Schizophrenia Susceptibility Genes: Emergence of Positional Candidates and Future Directions

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Schizophrenia is a devastating psychiatric disorder that affects ~1% of the population worldwide. It is characterized by so-called 'positive symptoms'–including delusions and hallucinations–'negative symptoms'–including blunted emotions and social isolation–and cognitive deficits–including impairments in attention and working memory. Studies of the inheritance of schizophrenia have revealed that it is a multifactorial disease that is characterized by multiple genetic susceptibility elements, each contributing a modest degree of risk. Linkage studies have identified several potential schizophrenia susceptibility loci, and in recent years major progress has been made in the identification of positional candidate susceptibility genes from these loci. A central goal of future research will be to use this genetic knowledge to generate specific animal models, characterize genetic interactions, investigate the disease pathophysiology and assist drug-discovery efforts.

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THE GENETIC COMPONENT OF SCHIZOPHRENIA

Schizophrenia is a severe psychiatric disorder that has a lifetime prevalence of ~1% in most of the populations studied (1). Similar to many common complex disorders, schizophrenia is a multifactorial disorder that is characterized by the contribution of multiple susceptibility genes that could act in conjunction with epigenetic processes and environmental factors (1). More than 20 genome-wide scans aiming to localize genes for this disorder have been reported. Two recent meta-analyses (2, 3) of the combined results of several genome-wide studies were performed to attempt to clarify inconsistencies among individual studies (meta-analytic approaches tend to amplify signals that are weak but consistent among studies and to attenuate signals that are strong but non-reproducible). One meta-analysis study confirmed 8p, 13q and 22q as valid linkage regions that probably contain one or more susceptibility genes. The second meta-analysis study (using a different statistical methodology) implicated 2p12–q22.1 (under stringent criteria), in addition to loci at 5q, 3p, 11q, 2q, 1q, 22q, 8p, 6p, 20p and 14q (under less-stringent thresholds). One interpretation of these findings is that approximately ten regions of the genome are likely to contain schizophrenia susceptibility genes, although this is almost certainly an underestimate because: (i) meta-analytic approaches tend to attenuate true strong signals that are population specific; and (ii) it is expected that many schizophrenia susceptibility genes are undetectable using linkage studies. The ultimate validation of the linkage results will be the identification of the susceptibility genes themselves. Indeed, in the past three years, the field of schizophrenia genetics has moved to the systematic positional cloning of susceptibility genes from chromosomal regions that were first identified by linkage approaches. These systematic efforts employ the genotyping of relatively large numbers of markers, including single nucleotide polymorphisms (SNPs) and linkage disequilibrium (LD) assays in family-based or case–control samples, and have resulted in the identification of strong positional candidate genes.

In this article, we discuss the genetic data regarding these strong positional candidate genes that were identified through the systematic follow-up of linkage signals (in chronological order of publication of the reports), in addition to their possible biological functions. We also discuss the genetic data for three candidate genes that are

Table 1. Schizophrenia positional candidate genes: chromosomal location and potential function

Gene symbol	Locus	Function	Refs
PRODH	22q11	Metabolism of L-proline; potential indirect influence on glutamate-mediated transmission	(5, 26)
DTNBP1	6p22	Member of dystrophin protein complex and biogenesis of lysosome-related organelle complex; potential presynaptic effects on glutamate release at excitatory synapses	(6, 37)
NRG1	8p12	Broad involvement in neuronal development, survival and synaptic function	(7, 44)
G72	13q34	Potential modulation of DAAO; indirect effects on glutamate-mediated signaling	(8, 54)
DISC1	1q42.1	Multifunctional; possible involvement in cytoskeletal and centromere function and in cell membrane receptor localization and signal transduction	(9, 61)
CAPON	1q22	Potential regulator of neuronal nitric oxide synthase association with PSD-95; implications for NMDA-receptor-coupled nitric oxide signaling	(10, 66)
ZDHHC8	22q11	Palmitoylation of PSD-95 and other substrates, potential implications for synaptic architecture and plasticity	(11, 69)
TAAR6	6q23	G-protein-coupled receptor for trace amines; potential role in neurotransmission	(12, 74)
EPN4	5q33	Clathrin-mediated pit formation and endocytosis; potential role in reuptake and storage of neurotransmitters	(13, 75)
GAB(A) receptors	5q34	GABA-mediated transmission	(14, 77, 78)
COMT	22q11	Metabolism of dopamine; regulation of extracellular dopamine levels in prefrontal cortex	(26, 79, 86)
RGS4	1q23	GTPase activator that modulates signal transduction through dopamine, metabotropic glutamate and muscarinic receptors	(91, 95, 96)
PPP3CC	8p21 g	Catalytic subunit of protein phosphatase calcineurin; subunit-specific function unknown; calcineurin is involved in synaptic plasticity and D1 receptor signaling	(97, 98)

