Review

Biological Psychiatry

Genetic Risk for Schizophrenia: Convergence on Synaptic Pathways Involved in Plasticity

Jeremy Hall, Simon Trent, Kerrie L. Thomas, Michael C. O'Donovan, and Michael J. Owen

ABSTRACT

Recent large-scale genomic studies have revealed two broad classes of risk alleles for schizophrenia: a polygenic component of risk mediated through multiple common risk variants and rarer more highly penetrant submicroscopic chromosomal deletions and duplications, known as copy number variants. The focus of this review is on the emerging findings from the latter and subsequent exome sequencing data of smaller, deleterious single nucleotide variants and indels. In these studies, schizophrenia patients were found to have enriched de novo mutations in genes belonging to the postsynaptic density at glutamatergic synapses, particularly components of the *N*-methyl-D-aspartate receptor signaling complex, including the PSD-95 complex, activity-regulated cytoskeleton-associated protein interactors, the fragile X mental retardation protein complex, voltage-gated calcium channels, and genes implicated in actin cytoskeletal dynamics. The convergence of these implicated genes onto a coherent biological pathway at the synapse, with a specific role in plasticity, provides a significant advance in understanding pathogenesis and points to new targets for biological investigation. We consider the implications of these studies in the context of existing genetic data and the potential need to reassess diagnostic boundaries of neuropsychiatric disorders before discussing ways forward for more directed mechanistic studies to develop stratified, novel therapeutic approaches in the future.

Keywords: Actin, ARC, Genetics, NMDA, Schizophrenia, Synapse.

http://dx.doi.org/10.1016/j.biopsych.2014.07.011

The primary motivation for studying the genetics of schizophrenia has been to illuminate the biological and pathological processes underlying this debilitating condition (1). Schizophrenia is a highly heritable disorder (2), leading to the hypothesis that genomic studies provide the best route to uncovering etiology and advancing treatment (3). However, the complex genetics of schizophrenia have proved remarkably difficult to unravel, requiring major technical advances and the collaborative efforts of many investigators to yield progress (4). As a result of these efforts, recent years have seen substantial progress in understanding the genetic architecture of schizophrenia, primarily through large-scale genomic approaches (5,6). While many aspects of genetic risk for the disorder remain to be fully interpreted, it has become increasingly clear that genes expressing proteins involved in the regulation of synaptic plasticity are particularly affected in schizophrenia (7) and also in related disorders such as autism spectrum disorder (ASD) (8,9). Here, we briefly review our current understanding of the genetic architecture of schizophrenia, before concentrating on recent studies of de novo and rare mutations in schizophrenia that have begun to implicate specific biological pathways at the synapse in pathogenesis.

THE EMERGING GENETIC ARCHITECTURE OF SCHIZOPHRENIA

Major progress in understanding the genetic architecture of schizophrenia has been leveraged through the application of whole-genome array-based association methods in large-scale collaborative studies. One of the early major insights into the genetics of schizophrenia was the significant polygenic component of risk for the disorder mediated through multiple risk variants, each individually contributing a very small component of risk but collectively accounting for a significant proportion of overall burden for the disorder (10–15). Moreover, an appreciable fraction of that risk was shared with other psychiatric disorders, in particular bipolar disorder (10). The second is that individually rare submicroscopic chromosomal deletions and duplications, known as copy number variants (CNVs), can also increase risk for the disorder in affected individuals with odds ratios that are much higher than those seen for common risk variants (16–19). While these array-based approaches fundamentally advanced our understanding of the genetic architecture of the disorder, the current robustly implicated loci collectively account for only a small proportion of overall risk (20,21). Notably, array-based methods are not well powered to detect the effect of rare, but potentially impactful, deleterious single nucleotide variants (SNVs) and other small variants such as indels (22). This issue has recently begun to be addressed by large-scale sequencing approaches, currently restricted to exome sequencing, which have the resolution to detect rare SNVs and indels contributing to risk (6,23). One particularly illuminating method to study rare variants (including both CNVs and SNVs) has been the use of case-parent trio designs to identify rare de novo variants that occur in patients but not in either of their

SEE COMMENTARY ON PAGE 3

 ISSN: 0006-3223

biological parents, potentially enhancing the likelihood that they are of pathogenic importance. This approach has been successful at implicating a role for loss-of-function de novo mutations in ASD and intellectual disability (ID) (24–27). The conclusions that can be derived from similar studies in schizophrenia populations have previously been less clear, possibly due to limited sample sizes and/or differences in analysis methods (28,29). However, very recently, the study of de novo variants in schizophrenia using trios and exome sequencing in case-control samples, combined with pathway-based analysis, has begun to produce some strikingly convergent findings regarding genetic pathways implicated in schizophrenia and these studies will be the focus of this review (30,31).

