CHAPTER

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The Neurochemistry of Schizophrenia

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OUTLINE

CLINICAL ASPECTS OF SCHIZOPHRENIA

Schizophrenia is a severe, chronic disabling mental disorder

Schizophrenia affects approximately 1% of the population worldwide (Lewis & Lieberman, 2002). The disease has its age of symptomatic onset in late adolescence and early adulthood in males and somewhat later in females, who tend to be less severely affected. Since schizophrenia starts in young adulthood and is typically a lifelong disorder, it produces a substantial degree of persistent disability. Schizophrenia is ranked among the top 10 causes of disability-adjusted life years and reduces life expectancy by 10 years, with death often as a result of suicide.

The conceptualization of schizophrenia as a specific disease occurred at the turn of the last century. The German psychiatrist Emil Kraepelin identified a group of psychotic patients characterized by the onset of symptoms in early adulthood, impaired cognition and poor outcomes, whose condition he labeled *dementia praecox*. The term *schizophrenia* was introduced a decade later by Eugen Bleuler, who characterized the splitting ("schizo") of affect (i.e., emotional tone) from cognition. He further identified four characteristic symptoms: autism, ambivalence, blunted affect and disturbances of volition. While these early clinicians readily accepted that schizophrenia was a brain disease with heritable vulnerabilities, this biological conceptualization of the disease was eclipsed by the rise of psychoanalysis in the 1930s and the dominance for the next 50 years of its theoretical conceptualization that intra-psychic conflicts are the cause of psychiatric disorders. Thus, until recently, many clinicians viewed schizophrenia as a consequence of pathologic maternal–child interactions and deviant family communication.

This chapter will review the compelling evidence that schizophrenia is a brain disease with a high degree of heritability. It will draw upon four major research strategies that have transformed our understanding of this disease,

including neuropsychopharmacology, brain imaging, genetics and postmortem studies of the brains of individuals affected with schizophrenia. Over the last decade, the convergence of findings from these different approaches is pointing to plausible final common pathways accounting for the pathophysiology of schizophrenia.

Schizophrenia is characterized by three independent symptom clusters

These symptom clusters are designated *positive symptoms*, *negative symptoms* and *cognitive impairments* (Liddle et al., 1989). The positive symptoms are the most dramatic and are a manifestation of psychosis. They include hallucinations in which the patient has sensory experiences in the absence of external stimuli. Hallucinations are typically auditory, but can be visual, tactile, gustatory or olfactory. A patient can also experience delusions, which are fixed, false beliefs that remain intact even in the face of contradictory external evidence. Delusions can range from the mundane (e.g., "my family has placed cameras in my house to watch me") to the bizarre (e.g., "aliens control my thoughts using a chip implanted in my brain"). Cognitive processes can become quite disorganized, resulting in loose associations with tangential thinking, illogical reasoning and emotional states inconsistent with thought content.

The positive symptoms are the most responsive to antipsychotic medications such as haloperidol and olanzapine. Initially, these drugs were thought to be specific for schizophrenia. However, psychosis is not unique to schizophrenia and frequently occurs in bipolar disorder and in severe major depressive disorder, in which paranoid delusions and auditory hallucinations are not uncommon. Furthermore, in spite of early hopes based on the efficacy of antipsychotic drugs in treating the positive symptoms, few patients are restored to their previous level of function with the typical antipsychotic medications (Lewis & Lieberman, 2002).

The more enduring and disabling components of the disease, which remain after effective treatment of the positive symptoms, are the negative symptoms and cognitive impairments. The term "negative" refers to a loss or deficit and includes decreased emotional reactivity, a paucity of speech, loss of motivation, and an inability to experience pleasure. The cognitive impairments, which are associated with a modest reduction in IQ, disrupt attention, problem-solving abilities, verbal declarative memory, and verbal fluency and cause delayed word recall. While clearly disabling, the cognitive deficits do not achieve the severity associated with dementia or delirium. It is not the group of positive symptoms, but cognitive impairment along with negative symptoms, which are the best predictors of functional outcome (Green, 1996).

Retrospective and prospective studies of children who are at genetic high risk for schizophrenia, indicate that subtle abnormalities in cognitive function and attention, social oddness and motor clumsiness are often present years before the onset of psychosis (Cornblatt et al., 2003). These observations on the developmental aspects of schizophrenia, as well as the enduring nature of the negative symptoms and cognitive impairments over the lifetime of the schizophrenic patient, have led to the conceptualization of these symptoms as the *endophenotype* of schizophrenia—i.e., the core symptomatic features that are observed in first-degree relatives. The variation in symptomatic features of schizophrenia across individuals and the common nature of the disorder strongly suggest that there will likely be multiple causes that result in the phenotype.

Schizophrenia is a disorder of complex genetics

Adoption, twin and family studies carried out over the last 50 years have provided compelling evidence for the heritability of schizophrenia (Tiwari et al., 2010). Meta-analyses of the extant twin studies indicate a concordance rate of approximately 50 percent in identical twins if one twin is affected, which is five-fold greater than the concordance rate in fraternal twins or siblings. The absence of complete concordance in identical twins points to epigenetic factors, which can transform genotype to phenotype. Perinatal insults such as second-trimester viral infection and anoxia at birth figure prominently as environmental factors enhancing the risk for schizophrenia. Factors in the family environment such as communicational deviancy are unlikely to contribute to risk, because adoption studies indicate that the risk for schizophrenia in offspring of schizophrenics (i.e., first-degree relatives) is unchanged by a subject being adopted into a family without schizophrenia. Nevertheless, adverse family environments characterized by high levels of expressed emotions negatively affect the course of schizophrenia for those who are already symptomatic.

Schizophrenia does not follow a Mendelian inheritance pattern and cannot be attributed to a single gene or genetic abnormality. The pattern of distribution of schizophrenia within affected families, with a rapid tailing off of concordance in identical twins (approximately 60%) versus fraternal twins and first-degree relatives (10%) versus second-degree relatives (3%), is consistent with complex genetics, in which many genes of small effect interact to contribute to the phenotype. Association studies carried out with putative risk genes have yielded a number of enticing leads, implicating genes involved with brain development, neurotransmission and myelination. However, genome-wide association studies that are "agnostic" have not yet confirmed most of these risk genes. Such studies, however, have revealed an excess of copy number variants associated with an increased risk for schizophrenia involving chromosomes 1q, 2p, 15q, and 22q. A copy number variant occurs when the number of copies of a DNA region differs in the probands relative to the controls.

