Genetic Analysis of Schizophrenia and Bipolar Disorder Reveals Polygenicity But Also Suggests New Directions for Molecular Interrogation

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Introduction

Schizophrenia and bipolar disorder are among the most debilitating psychiatric illnesses and represent a tremendous public health burden. Nearly a century ago, Emil Kraepelin delineated two forms of mental illness as "dementia praecox" (now called schizophrenia) and "manic-depressive illness" (now called bipolar disorder). This dichotomy has been one of the founding principles of modern Western psychiatry, but in recent years, analysis of new genetic data is leading to its reexamination. In defining "dementia praecox," Kraepelin reviewed its apparently familial nature, noting a high degree of sibling sharing as well as parent offspring sharing, albeit to a lesser extent (Kraepelin, 1919). Thus, even a century ago, researchers were observing inheritance in families for severe mental illness.

These early views of schizophrenia and bipolar disorder as inherited disorders have been refined by almost one hundred years of twin and family studies. These studies demonstrated substantial heritability (the proportion of disease liability due to genetic factors) for both schizophrenia (estimates ranging from 60% to 90%) and bipolar disorder (estimates ranging from 60% to 80%) and showed that these disorders can co-occur in families (Berrettini, 2000; McGuffin et al., 2003; Sullivan et al., 2003; Lichtenstein et al., 2009). The strong and consistent evidence for high heritability suggested that disease genes might be identified using genetic approaches. Linkage, which is one of the earliest methods of genetic analysis, works by scanning the genome in search of regions that are shared by family members who are affected by the disease under study. This development spurred many linkage studies to search for schizophrenia and bipolar disorder regions and the genes within them (Levinson et al., 2003; Lewis et al., 2003; and Segurado et al., 2003). However, linkage is effective only when there is limited "locus heterogeneity" (e.g., if the genetic variation that influences schizophrenia is restricted to a few regions of the genome) or when there are large pedigrees with a nearly Mendelian cause (i.e., a genetic variant is nearly sufficient to cause disease in all affected members of the pedigree). To date, for schizophrenia and bipolar disorder, linkage has met with no clear success despite the meta-analysis of thousands of samples. These findings indicate that risk variants are not fully causal and that many regions in the genome are likely relevant to both schizophrenia and bipolar disorder.

The past decade of human genetics research has seen a staggering technological revolution in our ability to gather information about the genome. The SNP Consortium (Sachidanandam et al., 2001; Thorisson and Stein, 2003) and International HapMap project (International HapMap Consortium, 2003, 2005, 2007) created a catalog of common DNA variations and characterized the genome-wide patterns of linkage disequilibrium (LD). LD is a term used to describe the correlation between genetic variations, i.e., two variants are said to be in LD if the genotypes correlate. The HapMap project, in particular, was a central community-wide resource that made genotype data available for individuals from multiple ethnicities, providing information on allele frequency and LD across different continental populations. These large-scale evaluations of genetic variation also laid the groundwork for genome-wide association studies (GWAS) by providing a sufficiently comprehensive set of genetic markers to effectively test genome-wide.

The most pervasive type of genetic marker is the single nucleotide polymorphism (SNP), which is a single base change in the DNA sequence. GWAS enables a systematic and unbiased population-based evaluation of individual DNA variants for association with disease across the genome. In GWAS, the classes of genetic variations best explored have been those that are common in the population (common genetic variants, typically with minor allele frequency > 1–5%, depending on the study), and this technique has been successfully applied across a wide range of complex traits. The array technology used for GWAS also revealed copy number variation (large deletions or duplications of DNA from individual chromosomes), which has been shown to confer risk of several psychiatric illnesses including autism, schizophrenia, bipolar disorder, and attention deficit hyperactivity disorder (GAIN Collaborative Research Group et al., 2007; International Schizophrenia Consortium, 2008; Williams et al., 2010; Levy et al., 2011; Sanders et al., 2011; Malhotra and Sebat, 2012; Ramos-Quiroga et al., 2014; Rees et al., 2014; Stefansson et al., 2014; Szatkiewicz et al., 2014). Beyond GWAS, next-generation sequencing technologies have been developed that enable the discovery of rare (< 1% minor allele frequency) and private variation (effectively specific to individuals or families), thus extending the application of association tests into this range.