located in the vicinity of linkage signals and are identified through multipronged candidate gene approaches, rather than systematic positional cloning approaches. Finally, we briefly comment on the statistical support for these findings and the future directions of genetic research in the context of advancing the understanding of how genetic factors contribute biologically to the disease process.

Owing to space limitations, other genes that could be good candidates (e.g. *DRD3*, *CHRNA2*, *BDNF*, *GAD2* and *AKT1*) but do not conform to the criteria outlined in the previous paragraph are not discussed in this article. Furthermore, we cite a limited number of follow-up genetic studies for each genetic finding, prioritizing the ones that use relatively large family-based samples. Such samples are considered more reliable than case–control samples in which factors such as hidden population stratification can confound the interpretation of a positive or negative finding (4).

GENES IDENTIFIED THROUGH THE SYSTEMATIC FOLLOW-UP OF LINKAGE SIGNALS

The first report of a strong positional candidate schizophrenia gene that was identified by a systematic fine-mapping approach within a region implicated by linkage analysis was published in 2002 (5). Soon thereafter, three additional reports described three new susceptibility genes that were identified using similar approaches (6–8). More recently, additional genes have been reported based on the systematic SNP-based follow-up analysis of linkage peaks (9–14) (Table 1).

PROLINE DEHYDROGENASE

The proline dehydrogenase (*PRODH*) gene is located on chromosome 22q11, which is a region implicated by some linkage studies (2, 3) and also frequently deleted in patients with schizophrenia (15). Several studies have established that the risk of schizophrenia for a patient with a 22q11 microdeletion is ~25–31 times the general population risk of 1% (16, 17) and that the rate of 22q11 microdeletions in schizophrenia, although relatively low, is ~12–80 times the estimated general population rate (15). This first unequivocal association between a well-defined genetic lesion and schizophrenia facilitated fine-mapping efforts at this locus. LD analysis in family samples (triads) that tested for preferential transmission of 72 SNPs and multi-SNP haplotypes from parents to affected (non-deleted) individuals identified the over-transmission

of a gene variant located at the 30 end of the *PRODH* gene (5, 18). This finding was recently replicated in two independent family-based samples, including a large collection of 528 families from China (19) and 274 families of Ashkenazi Jewish origin (20), although one negative family study has also been reported (21). Moreover, 30-end variants of the gene were also identified as being a risk factor for the development of psychotic symptoms during adolescence in children with 22q11 microdeletions (22). Although the implicated variants are consistently located at the 3′ end of the gene, their functional consequences are still unknown. However, rare variants of the *PRODH* gene affecting highly conserved amino acids (generated through gene conversion from a nearby pseudogene) that are enriched to various degrees in samples of individuals with schizophrenia have been identified (5). This discovery gained additional support in an independent set of studies (23, 24), and functional analysis has linked several of these variants with marked reductions in enzymatic activity (25). *PRODH* encodes an enzyme that metabolizes L-proline—a putative neuromodulatory amino acid that could directly influence glutamate-mediated transmission, which is believed to have a crucial role in the pathophysiology of schizophrenia (26). In addition, *Prodh*-deficient mice show dysregulation of cortical dopamine turnover and transmission that is reminiscent of schizophrenia in humans (26).