Current treatment of schizophrenia relies on atypical antipsychotic drugs

The antipsychotic efficacy of chlorpromazine for the treatment of schizophrenia was discovered serendipitously by Laborit, Delay and Denicker in France in 1953 after the observation of the peculiar calming and poikilothermic effects of chlorpromazine in experimental animals (Deniker, 1978). In the early 1960s, placebo-controlled, double-blind studies demonstrated that chlorpromazine was superior to placebo and to the sedative phenobarbital in reducing

psychosis in schizophrenic patients. A number of other effective antipsychotic drugs were subsequently discovered over the next 30 years on the basis of animal behavioral screens, which we now know selected for the ability of antipsychotics to block dopamine D2 receptors, such as their ability to prevent apomorphine-induced vomiting in dogs and amphetamine-induced hyperactivity in rodents. Fifty years ago Nobel laureate Arvid Carlsson proposed that antipsychotic drugs exert their therapeutic effects by blocking dopamine receptors, based on the observations that they cause Parkinsonian side effects, which were known to be associated with loss of striatal dopamine, and that they increased the turnover of dopamine in the striatum (Carlsson, 2001).

Fifteen years later, following the development of radioligand binding assays, the dopamine D2 receptor was identified by the specific, high-affinity binding of [3H]haloperidol to brain membranes that displayed the requisite regional and pharmacologic characteristics for a dopamine receptor distinct from the dopamine receptor associated with adenylyl cyclase activity (D1 receptor) (Seeman & Van Tol, 1994). Furthermore, a compelling correlation between the affinity of antipsychotic drugs, regardless of structural class, for the D2 receptor and their clinical potency was demonstrated. Abuse of stimulants like amphetamine, which releases dopamine, was known to cause psychotic symptoms, especially paranoid delusions, similar to those observed in schizophrenia. Given the antipsychotic effects of these D2 receptor–blocking drugs in schizophrenia, the *dopamine hypothesis* was proposed, according to which schizophrenia was caused by excessive dopaminergic neurotransmission (Snyder, 1976).

The behavioral symptoms that accompany psychosis, such as agitation or profound withdrawal, respond to antipsychotic drugs within a period of hours to days after the initiation of treatment. The cognitive symptoms of psychosis such as the delusions and hallucinations, however, tend to resolve more slowly. In fact, for many patients, the hallucinations and delusions may persist but lose their emotional salience and intrusiveness. The positive symptoms tend to wax and wane over time, are exacerbated by stress, and generally become less prominent as the patient ages. Among the commonly used typical antipsychotic drugs are chlorpromazine, haloperidol, fluphenazine, molindone and thioridazine. In spite of their structural heterogeneity, there is no evidence of differential efficacy in the treatment of psychosis, although they do differ in their clinical potency and in their side effect profiles.

A major limitation of the typical antipsychotic drugs is the high risk of extrapyramidal side effects due to a blockade of striatal dopamine D2 receptors (Coyle, 1982). Manifestations of these side effects include Parkinson's syndrome characterized by bradykinesia, tremor and rigidity. The associated mask-like face and "zombie-like" gait are stigmatizing for patients. Younger patients are particularly prone to the development of dystonic reactions with cramping of tongue, neck or back muscles causing grotesque posturing. Akathisia causes a sense of inner tension that is relieved only by movement (i.e., pacing). As akathisia is quite uncomfortable, it is a major cause of noncompliance in taking antipsychotics. Finally, with long-term use of the antipsychotics, a relatively irreversible neurologic syndrome known as *tardive dyskinesia*, can occur, which is characterized by writhing movements of the tongue and choreiform movements of the extremities that persist long after the discontinuation of the antipsychotic drugs.

Additional side effects reflecting the various degrees of muscarinic receptor antagonist actions of the antipsychotic drugs result in dry mouth and constipation. Alpha-adrenergic receptor antagonism is associated with hypotension, tachycardia and impotence. Histamine H1 antagonism contributes to sedation. Needless to say, this panoply of side effects has been associated with serious problems in compliance with taking medications, resulting in their discontinuation and recurrence of psychosis.

Because of the problems with compliance and stigma, neuropharmacologists have long sought antipsychotic drugs that are devoid of neurologic side effects. The first such agent was clozapine (Meltzer, 2004). It does not produce catalepsy in rodents, nor does it cause acute extrapyramidal side effects or tardive dyskinesia in humans.

Furthermore, low doses of clozapine have been used effectively in Parkinson's disease to treat psychotic symptoms induced by dopamine replacement therapy without exacerbating the underlying neurologic symptoms. The clinical use of clozapine has been limited because of a 1% risk for a fatal hematologic complication known as *agranulocytosis*. To avoid this complication, patients receiving clozapine are subject to regular hematologic studies to detect early signs of it. Clozapine has been found to be particularly effective in treating a subgroup of schizophrenic patients who respond poorly to typical antipsychotic drugs, because it reduces negative symptoms, improves cognition, and is associated with a marked reduction in suicide (the lifetime risk for suicide in schizophrenia is 10%).

Deciphering the mechanism of action of clozapine has been difficult because clozapine and its metabolites interact with several neurotransmitter receptors aside from the D2 receptor, including the dopamine D4 receptor, which is predominantly expressed in corticolimbic regions, muscarinic receptors, alpha-adrenergic receptors, histamine receptors and the serotonin 5-HT2A receptor. Since blockade of the 5-HT2A receptor appears to mitigate against extrapyramidal symptoms resulting from D2 receptor blockade, a number of effective antipsychotic drugs have subsequently been developed that have dual D2 and 5-HT2A receptor antagonism. These include risperidone, olanzapine, quetiapine and ziprasidone. Since these newer agents have a substantially lower propensity for causing extrapyramidal symptoms, they have been designated "atypical" antipsychotic drugs. These "second-generation" or "atypical" antipsychotic drugs have largely replaced the typical antipsychotic drugs in the management of schizophrenia. Nevertheless, the atypical antipsychotics may cause substantial weight gain, hyperlipidemia and type II diabetes, a new category of medically serious side effects. Recent efficacy studies in real-world clinical settings indicate that the newer atypical antipsychotic drugs exhibit negligible clinical superiority over the older typical antipsychotic medications and that both groups fail to improve the negative symptoms and cognitive impairments that account for the persistent disability of schizophrenia (Lewis & Lieberman, 2008). Thus, the current challenge is to develop drugs that address negative symptoms and cognitive impairments in schizophrenia.