In general, the results from the current GWAS and sequencing studies of schizophrenia and bipolar disorder clearly show that a great many genetic variants influence the risk of schizophrenia and

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bipolar disorder. Both common and rare variants contribute to the genetic architecture of these diseases, and most effect sizes are small to modest, necessitating large-scale genetic studies to robustly identify novel risk factors. The identification of these genetic variants and the interpretation of the biological consequences of said variants will form the basis for new insights into the pathogenic processes that underlie schizophrenia and bipolar disorder.

Many Common DNA Variants Play a Large Role in Schizophrenia and Bipolar Disorder

The first GWAS of common variants in schizophrenia and bipolar disorder identified only a small handful of genome-wide significant loci (GAIN Collaborative Research Group et al., 2007; O'Donovan et al., 2008; International Schizophrenia Consortium et al., 2009). This limited initial success was driven mainly by the small effect size each variant is likely to have (< 1.5-fold influence on risk) and the comparatively modest sample sizes (< 10,000 individuals). In spite of a relative paucity of strongly associated loci (compared with some other traits, like those of Crohn's disease [Barrett et al., 2008] or age-related macular degeneration [Klein et al., 2005]), one of the first large-scale GWAS of schizophrenia demonstrated a myriad of DNA variants whose effects are too small to detect individually, but when summed together clearly contributed to schizophrenia risk (International Schizophrenia Consortium et al., 2009). This GWAS provided the first molecular evidence that schizophrenia is highly polygenic, and subsequently, it has been widely validated in many independent samples.

The many risk variants, when summed in this manner, essentially form a polygenic risk score (PRS). That is, based on a person's genotype, it is possible to count the number of risk alleles he or she has and to use that count to predict risk for diseases such as schizophrenia. This prediction can be improved by weighting each variant's contribution to the score based on the strength of association between that allele and the disease outcome, so that alleles that show larger effect sizes are counted more heavily in creating the score. Based on part of the early schizophrenia GWAS sample, such PRSs were then used to predict the risk of disease in an independent subsample. This procedure consistently demonstrates a minimal predictive ability but strong evidence for the combined role of common variation. Ensuring that there is no overlap between the discovery sample (the sample used to estimate the genetic effects across the genome) and the testing sample (the sample used to evaluate the predictive validity of the PRS) is essential to avoid false-positive associations and overestimation of the predictive validity of such a score. The PRS from the early GWAS explained only a small amount of the variance in liability to becoming ill with schizophrenia. At present, the PRS score is not sufficiently specific for clinical use. One of the predictions from the work suggested that increasing sample size would increase the explanatory power of such scores because it would more accurately estimate the true effect size of these genetic variants.

Not only did the schizophrenia PRS predict schizophrenia, but perhaps more surprisingly, it also showed predictive ability for bipolar disorder, implying that these two disorders share many more genetic risk factors than had been expected. In order to quantitate how much genetic overlap exists between schizophrenia and bipolar disorder (and four other majors forms of psychopathology), genome-wide complex trait analysis (GCTA) was performed (Yang et al., 2011). The basic principle behind GCTA is that if a trait has a genetic component, then people who are more phenotypically similar (e.g., both have schizophrenia) will tend to be more genetically similar (i.e., they will tend to share risk alleles for the disease in question). This framework enables not only the assessment of evidence for heritability from common DNA markers across the genome (rather than using twin and family relationships to tease out genetic contribution to disease) but also a multivariate approach for comparing different diseases. Consistent with the polygenic prediction work, when schizophrenia and bipolar disorder were analyzed jointly using GCTA, there was a substantial overlap in their genetic basis, with a genetic correlation of ~0.7 (Cross-Disorder Group of the Psychiatric Genomics Consortium et al., 2013). This genetic overlap highlights that there may be biological pathways that do not respect the traditional clinical boundaries but rather confer risk of both schizophrenia and bipolar disorder.