GENETIC FINDINGS FROM STUDIES OF DE NOVO AND RARE MUTATIONS IN SCHIZOPHRENIA

To investigate de novo CNV mutations in schizophrenia, Kirov *et al.* (7), building on previous evidence of elevated rates of CNVs (16,17,32), investigated de novo CNV mutations in over 600 schizophrenia parent-proband trios using array-based methods (7). The study identified de novo copy number variants in approximately 5% of cases, a rate 2 to 3 times higher than in control subjects depending on the size of the CNV. Mutations occurring de novo included previously identified risk CNVs at 3q29, 15q11.2, 15q13.3, and 16p11.2, as well as recurrent likely pathogenic CNVs affecting discs large 2 (*DLG2)* and Eu-HMTase1 (*EHMT1)*. However, the majority of the identified CNVs affect more than one gene, and along with the rarity of most individual CNVs, this makes it difficult to determine the biological processes implicated by these mutations. To overcome this problem, Kirov *et al.* (7) used a systems-based statistical approach to identify biological pathways enriched for genes within the de novo CNV loci. Using gene sets constructed from experimental proteomics, they identified significant enrichment at the CNV loci for genes involved in the postsynaptic density (PSD) proteome at synapses. This enrichment was largely explained by genes encoding components of the *N*-methyl-D-aspartate (NMDA) receptor signaling complex, including members of the disks large family of membrane associated guanylate kinases, and synaptic protein interactors of the activity-regulated cytoskeleton-associated protein (ARC) (also known as Arg3.1) (7,33,34). Other genes within schizophrenia-implicated CNVs included CYFIP1, a recently characterized protein binding partner of the fragile X mental retardation protein (FMRP), which together target and regulate the protein translation of a number of identified messenger RNAs (mRNAs) including ARC itself (35,36). Enrichment for genes encoding NMDA receptor complex proteins was further substantiated in analysis of CNVs from a large case-control dataset, although association with *ARC* was not seen in that analysis (7).

More recently, the same trio sample analysis has been coupled with exome sequencing to identify smaller rare de novo mutations associated with schizophrenia (30). Notably, this approach also revealed an excess of smaller deleterious de novo mutations in schizophrenia affecting the components of the NMDA receptor signaling complex and synaptic protein interactors of ARC, as well as an enrichment of mutations in the targets of the FMRP complex. A genome-wide, hypothesis-free analysis of this dataset based on gene ontology annotations additionally revealed a significant enrichment in schizophrenia for mutations in genes involved in actin filament bundle assembly (F-actin polymerization), a highly dynamic process implicated in the regulation of dendritic spine morphology and synaptic plasticity (37), which interacts with, and is regulated by, NMDA receptor-associated signaling and ARC. The finding of an enrichment of rare variants in PSD complex genes in schizophrenia was further supported in an independent large case-control exome sequencing study in which rare disruptive mutations were enriched in the NMDA receptor-associated PSD-95 protein complexes, ARC-interacting proteins, and targets of the FMRP (31). The latter study by Purcell *et al.* (31) also identified a significant enrichment of rare mutations in voltage-gated calcium ion channels (VGCCs), a class of genes known to also be central to the regulation of plasticity and previously strongly implicated in the pathogenesis of schizophrenia and related disorders through genome-wide association studies of common variants (38–41).

TOWARD MECHANISMS OF DISEASE

While the currently identified rare variants in schizophrenia still only account for a small proportion of overall risk, the convergent findings in these studies point strongly to the involvement of a related group of genes and proteins in the pathogenesis of schizophrenia. It is unlikely that these represent the only route to increased risk for this highly complex disorder, as the identified pathways were largely identified on the basis of empirical testing of prior hypotheses regarding the importance of synaptic processes in disease. However, the emergence of one clear pathway of risk would be a significant advance in understanding pathogenesis and point to new targets for biological investigation.

A notable feature of these findings is not only their consistency across different studies but also their convergence into a coherent set of biological processes involved in the regulation of plasticity, particularly at glutamatergic synapses (Figure 1). It is worth, therefore, considering briefly some ways in which the implicated genes and proteins are functionally interconnected. The NMDA receptor complex at the PSD, as well as VGCCs, play a central role in the control of associative plasticity at the synapse through the regulation of calcium entry (42–44). The entry of calcium ions into the postsynaptic dendrites through NMDA receptors and VGCCs leads to the activation of a series of second messenger systems, one consequence of which is altered regulation of gene expression through the activation of transcription factors including cyclic adenosine monophosphate response element-binding protein (45,46). Through this mechanism, the activation of NMDA receptor and VGCC signaling results in the onset of gene expression at activated synapses, including rapid transcription of the *Arc* gene, as well as other regulated genes, including *BDNF* (47,48). In rodents, *Arc* mRNA is one of a relatively small number of dendritically targeted mRNAs that accumulate in the region of activated synapses (47,49–51). Translation of Arc can thus occur in response to local demands by a process that is regulated by translational