BRAIN IMAGING

Brain imaging studies provide unequivocal evidence that schizophrenia is a brain disease

Brain imaging exploits three different strategies: morphometric analysis based on computer assisted tomography (CAT) or magnetic resonance imaging (MRI) to reveal brain structure; functional brain imaging, which monitors blood flow or glucose utilization through Positron Emission Tomography (PET) or functional (f) MRI; and measurement of brain neurochemical features by means of PET ligand-binding studies or magnetic resonance spectroscopy (MRS), which can quantify chemicals of high concentration in the brain.

The first report of cortical volume loss in schizophrenia is credited to Alois Alzheimer, who is most noteworthy for his clinical and neuropathologic descriptions of the primary dementia that bears his name. However, the unequivocal establishment of the veracity of his initial observations awaited the application of CAT scanning and quantitative morphometric MRI nearly 100 years later. Initial findings to support this inference came from CAT scans documenting an increase in the size of the lateral ventricles in schizophrenia. However, more recent MRI studies that exploit sophisticated statistical analyses demonstrate unequivocally reduced volume of the cerebral cortex, which reduction is unevenly distributed and affects predominantly the frontal cortex and temporal lobe (Kuperberg et al., 2003). Segmentation studies indicate that these effects are borne predominantly by the gray matter, although some reductions in white matter volume have been demonstrated. Magnetic tensor imaging studies also indicate disruption of axon terminals in the cerebral cortex in schizophrenia.

The reduction in cortical volume observed in schizophrenia is substantially less than that found in the adult-onset primary dementias such as Alzheimer's disease and Huntington's disease. A subject of controversy has been whether the reduction in cortical volume in schizophrenia is the result of cortical hypoplasia or a progressive atrophy. Studies of patients in the earliest stages of schizophrenia do indicate cortical volume reduction as well as functional abnormalities. Nevertheless, prospective studies have revealed increases in ventricular volume, progressive loss in cortical volume and progressive impairments in cortical function in a substantial portion of, but not all, subjects with schizophrenia (Puri, 2010). Notably, the degree of cortical volume reduction correlates with the severity of cognitive impairments and negative symptoms in patients suffering from chronic schizophrenia. A longitudinal study provides convincing evidence that antipsychotic treatment contributes to cortical atrophy.

Magnetic resonance spectroscopy (MRS) has demonstrated significant persistent neurochemical abnormalities in the brains of individuals with schizophrenia (Dager et al., 2008). N-acetylaspartate (NAA), an amino acid of poorly understood function, is concentrated in neurons, including their somata, axons and dendrites. NAA appears to be a marker of neuronal functional status. A number of studies have demonstrated small but highly significant reductions in the levels of NAA in the prefrontal cortex, temporal lobe and hippocampal formation in patients with schizophrenia. Prefrontal cortical reductions in NAA correlate significantly with symptom severity. Furthermore, NAA deficits in the hippocampal formation and in the dorsolateral prefrontal cortex correlate with working memory impairments. In contrast, the level of glutamine, a precursor to the neurotransmitter glutamate, has been reported to be elevated in the anterior cingulate cortex and in the thalamus in patients with schizophrenia who have never been treated (Kristiansen et al., 2007). Phosphorus MRS studies have consistently shown membrane phospholipid abnormalities in individuals with schizophrenia (Smesny et al., 2007). For example, glycerophosphoethanolamine has been found to be decreased in the anterior cingulate, prefrontal cortex and thalamus in schizophrenia. There have been consistent reports of a decrease in phosphomonoester and an increase in phosphodiester in prefrontal cortex.

Functional imaging studies have consistently shown corticolimbic abnormalities in schizophrenia

Two noninvasive brain-imaging techniques have been used to understand neural function in schizophrenia: surface electroencephalographic (EEG) recordings such as event-related potentials (ERP) and brain hemodynamic activity as measured by $^{15}O_2$, regional cerebral blood flow measured with PET, or blood oxygen level desaturation (BOLD) measured with fMRI. The EEG allows real-time measures of neural activity with millisecond (msec) temporal resolution. When coupled with the repeated presentation of a visual stimulus, the electroencephalograms can be averaged to produce an ERP whose components develop and resolve within tens or hundreds of msec. These potentials are designated by the msec lag between presentation of the stimulus and the appearance of the ERP with the P50 (i.e., potential at 50 msec after the stimulus) and P300 being the subject of most study of schizophrenia.

The P300 ERP is smaller in amplitude and longer in latency in patients with schizophrenia as compared to controls (Thaker, 2008). The latency is increased with the duration of disease, although the P300 amplitude is unaffected by the severity of symptoms or administration of antipsychotic medications. The P50 is a measure of sensory gating. The subjects receive two brief auditory stimuli separated by 400 msec. A negative wave at 50 msec after the second stimulus is suppressed in control subjects; this is not the case in subjects with schizophrenia. An allelic variant in the promoter region of the $\alpha 7$ nicotinic receptor gene (*CHRNA7*), which has been linked to schizophrenia, is associated with abnormal P50 potential (Leonard et al., 2002). Notably, the atypical antipsychotic clozapine corrects this abnormality in schizophrenic patients, whereas other typical and atypical antipsychotic drugs do not. In a related but auditory task, pre-pulse inhibition (PPI), the subject is given a warning low-volume tone followed by a loud noise, which causes a startle response. After a few presentations, normal subjects recognize the low-volume warning tone and suppress the startle response, whereas schizophrenics fail to suppress the startle response.

Early functional brain imaging studies performed in subjects at rest suggested that blood flow is reduced

somewhat in the frontal cortex in patients with schizophrenia. Moreover, when subjects with schizophrenia are compared to normal controls while performing cognitive tasks that require the engagement of the frontal cortex, such as the Wisconsin Card Sort Task, robust differences between normal controls and the patients are observed (Weinberger et al., 1986). Similarly, when patients with schizophrenia are compared to controls on a difficult memory recall task, patients perform more poorly than the controls and exhibit little increase in blood flow to the hippocampus (Heckers, 2001). Measurement of the absolute blood flow to the hippocampus indicates a significantly elevated blood flow at rest in the schizophrenic subjects, suggesting that the failure of the task to activate blood flow reflects a "ceiling effect." Functional imaging during the performance of cognitive tasks such as word recognition demonstrates regions of reduced blood flow in schizophrenic subjects as compared to controls: these are the dorsolateral prefrontal cortex and paralimbic regions. However, in schizophrenic patients compared to normal subjects, there are also areas of increased blood flow, such as the anterior prefrontal cortices, indicating that schizophrenic subjects engage alternative neuronal systems in their attempt to perform such tasks.