In the past few years, there has been a massive increase in the sample sizes for schizophrenia GWAS owing to the efforts of the Psychiatric Genomics Consortium and others (Ripke et al., 2013). As sample sizes have increased, the number of identified loci has increased as expected, most recently with 108 risk loci identified at genome-wide significance in a sample of more than 36,000 cases (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Furthermore, as predicted, the specificity of the PRS also improved, in line with the initial PRS work, though it remains of limited

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clinical utility. As with other complex traits, the effect size of the genetic variants in these regions is quite modest (odds ratios ≈ 1.2). In most loci, the strongest associated variant is noncoding, consistent with the underlying causal alleles having a regulatory impact on disease. Furthermore, many genome-wide significant loci harbor multiple genes, any of which could be driving the association. A number of these loci contain genes that code for proteins supporting several prior biological hypotheses, such as *DRD2* (the D2 subtype of the dopamine receptor, thought to be the antipsychotic drug target); *GRM3*, *GRIN2A*, *SRR*, and *GRIA1* (involved in glutamatergic neurotransmission and synaptic plasticity); and *CACNA1C*, *CACNB2*, and *CACNA1I* (calcium-channel signaling). Because the majority of risk variants are found in noncoding regions, the precise biological mechanisms will be harder to uncover; however, many will be regulatory in nature.

At a global level, a series of analyses examining different biological annotations has been performed to evaluate whether further insights could be gleaned from these results. One of the most important sources of genomic annotations is the ENCODE/Roadmap Epigenomics Project (www.roadmapepigenomics.org) (ENCODE Project Consortium, 2011, 2012). This international collaborative effort is focused on measuring different functional and regulatory features of the genome. These features range from chromatin state (i.e., how exposed DNA is in the cell, which can be assayed through DNase I hypersensitivity) to histone modification (which relates to how DNA is bound and packaged in the cell) to DNA methylation (which may play a role in epigenetic regulation of gene expression, as a mechanism for silencing genes). These annotations were leveraged in the analysis of the most recent schizophrenia GWAS to demonstrate excess association in regions containing neuronal enhancers, as well as immune enhancers, an association that persists even after controlling for neuronal enhancers (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). Similarly, GWAS loci in schizophrenia are enriched for variants that influence gene expression (expression quantitative trait loci [eQTLs]), some of which are located in cis-regulatory elements, including promoters (Richards et al., 2012). These global analyses suggest that information on functional annotations can be used to prioritize genes in a risk locus for biological follow-up. One key example has been *CACNA1C*, which encodes the alpha subunit of the L-type calcium channel. In *CACNA1C*, the schizophrenia risk variant is associated with transcriptional regulation in the brain and is positioned within an enhancer sequence that physically interacts through chromosome loops with the promoter region of the gene (Roussos et al., 2014). This example demonstrates one paradigm for moving from DNA variant to biology, although many others are expected to emerge.

Available GWAS of bipolar disorder have used smaller sample sizes and identified approximately 10 genome-wide significant loci (Ferreira et al., 2008; Sklar et al., 2008; Chen et al., 2013; Green et al., 2013; Mendenhall et al., 2013). Explorations of the overlap of bipolar disorder–associated loci demonstrate that many, although not all, have shared effects in schizophrenia.

Rare *De Novo* and Inherited Variation

Copy number variants (CNVs) were the first rare variants found to be associated with psychiatric illness because they were large, easily detectable, and thus amenable to a wide variety of microarray technologies, including GWAS. A large body of work has shown that the rates of inherited and *de novo* (newly arising) CNVs are elevated in schizophrenia and, to a lesser extent, bipolar disorder. Many CNVs confer high risks (2.1–49.5), but none are determinative, and several genomic regions are frequent targets (International Schizophrenia Consortium, 2008; Malhotra and Sebat, 2012; Rees et al., 2014). Notably, these CNVs can produce a wide variety of neuropsychiatric phenotypes (most commonly, autism spectrum disorder, learning disability, and epilepsy) and are enriched for genes involved in synaptic processes and neuronal development (Kirov et al., 2012).

