor reproducibility across studies, data-driven approaches have been able to provide us with a more reproducible list of gene expression network disruptions that are related to schizophrenia. While the cascade of causality remains uncertain, it appears that synaptic (7,8), mitochondrial (9,10), immune system (6,11), gamma-aminobutyric acid (GABA)ergic (12,13), and oligodendrocytic (14,15) messenger RNA (mRNA) changes are all integral parts of the disease process (1,16). However, it is important to point out that not all patients show deficits in all molecular domains—there is a clear molecular substratification of patients (6,11)—and that synaptic, immune, oligodendrocytic, GABAergic, or other, etiologically diverse processes might give rise to the same behavioral disturbance. Thus, schizophrenia is not a disease of a single molecular pathway—rather, transcriptome changes argue for the existence of predominantly synaptic, oligodendrocytic, and multiple other molecular subtypes of schizophrenia (17) that sort along a continuum in a complex, partially overlapping pattern: each subject with schizophrenia might show a dominant deficit in one of the molecular domains, yet the overall molecular deficit might also encompass elements from other molecular pathways.

The current review is focused on mRNA changes; however, it is clear that other, noncoding RNA species also play a critical role in regulating gene expression and appear to significantly contribute to the disease process of schizophrenia (18,19).

SMALL SIGNALS IN GENETICS VERSUS STRONG SIGNALS IN TRANSCRIPTOME

Postmortem gene expression studies are typically performed on dozens of brains, while genome-wide association studies (GWAS) include thousands of patient samples. To date, GWAS identified a number of genetic elements that predispose to schizophrenia (20–23). It appears that two different but

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interrelated mechanisms are at work: common alleles conferring small, cumulative risk to the disease through single nucleotide polymorphisms (SNPs) and low-frequency large effect structural chromosomal abnormalities known as copy-number variants (CNVs). Yet, common SNPs with relatively small effect sizes that can only partially explain the strong heritability of the disease (20–24) and defining a CNV as causal to the disease is even more challenging. In contrast, postmortem gene expression studies reveal much stronger disease-associated signals: even with small sample sizes, there are well-replicated gene expression disturbances that are present in a significant subpopulation of subjects with schizophrenia. For example, glutamate decarboxylase 1 (*GAD1*)/glutamate decarboxylase 67 (GAD67) underexpression appears to be a hallmark of the illness (25), and present in the majority of the subjects with the disease—yet, this cannot be explained by genetic susceptibility in the *GAD1* gene itself—lifestyle, medication history, or other confounds. Similarly, immune system disturbances can be identified in >20% of the postmortem brains of diseased subjects (6,11,26), but these changes cannot be traced back to a specific genetic predisposition. Thus, this strongly suggests that gene expression changes are a cumulative readout of different genetic susceptibilities and environmental insults, which converge onto common molecular (and ultimately functional cellular) pathways (5,27).

Expression quantitative trait loci (eQTL) studies of schizophrenia also support the notion of common, converging expression readout of genetic vulnerabilities. Expression quantitative trait loci are genomic loci that regulate expression levels of mRNAs or proteins. In the context of schizophrenia, one would predict that schizophrenia susceptibility alleles are enriched for those that can affect gene expression and that eQTLs should carry more true association signals. Recent studies provide strong support for this prediction: schizophrenia susceptibility alleles are enriched for SNPs that affect gene expression in adult human brain. Furthermore, higher probability eQTLs predict schizophrenia better than those with a lower probability for being an eQTL (21,28).

Further evidence for genome-transcriptome convergence comes from an interesting relationship between genetic susceptibility found in patient DNA and gene expression changes seen in postmortem brains of subjects. The vast majority of the schizophrenia susceptibility genes also show altered transcript expression in the postmortem brain, even in subjects that do not harbor these particular disease-predisposing variants (5). This is true for both the previously identified candidate gene studies and the GWAS uncovered susceptibility genes. For example, the major histocompatibility complex locus confers genetic susceptibility to schizophrenia (29), and transcripts originating from this cytogenetic region also show dysregulation in postmortem transcriptome studies (30,31)—even in the brains with no apparent genetic susceptibility in the same locus. Similarly, risk-associated SNP of the alpha-1C subunit of the L-type voltage-gated calcium channel shows mRNA expression changes in the brain of subjects with schizophrenia (32), and a similar relationship is observed for transcription factor 4 (33) and possibly other GWAS-uncovered schizophrenia-predisposing genes. This can be explained by the above-discussed pathway view of schizophrenia: there might be various, patient-specific disease-predisposing genetic elements in the pathways upstream of the changed transcript. Yet, the different genetic susceptibilities are likely to give rise to a common expression change at points of molecular convergences.

ENVIRONMENTAL INFLUENCES AND GENETIC VULNERABILITY CONVERGE ON THE TRANSCRIPTOME

As mentioned above, genetic susceptibility can be strongly potentiated by environmental factors. Increased incidence of schizophrenia has been associated with urban lifestyle, prenatal infections, malnutrition, adolescent cannabis abuse, perinatal hypoxia, and other factors (34). These adverse events act in concert with genetic predisposition, and the transcriptome changes represent a sum of gene × environment interactions that jointly tip the balance of the transcriptome. Ultimately, the transcriptome changes will affect growth, axonal pathfinding, neuronal arborization, synapse formation and pruning, energy metabolism, and many other developmental and cellular processes. Once the compensatory mechanisms are exceeded, these changes manifest themselves as a specific behavioral phenotype, which we define as the symptoms of the disease.

Many human and transgenic mouse studies demonstrate this very eloquently. However, while human evidence comes from epidemiologic studies (34) and can be considered only indirect proof, the animal studies provide clear evidence that the effect of putative schizophrenia susceptibility genes is greatly influenced by environment. *Disc1* mutant mice, when exposed to early immune activation or social paradigms, show more pronounced behavioral and molecular deficits not observed in the unchallenged mutants (35). In addition, a mild isolation stress affects the mesocortical projection of dopaminergic neurons, but only when combined with a relevant genetic risk for neuropsychiatric disorders (36). Furthermore, *Ifitm3*$^{-/-}$ mice do not develop the characteristic cellular-molecular-behavioral phenotype after maternal immune activation (e.g., impaired neurite outgrowth and dendritic spine formation, diminished microtubule-associated protein 2 expression, altered object recognition, and exploratory behavior), underscoring that the gene × environment interaction can act both as detrimental and protective factors (37).

It is important to note that the environmental modification of genetic disease predisposition is not only related to schizophrenia but appears to be a universal theme across many neurological and psychiatric disorders. For example, when coupled with adverse life experiences, individuals with one or two copies of the short allele of the serotonin transporter promoter polymorphism show more depressive symptoms, diagnosable depression, and suicidality than individuals homozygous for the long allele (38,39). In contrast, Alzheimer's disease mutant mice models, when exposed to enriched environment, show significantly reduced amyloid deposition in the brain and remarkable sparing of cognitive functions (40).

ENVIRONMENT PREDISPOSES, GENETIC SUSCEPTIBILITY SPECIFIES DISEASE

It is well established that environmental influences protect or predispose to disease (34). Yet, environmental factors appear