One of the most prominent positive symptoms in schizophrenia is the auditory hallucination that is perceived as distinct voices emanating from outside the individual. Regional cerebral blood flow studies in patients experiencing auditory hallucinations reveal activation of the associational auditory cortex during the episodes of hallucinations but not in their absence (Jardri et al., 2011). One theory holds that auditory hallucinations occur as a consequence of the inability of individuals with schizophrenia to monitor their inner speech effectively. fMRI studies suggest that this monitoring function requires activation of the temporal, parietal and parahippocampal cortices. Schizophrenic subjects exhibit much lower activation of these regions when generating inner speech.

PET studies are helpful in measuring trace markers such as receptors and transporters in the brains of living subjects (Howes et al., 2009). Because antipsychotic drugs all block dopamine D2 receptors, the status of the forebrain dopaminergic system has been of particular interest with regard to schizophrenia research. Quantifying dopamine transporters (DAT), a marker thought to be an index of dopaminergic terminal density, with [^{123}I]β-labeled N-delta-(fluoropropyl)-2 beta-carbomethoxy-3beta-(4-iodophenyl)tropene has revealed no apparent difference in DAT density in schizophrenic subjects as compared to controls. Most evidence indicates no difference in the density of D2 receptors in the striatum in schizophrenic subjects as compared to controls in PET-scan studies. Investigators have exploited the PET ligand [^{11}C] raclopride, which has high specificity but low affinity for the D2 receptor, to monitor endogenous dopamine release through DA displacement of the ligand from the D2 receptor (Howes et al., 2009). Challenge with D-amphetamine, which releases endogenous dopamine, causes a greater displacement of [^{11}C]raclopride in schizophrenic subjects than controls, thus indicating greater DA release from dopaminergic terminals in the schizophrenic subjects. Consistent with this finding, several studies using [^{18}F] or [^{11}C]DOPA have documented an increased incorporation of the dopamine precursor in the striatum in schizophrenic subjects as compared to controls, indicating increased dopamine release in schizophrenic subjects. Hypofunction of the NMDA subtype of glutamate receptors has also been hypothesized as a core feature of schizophrenia. Studies of striatal D2 receptor occupancy with [^{11}C]raclopride show that normal subjects receiving the NMDA receptor antagonist ketamine exhibit a more robust release of dopamine with an amphetamine challenge than do ketamine-naïve subjects, replicating the findings from schizophrenic subjects.

A recent modification of the dopamine hypothesis proposes that dopaminergic neurotransmission is impaired in the prefrontal cortex although elevated in subcortical regions. Studies with ligands for the dopamine D1 receptor have revealed an increase in the density of these receptors in the frontal cortex, with the increase correlating with the severity of negative symptoms.

In summary, brain-imaging studies have provided compelling evidence of structural, neurochemical and functional abnormalities in the cerebral cortex, limbic system and thalamus in patients suffering from schizophrenia. The structural abnormalities, primarily loss of gray matter in discrete cortical regions and in the thalamus, correlate with the impairments in the cognitive functions associated with these regions. Finally, these cognitive impairments correlate with regionally specific abnormalities in neural processing. These findings provide a solid foundation for inferring neurochemical abnormalities in the brains of individuals with schizophrenia.

CELLULAR AND MOLECULAR STUDIES

The dopamine hypothesis has dominated schizophrenia research for 40 years

Given the current evidence that schizophrenia is a highly heritable disease with complex genetics involving multiple genes of small or modest effect interacting to produce the phenotype, it is now clear that dysfunction of a single neural system would unlikely account for the pathophysiology of the disease. Rather, the emerging evidence from postmortem studies in the context of the accumulating genetic findings is that there are a number of neural systems that can be affected in schizophrenia. Postmortem tissue studies have become much more comprehensive with the introduction of DNA microarray analyses capable of measuring thousands of transcripts. Cellular specificity has also been markedly increased with the use of laser capture single-cell analysis as well as *in situ* hybridization. The extensive array of antibodies against specific proteins augurs well for proteomic strategies to measure proteins as well as their post-translational modifications such as glycosylation and phosphorylation. Finally, informatics can identify interrelationships among proteins and families of proteins involved in common functional or structural roles. Freeware such as Expression Analysis Systemic Explorer (EASE), available through the NIH Database for Annotation, Visualization and Integrated Discovery (DAVID), facilitate such analyses. This strategy assists in linking

seemingly isolated abnormalities in gene or protein expression that may be involved in related functions such as myelinization or mitochondrial oxidative metabolism (Lencz & Malhotra, 2009).

Despite the ability to measure dopamine, its metabolite homovanillic acid (HVA), and other presynaptic markers for dopaminergic neurons in the striatum and corticolimbic regions for 40 years, most postmortem findings of dopaminergic markers in schizophrenia have been inconsistent and largely negative. The most reproducible finding is that antipsychotic drugs increase brain, cerebrospinal fluid and plasma HVA, consistent with preclinical studies demonstrating that D2 blockade causes an increase in dopamine turnover.

The dopamine receptors, numerically designated by the sequence of their discovery, consist of the DA1 receptor, which is positively coupled to adenylyl cyclase, and the DA 2 receptor, which is negatively coupled to adenylyl cyclase (Seeman & Van Tol, 1994) (see Ch. 14). Cloning strategies yielded three additional DA receptors: D3 and D4, which are D2-like, and D-5, which is D1-like. Given the remarkable correlation of D2 receptor affinity and clinical potency of antipsychotic drugs, perhaps it is surprising to note that over a generation of studies have revealed no consistent evidence of alterations in D2 receptors as measured by ligand binding, receptor autoradiography and *in situ* hybridization in brains from schizophrenic patients who were untreated or not recently treated with antipsychotic drugs (to avoid the confounding effects of D2 antagonists on D2 expression). D3 receptors are concentrated in the ventral striatum and their expression is regulated by brain-derived nerve growth factor (BDNF) (Ch. 29). Studies of the expression of D3 receptors in schizophrenia have been largely inconclusive. However, results of studies analyzing the serine-9-glycine polymorphism of the D3 receptor suggest that the glycine-9 allele may confer susceptibility to tardive dyskinesia (Lencz & Malhotra, 2009).