Beyond the GWAS of schizophrenia and bipolar disorder, the advent of next-generation sequencing technologies has enabled the assessment of rare and *de novo* variation for novel risk factors at the single-base level. The analysis of rare variation is in many ways more challenging than GWAS. For rare variation, case control study designs are one of the primary approaches, much in the same manner as GWAS. In contrast to GWAS, a direct test of each variant is effectively impossible because the number of copies of any allele is quite small. To overcome the limited power of testing individual rare variants, it is necessary to sensibly group them together to identify risk factors (Li and Leal, 2008; Price et al., 2010; Neale et al., 2011; Li et al., 2013). For the coding region, grouping variation is comparatively straightforward, as the gene is a natural analytic unit. Grouping together genetic variation outside the coding region is more challenging as our ability to functionally annotate noncoding genetic variation

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remains limited compared with the coding region, and currently appreciated functional units are typically too small to encompass a sufficient number of variants to perform a test (Zuk et al., 2014).

Thus far, the majority of exome sequencing completed for psychiatric illness has been for schizophrenia. The largest effort to date is an exome sequencing project of 2536 schizophrenia cases and 2543 controls in Sweden (Purcell et al., 2014). Notably, no single gene or rare variant has been associated with the disease, beyond chance expectation. Perhaps unexpectedly, however, a high degree of polygenicity was also observed for rare variants, meaning that rare variants scattered across a large number of genes likely influence the risk of schizophrenia. In many ways, this work echoes the earlier GWAS findings, with the implication that expanded sample sizes may yield significant loci and variants as they have for GWAS. Even though no single gene was identified, mutations predicted to disrupt gene function were found in sets of genes implicated in the CNV and GWAS studies described above. These sets included voltage-gated calcium channels and the signaling complex formed by the activity-regulated cytoskeleton-associated scaffold protein of the postsynaptic density.

The other major way to discover rare variation that increases the risk of disease is to search for *de novo* mutations by sequencing both parents and an affected child (also termed a proband), seeking to identify newly mutated DNA variants found only in the child. This approach has been successfully applied to severe, single-gene phenotypes found in intellectual disability and Kabuki syndrome to map novel risk genes (Ng et al,. 2010; Veltman and Brunner, 2012). The motivations for searching for *de novo* mutations that influence risk are based on the low background rate of such mutations (approximately one per offspring in the coding region) and the reduced fecundity that has been observed for individuals diagnosed with schizophrenia (Power et al., 2013).

More than 900 schizophrenia patients and their parents' exomes or coding regions have been sequenced to identify *de novo* mutations that might exert a strong influence on the risk of disease (Girard et al., 2011; Xu et al., 2012; Gulsuner et al., 2013; Fromer et al,. 2014; McCarthy et al., 2014). Even with this number of trios, however, few examples exist in which *de novo* mutations occur more than once in the same gene. In contrast to intellectual disability and autism, for which definitive genes have been identified (the discovery of which was driven by highly penetrant alleles), the results of the work on schizophrenia suggest few such strong-acting alleles. However, there is evidence for an enrichment of mutations in sets of genes, consistent with the model that the set of *de novo* mutations identified represents a mixture of variants that confer risk as well as those that are simply background events (Fromer et al., 2014). To distinguish the risk-conferring genes, here too it will be necessary to further expand the sample size.

Convergence on Pathways and Implications for Neurobiology

For schizophrenia and bipolar disorder, strong evidence favors a genetic component to risk. Overall, emerging evidence favors some shared genetic risk across the allele frequency spectrum, from rare to common, although the overlap is far from complete (Fig. 1). As described earlier, rare and common variants are enriched in several synaptic components, including calcium-channel subunits and postsynaptic elements. In fact, multiple independent lines of genetic data point to voltage-gated calcium channels. For example, common variation in the pore-forming alpha subunit *CACNA1C* is significantly associated with both schizophrenia and bipolar disorder, whereas variation in *CACNB2* is significantly associated with schizophrenia and at 10^{-4} in bipolar disorder (Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011; Ripke et al., 2013). As a set, voltage-gated calcium-channel genes were found to be enriched in rarer variants in the Swedish exome study (Purcell et al., 2014). Furthermore, in the severe Mendelian disorder Timothy syndrome, which results from single-base mutations in *CACNA1C* (the subunit most associated with schizophrenia), cases often present with autism spectrum phenotypes (Splawski et al., 2005). Similarly, multiple independent lines of genetic evidence converge on postsynaptic components and glutamate signaling. In the Psychiatric Genomics Consortium schizophrenia GWAS, genomic loci containing several NMDA and AMPA receptor subunits were associated, and in the *de novo* CNV and sequencing studies, postsynaptic density components such as *DLG1* and *DLG2* were found to be enriched.