The D4 receptor differs from D2 and D3 receptors by having a disproportionately high affinity for the atypical antipsychotic clozapine as well as for some other antipsychotic drugs. The D4 receptor is relatively enriched in corticolimbic regions, including the hippocampus in humans, but is expressed at very low levels in the striatum. PCR and *in situ* hybridization studies have not revealed consistent alterations in D4 receptors in striatum or in the cortex in schizophrenia. The D1 receptor, for which antipsychotic drugs have a quite variable affinity, are highly expressed in frontal cortex and play an important role in cognitive functions.

Since the ventral tegmental area (VTA) cortical dopaminergic fibers express negligible amounts of DAT, the inactivation of extracellular dopamine in cortex occurs primarily through catabolism by catechol-O-methyltransferase (COMT) (see Ch. 14). In this regard, a common allelic variant of COMT, valine-158-methionine, is associated with substantial differences in COMT activity, as the methionine allele is much more temperature sensitive, resulting in lower enzymatic activity than does the valine allele (Akil et al., 2003). Cognitive testing of normal subjects matched for IQ demonstrate that those with the least-active COMT genotype (methionine), which would enhance cortical dopaminergic neurotransmission, perform better on a task that requires prefrontal cortical function as compared to those with the valine allele. Schizophrenics with valine genotype for COMT exhibit greater impairments in cognitive functioning, suggesting that this may be a risk gene for the disease. These genotype differences have been confirmed in postmortem studies.

Hypofunction of NMDA receptors may contribute to the endophenotype of schizophrenia

The hypothesis that hypofunction of a subpopulation of *N*-methyl-D-aspartate (NMDA) receptors contributes to the pathophysiology of schizophrenia has gained considerable support over the last decade (See Figure 58-1). The dissociative anesthetics, including phencyclidine (PCP) and ketamine, were noted 40 years ago to produce a syndrome that was difficult to distinguish from schizophrenia. These agents are noncompetitive open-channel blockers of the NMDA receptor. Careful studies with titration of doses in normal subjects demonstrate that ketamine produces negative symptoms such as withdrawal and the subtle cognitive impairments associated with schizophrenia (Krystal et al., 2003). As is the case for schizophrenia, these symptoms occur without clouding of consciousness or frank dementia. Positive symptoms with auditory hallucinations and fully formed delusions do not typically occur with acute administration of ketamine to normal subjects but are seen with chronic abuse of the drug. Furthermore, low-dose ketamine produces several physiologic abnormalities associated with schizophrenia such as abnormal eye tracking, enhanced subcortical dopamine release, impaired prepulse inhibition, hypofrontality and abnormal ERPs. *In vivo* dialysis studies indicate that acute blockade of the NMDA receptor in experimental animals results in a disinhibition of forebrain dopamine release, suggesting that the dopamine-mediated psychosis could be secondary to a more primary defect in cortical glutamatergic signaling.

Postmortem studies combined with genetic findings have generated compelling evidence that disruption in the modulation of subtypes of NMDA receptors contribute to the psychopathology in schizophrenia (Coyle et al., 2010). Glutamate carboxy peptidase II (GCPII; folate hydrolase I or FOLH1) degrades the neuropeptide N-acetylaspartylglutamate (NAAG), which is colocalized to and released from glutamatergic neurons as well as from other neuronal systems (cholinergic motor neurons, noradrenergic locus ceruleus neurons). NAAG is an agonist at the metabotropic glutamate receptor (mGluR)3 receptor, which reduces glutamate release, and a reversible antagonist of glycine at hippocampal NMDA receptors (see Ch. 14) (Bergeron et al., 2007). Postmortem studies with different cohorts have documented significant reductions in GCPII enzymatic activity and in its mRNA levels in frontal and temporal cortex/hippocampus. A translocation associated with schizophrenia affects a locus on chromosome 11q13 in close proximity to the gene encoding GCP II.

D-serine is a full agonist at the glycine modulatory site on the NMDA receptor. D-serine levels are determined in part by the catabolic enzyme D-amino acid oxidase (DAAO), since the levels of the former are inversely correlated with the activity of the latter. A polymorphism in the gene encoding a protein

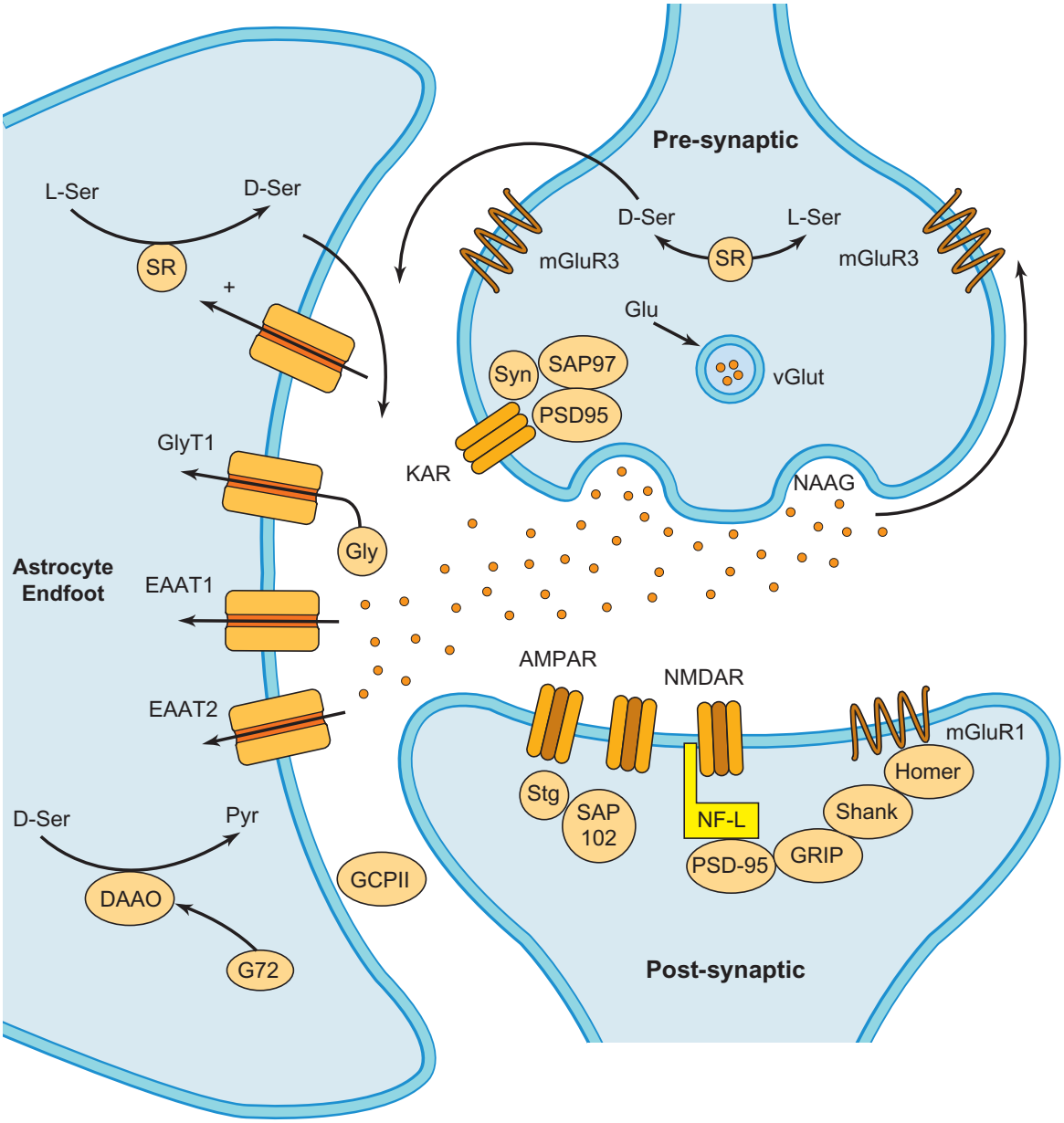

FIGURE 58-1 **Schematic relationship of the tripartite glutamatergic synapse.** This synapse consists of a presynaptic glutamatergic bouton, the postsynaptic spine and the astrocytic end-foot. Presynaptic mGluR3 receptors inhibit the release of glutamate and are activated by N-acetyl aspartyl glutamate (NAAG). Presynaptic kainite receptors (KAR) regulate glutamate release. Postsynaptic AMPA receptors (AMPAR) and NMDA receptors (NMDAR) generate excitatory postsynaptic currents when activated by glutamate. The astrocyte expresses two types of Na^+-dependent glutamate transporters (EAAT1 and 2); a glycine transporter (GlyT1), which maintains subsaturating concentrations of glycine at the synaptic NMDA receptor; serine racemase (SR), which synthesizes D-serine (ser); glutamate carboxypeptidase II (GCPII), which hydrolyzes NAAG; and D-amino acid oxidase (DAAO), which degrades D-serine. SR is also expressed in the glutamatergic neurons. The receptors are associated with a variety of structural proteins that anchor them in the synapse, including PSD-95 (postsynaptic density, kDa 95), stargazin (STG), SAP-97 and -102 (synapse associated protein, kDa 97 and 102), syntenin (SYN), glutamate receptor interacting protein (GRIP) and neurofilament light protein (NF-L).

designated *G72*, which inhibits DAAO, has been associated with schizophrenia in several studies (Sacchi et al., 2008). Single-nucleotide polymorphisms (SNPs) of DAAO have been associated with increased risk for schizophrenia. The cerebrospinal fluid (CSF) and plasma levels of D-serine are decreased in schizophrenic patients. Kynurenic acid is another endogenous antagonist of the glycine modulatory site on the NMDA receptor. Kynurenic acid is significantly elevated in cortex and in CSF in schizophrenic patients as compared to controls, a finding that appears to be unrelated to antipsychotic drug

exposure. Tryptophan-2,3-dioxygenase, an upstream enzyme in kynurenic acid synthesis, is also upregulated in cortex in schizophrenia (Wonodi & Schwarcz, 2010). Notably, double-blind, placebo-controlled clinical trials of agents that directly or indirectly enhance NMDA receptor function at the glycine modulatory site show that they significantly reduce negative symptoms, enhance cognitive function and decrease positive symptoms in patients with chronic schizophrenia receiving antipsychotic drugs (Tsai & Lin, 2010).

NMDA receptors are anchored in the postsynaptic density (PSD), a multimolecular complex with which over 80 proteins have been associated. Postmortem studies have examined the expression of the subunits of the NMDA receptors as well as components of the PSD (Kristiansen et al., 2007). In the thalamus, the NR1 and NR2B subunits (see Ch. 14) were reduced in schizophrenia, and PSD95 (post-synaptic density, kDa 95), SAP102 (synapse associated protein kDa 102) and NF-L (neurofilament-light), components of the PSD, were also significantly reduced, with the latter reduction also found in bipolar disorder. Decreased phosphorylation of NR1 at serine 897, which impairs NMDA receptor function, has been found in prefrontal cortex in two cohorts of schizophrenic subjects (Li et al., 2009). That the postsynaptic density complex may be an important site for the pathophysiology of schizophrenia is reinforced by the association of SNPs for distobrevin and dysbinden, two proteins in the PSD complex, with the risk for schizophrenia. Notably, dysbinden expression is reduced in prefrontal cortex.

Nitric oxide (NO) synthesis in brain is partly driven by NMDA receptor activation through Ca^{++}/calmodulin-dependent activation of NO synthase (NOS). NO interacting with NMDA receptor thiols causes inhibition of the NMDA receptor. Notably, arginine, the substrate for NOS, was found to be elevated by three-fold and the expression of endothelial and inducible NOS was significantly elevated in the frontal cortex in schizophrenia (Brzustowicz, 2008). While these alterations would be expected to be associated with oxidative stress, which promotes DNA damage and apoptosis, DNA fragmentation is markedly reduced in prefrontal cortex in schizophrenic subjects as compared to both controls and bipolar subjects.

Other components of the glutamatergic signaling system are also affected. For example, the glutamate transporters EAAT (excitatory amino acid transporter) 1 and 2 and their interacting proteins are altered in the thalamus and prefrontal cortex, which should further compromise glutamatergic neurotransmission (Bauer et al., 2008). Consistent reductions in kainic acid receptor have been documented by ligand binding, *in situ* hybridization of subunits and DNA microarray studies in the prefrontal cortex and hippocampus (Harrison et al., 2003). SNPs for the mGluR3 receptor gene, which downregulates glutamate release, have been linked to the risk for schizophrenia in several studies, and its expression appears to be reduced in schizophrenia.

Neuregulins are cell–cell signaling proteins that are ligands for the tyrosine kinase receptor of the Erb family (see Chs. 26, 28, 29) (Buonanno, 2010). Several separate studies have associated allelic variants of neuregulin 1 with the risk for schizophrenia. Neuregulin has complex effects on glial differentiation, astrocyte function and synapse stabilization. Postmortem studies have revealed reductions in neuregulin expression in the cortex in schizophrenia. Mice homozygous for a null mutation of neuregulin or its ErbB4 receptor display hyperactivity that responds to clozapine and exhibit a reduced number of NMDA receptors. Individually and in concert, such alterations would be expected to attenuate NMDA receptor function in schizophrenia.