Tying these observations together, we know that calcium-mediated signaling has an important role in neuronal differentiation by regulating axonal growth and guidance, and this process is also controlled by glutamate signaling (Rosenberg and Spitzer, 2011). Thus, future investigations should explore the effects of disease variants in these groups of genes on neuronal development and the inefficient neuronal circuitry observed in schizophrenia. Intriguing evidence is also beginning to converge on several

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neurodevelopmental genes, such as *KCTD13*, the gene encoding the polymerase delta-interacting protein 1. Although previously not a strong biological candidate, this gene is found in a region with significant common variants associated with schizophrenia and lies in a schizophrenia and autism–associated duplication on chromosome 16p11.2. Remarkably, the overexpression or reduction of *KCTD13* mRNA in zebrafish produces significant changes in head size, and *Kctd13* knockdown in the embryonic mouse brain decreases neurogenesis (Golzio et al., 2012). A further intriguing example is the fragile X mental retardation protein, FMRP, encoded by the *FMR1* gene, that regulates translation and is needed at synapses for normal glutamate receptor signaling and neurogenesis (Callan and Zarnescu, 2011) as well as being a common cause of mental impairment. Rare disruptive mutations in FMR1 such as nonsense, essential splice site, or frameshift mutations are enriched in schizophrenia cases.

A natural question for genetics is the extent to which this work adds to our understanding of disease, as the emerging biological themes are relevant to major systems that have previously been implicated. However, prior to the genetics work, calcium-channel signaling and abnormalities were not heavily investigated as a pathogenic mechanism in schizophrenia or bipolar disorder. Similarly, *KCTD13* has emerged as a novel candidate for follow-up functional characterization. Furthermore, genetic risk is consistent with these dysfunctions playing an etiological role in schizophrenia and bipolar disorder, aiding in the resolution of whether these dysfunctions are pathogenic or sequelae of the disease process. These genetic data signal a sea change, in that there are now multiple avenues of statistically confident genetic observations implicating specific biological processes. An important limitation of these studies is, of course, that they have not yet identified the precise alleles and, in some cases, the precise genes to target in the future. Although most evidence currently involves schizophrenia, we expect similar findings to emerge for bipolar disorder as sample sizes increase. The individual genetic effects acting on these diseases can broadly be described as spanning the allele frequency spectrum, with generally modest effect sizes, and strongly suggest multiple complex biological processes that are relevant to disease and have relevance for downstream neurobiological experiments. Across the population, many different combinations of alleles—some rare and some common—will contribute to the ultimate phenotype.