GABAergic neurons are also implicated in schizophrenia

Early postmortem neurochemical studies revealed reductions in presynaptic markers for GABAergic neurons, such as glutamic acid decarboxylase (GAD) activity, in prefrontal cortex in schizophrenic subjects (Gonzalez-Burgos et al., 2010). However, the validity of these findings was undermined by the concern that agonal events, such as anoxia and a slow death, to which individuals with schizophrenia in particular are prone, could reduce the activity of GAD independent of diagnosis. Later studies, however, have profitably taken advantage of more discrete anatomical analyses of GABAergic interneurons. Disruption in the migration of these neurons in frontal cortex, a process occurring during the second trimester (see Ch. 28), has been noted in a subpopulation of patients with schizophrenia. Several studies have shown reduced expression of presynaptic markers including GAD67, parvalbumin and the GABA transporter (GAT-1) in interneurons in the intermediate layers of the cortex, particularly represented by the chandelier cells. In support of a discrete reduction in GABAergic innervation, increased ligand binding for the $GABA_A$ receptor and expression of $GABA_A$ $\alpha 2$ subunits have also been noted in the same sector, consistent with denervation supersensitivity. Most of these findings have been confirmed in several laboratories using different sources of brain material.

Increasing evidence suggests that the GABAergic alterations are downstream effects of impaired glutamate neurotransmission, especially at NMDA receptors (Coyle et al., 2010). GABAergic interneurons express a host of glutamate receptors. In the cingulate cortex, for example, the density of GAD67-positive neurons, which co-express the NMDA receptor subunits NR2A and 2B mRNA, are reduced in schizophrenia. Furthermore, chronic treatment with the noncompetitive NMDA receptor antagonist dizocilpine (MK801) results in a downregulation in the expression of GAD67 and GAT and upregulation of GABA-A receptors in rat cortex, mirroring the alterations in these parameters observed in the cortex in schizophrenic subjects. Electrophysiologic evidence indicates that limbic GABAergic interneurons may be differentially more sensitive to NMDA receptor antagonists than those located on pyramidal neurons. Subchronic treatment of mice with an NMDA receptor antagonist causes reduced inhibitory postsynaptic currents on prefrontal cortex pyramidal neurons (Lisman et al., 2008). In addition, several groups have demonstrated a significant reduction in the levels of reelin, a high-affinity peptide ligand for integrin receptors, in schizophrenia. The reelin/integrin system modulates early cortical development, especially neuronal migration (see Ch. 28)

(Tueting et al., 2006). Notably, reelin is expressed by virtually all GABAergic neurons throughout life.

The cholinergic system has also been implicated in schizophrenia

Cigarette smoking is remarkably prevalent among patients suffering from schizophrenia. Speculating that this addiction may reflect a form of self-medication, Freedman and colleagues demonstrated that nicotine improved the sensory gating abnormalities that occurs in the P50 potential in schizophrenia (described above) (Leonard et al., 2002). Further studies have also revealed similar P50 abnormalities in many first-degree relatives of schizophrenic probands, suggesting that the gating abnormality may be a heritable endophenotype for schizophrenia. Subsequent linkage studies identified a locus on 15q13 that was associated with increased risk for schizophrenia. This region contains the gene for the α7−nicotinic receptor (CHRNA7), a subtype of nicotinic receptor with intriguing parallels to the NMDA receptor because of its calcium conductance, sensitivity to kynurenic acid inhibition and involvement in neuroplasticity (Wonodi & Schwarcz, 2010). An allelic variant in the promoter region of CHRNA7, which reduces the expression of the receptor, is associated with P50 abnormalities and with increased risk for schizophrenia.

Some intracellular signal transduction molecules are reduced in schizophrenia

Neurotransmitter release is regulated by a family of proteins that coordinate vesicular trafficking (see detailed discussions in Chs. 7, 12). Of these, the expression of complexin I and II appears to be decreased in prefrontal cortex and subfields of the hippocampal formation, while the ratio of complexin I to complexin II is elevated in the hippocampus in schizophrenia (Sawada et al., 2002). SNAP-25 (synaptosomal associated protein, kDa 25) has inconsistently been found to be downregulated in both these regions. Synapsin expression is also reduced, but more robust decrements have been observed in bipolar disorder. Regulators of G protein signaling (RGS) comprise a large family of modulators of synaptic neurotransmission. RGS4 has been implicated in schizophrenia in part because its gene is located at 1q21-22, a locus associated with high risk for the disease (Cornblatt et al., 2003). The expression of RGS4 has been shown to be consistently downregulated in prefrontal cortex in schizophrenia. The AKT and glycogen synthase kinase-3beta (GSK3β) (Ch. 23 and 25) are downstream components of the neuregulin and wingless (WNT) signaling pathways (Ch. 28) (Freyberg et al., 2010). Association studies have implicated the neuregulin gene at 8p12 as a risk gene for schizophrenia. Post-mortem studies have revealed decreased levels of AKT1 protein and reduced phosphorylation of serine 9 on GSK3β, as well as decreased GSK3β expression in frontal cortex and hippocampus in schizophrenia (see kinases in Ch. 25).

Proteins involved in fundamental structure and function of neurons are decreased in schizophrenia

Several postmortem studies have identified significant reductions in the expression of mitochondrial-associated genes involved in oxidative metabolism such as cytochrome oxidase and cytochrome C reductase (Clay et al., 2011). Reduced oxidative metabolism is consistent with evidence of increased brain lactate documented with MRS and postmortem measures as well as decreased brain pH observed in postmortem studies in schizophrenia. Morphologic studies have also shown a decreased number of mitochondria in both frontal cortex and striatum. Robust decreases in the expression of the various proteosome subunits and ubiquitin-conjugating enzymes have been described in prefrontal cortex in schizophrenia (see in Chs. 7, 8). Neuronal ubiquitin and proteosomes play an important role in the assembly, function and plasticity of the synapse. Structural proteins including tubulin and α-spectrin also show decreased expression in prefrontal cortex. Consistent with these findings, Golgi studies reveal reduced dendritic complexity and spine number on cortical pyramidal cells in schizophrenia, supporting the notion that it is a "disconnection" syndrome (Coyle et al., 2010).