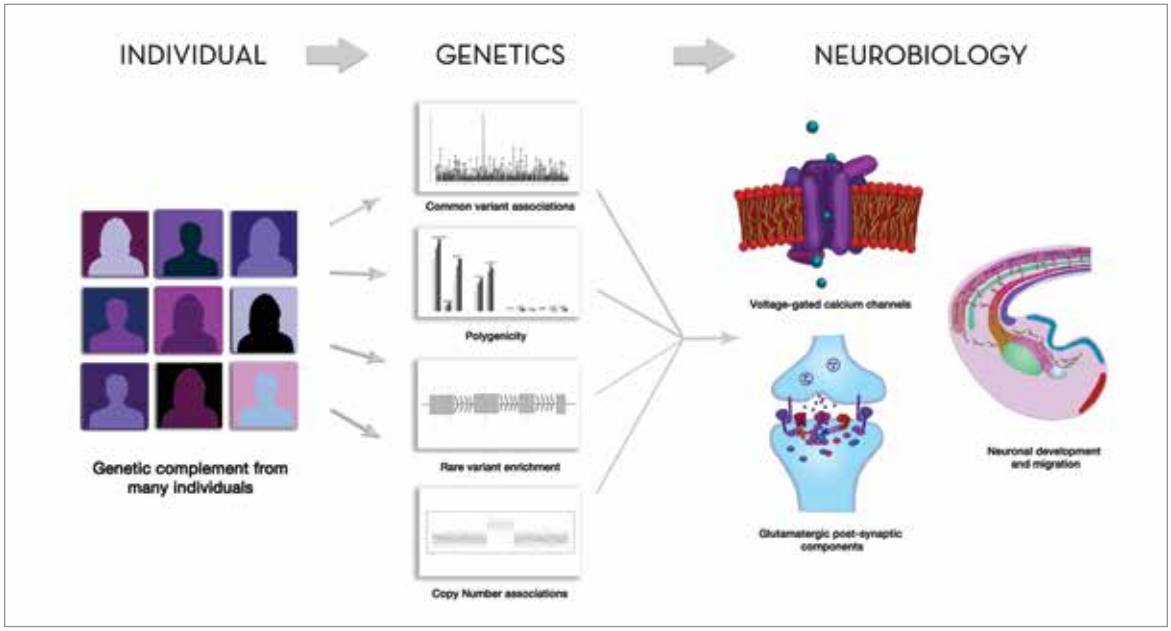

Figure 1. Moving from individuals to genetics to neurobiology: convergence of systems and pathways. Combining genetic information in many forms from large numbers of individuals can be used to identify specific genomic elements that contribute to disease. When integrated, these elements point to abnormalities in voltage-gated calcium channels, postsynaptic proteins, and neurodevelopmental molecules. Illustrations of common variant associations and polygenicity were adapted from International Schizophrenia Consortium et al. (2009), their Figs. 1 and 2, and the illustration of neuronal development and migration was adapted from Marin et al. (2010), their Fig. 5.

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Next Steps

The genetic analysis of schizophrenia, bipolar disorder, and other mental illnesses is yielding robustly significant genetic variants. These first unequivocal findings demonstrate that large-scale, unbiased screens of genetics can identify novel risk factors. However, these results are only the beginning of a long journey toward understanding the underlying biological processes involved in such diseases.

One prevailing paradigm for biological investigation proceeds in a gene-by-gene fashion, often necessitated by the difficulty and cost of the experiments. Unfortunately, this paradigm generally dictates that only variants conferring strong risk for a disease can be investigated. For schizophrenia, there are no such variants; rather, there is a plethora of variants that confer more moderate risk that need to be investigated. Thus, we will need to adapt methods that allow multiple genes and variants to be studied simultaneously in a more global, unbiased manner. The GWAS results also suggest a substantial role for regulatory variation in the pathogenesis of disease. To gain insight into how these regulatory variants influence risk, we will need to produce comprehensive maps of genomic gene expression and regulatory regions, such as enhancers and promoters in human brain tissue as well as in individual human neuronal subtypes. These efforts are gaining traction in several consortium projects, such as the CommonMind Consortium (commonmind.org), the Lieber Institute for Brain Development (www.libd.org), and the PsychENCODE project (psychencode.org). Given the limited availability of brain tissue, induced pluripotent stem cell–derived neuronal cell lines may provide another important resource for characterizing gene expression and regulatory regions (Brennand et al., 2011). Furthermore, these neuronal cell lines may form the basis for small-molecule screens to aid in the development of novel therapeutics. Integrative approaches that focus on developing biological networks from diverse sets of data can help focus attention on key biological drivers (Schadt and Bjorkegren, 2012). There are, or eventually will be, large-scale catalogues of gene expression, proteomics, protein interaction, drug interactions, and other data that will set the course for a more integrative biological approach.

Understanding how genetic variation influences gene regulation across developmental time points and in response to environmental stimuli is one of the key challenges for translating genetic discoveries into actionable biological hypotheses that can power a new round of therapeutic development. Fortunately, the current project of identifying genetic loci through GWAS and sequencing is moving forward at a rapid pace. This will lead to many more high-confidence loci that will more precisely pinpoint the most productive avenues for follow-up.

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