Glia may play a role in schizophrenia

Disruption in myelin is a novel pathophysiologic mechanism recently identified in schizophrenia (Karoutzou et al., 2008). Imaging studies reveal abnormalities in myelin tracks with reduction in the total white matter volume in whole brain, significant decreases in myelin or axonal membrane integrity as seen with magnetization transfer ratio and decreased anisotropy in cortical regions indicative of myelin disruption, as documented by diffusion tensor imaging (DTI). DNA microarray and RT-PCR studies have repeatedly shown reduced levels of mRNA encoding proteins associated with oligodendrogliacytes and myelinization. Among these are *myelin-associated glycoprotein* (MAG), *myelin lymphocyte protein* (MAL), the neuregulin receptor *ErbB3* and *transferrin* (see Ch. 10). The genes encoding these proteins are located at loci on the human genome that have been associated with heritable risk for schizophrenia. It is noteworthy that downregulation of the expression of several of these genes has also been observed in postmortem cortex from individuals with bipolar disorder. This suggests that these myelin abnormalities may account for features, such as psychosis, that are shared between schizophrenia and bipolar disorder.

Although the apparent number of glial fibrillary acidic protein (GFAP) positive astrocytes is unchanged in schizophrenia, the expression of GFAP is reduced in prefrontal cortex. While reduction in glial numbers has been associated primarily with affective disorders (see Ch. 60), reduced numbers of astrocytes have been reported for the anterior cingulate cortex in schizophrenia (Bernstein et al., 2009). Both the activities and the protein levels of glutamine synthetase and glutamate dehydrogenase, which are associated with astrocytes, are altered in prefrontal cortex in schizophrenia.

Notably, astrocytes play a critical role in regulating the synaptic availability of the NMDA receptor co-agonists glycine, D-serine and glutamate.

SUMMARY

Our understanding of the pathophysiology of schizophrenia has advanced remarkably over the last decade through the combined use of *in vivo* imaging techniques, genetic studies and neurochemical (in the broadest sense) analyses of post-mortem brain. Collating the findings from these different methods of analysis indicates that the disease is highly heritable and involves interactions of multiple genes of small effect, which likely affect several functional domains (see Box). Aside from dopamine, which may be central to the psychosis, the glutamatergic, GABAergic and cholinergic neurotransmitter systems are also credible participants. Since several of the affected proteins modulating NMDA receptor function are expressed in astrocytes and since multiple candidate genes and their products are involved in myelinization, non-neuronal cell types are also likely to be important participants in the pathophysiology of schizophrenia. A major challenge for the future will be to disentangle the alterations in gene expression that are primary from those that are secondary and relating these alterations in a meaningful way to the symptoms of schizophrenia. These advances will provide the hope for developing more effective treatments that address the persistent cognitive and negative symptoms that are not affected by current treatments targeted at the dopamine D2 receptor.

IT'S COMPLICATED: THE GENETICS OF SCHIZOPHRENIA AND RELATED SERIOUS MENTAL ILLNESSES

Joseph T. Coyle

Findings from family, adoption and twin studies have provided compelling evidence that serious mental disorders including schizophrenia, bipolar disorder and autism have high degrees of heritability. In the case of schizophrenia, concordance for the diagnosis of schizophrenia if one twin is affected is approximately 60%. However, the relative risk for fraternal twins falls to the level observed in first-degree relatives, which is 10–15%. This lack of complete concordance points to important environmental risk factors, and a relative risk well below 25% in first-degree relatives suggests non-Mendelian, i.e., complex, genetics. The high degree of heritability in schizophrenia held forth the promise that identifying risk genes for the disorder would shed light on the underlying pathophysiology and reveal potential targets for therapeutic intervention.

Initial forays into risk gene identification for schizophrenia in the 1990s exploited linkage strategies. Linkage analysis of families with multiple affected members exploited intrafamily correlations between illness and allelic markers that were thought to be close to the disease-related genes (Psychiatric GWAS Consortium Coordinating Committee, 2009). When the sequence of the entire human genome was complete early in this decade, it became possible to determine whether genes that encoded proteins implicated in the pathophysiology of schizophrenia, such as dopamine receptors, were in fact genes of risk. These studies were often carried out with 100 or fewer affected individuals and a comparable number of suitable controls to determine whether a known allelic variant was transmitted more frequently with the disorder. While hundreds of candidate genes appeared significantly associated with risk for schizophrenia, replication studies were often negative. It became clear that these studies were underpowered and were likely generating false positive findings. Meta-analysis of these studies identified only four "strong" potential gene associations. None involved dopaminergic-related genes, but the gene encoding the NMDA receptor NR2B subunit was implicated.

Lessons learned from research on medical disorders with complex genetics such as type II diabetes indicated that common alleles that conferred a relative risk between 1.1 and 1.4 required the analysis of thousands of subjects for genome-wide association studies (GWAS) to achieve statistical significance. Nevertheless, recent well-powered GWAS studies on schizophrenia and related serious mental disorders have been remarkably unrevealing of common alleles that confer significant risk, with the possible exception of *ZNF804A*, a zinc-finger protein. One possibility for the relatively negative findings is that there is a gene–gene interaction of common alleles such that the association is not detected by testing single nucleotide polymorphisms (SNPs) one at a time.

An alternative hypothesis that is gaining traction is that rare mutations with high penetrance might account for the heritable risk for schizophrenia. Consistent with this hypothesis, several GWAS studies have revealed rare copy number variants (CNVs) associated with the risk for schizophrenia. CNVs are structural genomic variants, stretches of DNA several hundred to several million base pairs in size, consisting of micro-insertions, micro-deletions and transpositions in the human genome (Gershon et al., 2011). Rare deletions were found at 1q 21.1, 15q13.3 and 22q 11. Notably, some of these CNVs are also associated with autism spectrum disorder and developmental disability and often occur *de novo*. Whereas GWAS studies indicated a significantly increased number of CNVs in the schizophrenia genome as compared to that of controls, the density of CNVs in bipolar disorder does not appear to differ from that of controls (Grozeva et al., 2010). Genes contained within the CNVs likely confer the risk, and understanding their function could reveal "final common pathways to vulnerability" that could be targets for pharmacologic intervention. This difference in CNV density may interact with shared risk alleles to distinguish schizophrenia from bipolar disorder.

IT'S COMPLICATED: THE GENETICS OF SCHIZOPHRENIA AND RELATED SERIOUS MENTAL ILLNESSES (*cont'd*)

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