DYSTROBREVIN-BINDING PROTEIN 1

The dystrobrevin-binding protein 1, or dysbindin, (*DTNBP1*) gene maps within a broad region on chromosome 6p where there is evidence of linkage to schizophrenia in Irish families (27). Genetic variants of this gene are associated with schizophrenia (6) in the same families. This finding has been replicated in several additional samples, including some family-based samples, but negative studies have also been reported and considerable putative allelic heterogeneity was evident among the positive studies (28–32). Initial expression and functional studies provide some additional support for a role for *DTNBP1* in schizophrenia. *DTNBP1* is a member of the biogenesis of lysosome-related organelles complex (33) and the dystrophin protein complex (34). It has a widespread distribution in the brain, including expression in pyramidal neurons in the hippocampus and the dorsolateral prefrontal cortex (DLPFC). Two recent studies provide evidence that *DTNBP1* expression is decreased in schizophrenia in both the DLPFC and the excitatory pathways of the hippocampus (35, 36). A substantial fraction of DTNBP1 is presynaptically localized, and preliminary *in vitro* evidence suggests that knockdown of endogenous dysbindin protein results in the reduction of presynaptic protein expression and glutamate release, indicating that dysbindin might influence exocytotic glutamate release (37).

NEUREGULIN 1

Neuregulin 1 (*NRG1*) was identified as being a susceptibility gene for schizophrenia following a genome-wide linkage scan of 33 Icelandic families with schizophrenia that highlighted a locus on chromosome 8p (7). Fine-mapping of the 8p locus, together with haplotype association analysis of a large number of patients with schizophrenia and control individuals, narrowed the region of interest to the 5′ end of the *NRG1* gene. A core haplotype at the 50 end of the gene comprising several markers within a 290-kb LD block showed highly significant association with schizophrenia (7). The functional consequences of this gene variant are still unknown. *NRG1* association with schizophrenia has been observed in several additional samples, including some reliable family-based samples, although considerable allelic heterogeneity was evident in these studies (38–41). Negative studies have also been reported (20, 42, 43). The *NRG1* gene encodes a well-characterized protein that is involved in many neuronal functions, ranging from neuronal survival to myelination and synaptic plasticity (44).

G72

The *G72* gene is located within a broad linkage peak that extends from 13q32 to q34, where there is evidence of linkage to both schizophrenia and bipolar disorder (2, 45). Significant association with schizophrenia was observed for several SNPs and haplotypes at the *G72* locus in a French–Canadian case–control sample, and the association for two SNPs was replicated in a Russian case–control cohort (8). Interestingly, a subsequent study provided evidence of an association between variants at the *G72* locus and bipolar disorder (46). The association of *G72* with schizophrenia has been observed in several additional samples with evidence of allelic heterogeneity, although negative studies have also been reported (47–53). Expression and functional studies indicate a potential interaction between G72 and D-amino acid oxidase (DAAO) that modulates the DAAO enzymatic activity and, thus, could indirectly affect glutamate-mediated signaling (8, 54). However, this interaction remains to be demonstrated *in vivo*.

DISRUPTED IN SCHIZOPHRENIA 1

A balanced translocation involving chromosomes 1 and 11 (1q42.1;11q14.3) was strongly linked to psychopathology, including schizophrenia, depression and mania in a large Scottish family. The 1q breakpoint was cloned and was found to involve two genes: disrupted in schizophrenia (*DISC)1* and *DISC2* (the latter is a noncoding, presumably regulatory, RNA) (55). Although *DISC1* was identified five years ago, it was a more recent large-scale linkage (56) and follow-up systematic LD analysis in families from Finland that identified *DISC1* as being a positional candidate from the 1q42 locus (9). *DISC1* association with schizophrenia has been observed in several additional samples with evidence of allelic heterogeneity, although negative studies have also been reported (20, 57, 58). Interestingly, a family afflicted with schizophrenia and schizoaffective disorder was recently shown to have segregated a rare frameshift variant of the gene (59). *DISC1* is a complex gene, the involvement of which in development and synaptic plasticity is poorly understood. The protein it encodes is associated with numerous cytoskeletal proteins and could be involved in centrosomal and microtubule function, cell migration, neurite outgrowth, membrane trafficking of receptors, mitochondrial function and, possibly, phosphodiesterase function (60, 61).

C-TERMINAL PDZ LIGAND OF NEURONAL NITRIC OXIDE SYNTHASE

LD analysis, using 14 microsatellite markers and 15 SNPs from a subregion of a previously reported linkage locus at 1q22 (62), in large Canadian families with schizophrenia produced nominally significant evidence of LD between schizophrenia and a subset of markers that is located within the genomic extent of the C-terminal PDZ ligand of neuronal nitric oxide synthase (*CAPON*) gene, making *CAPON* a prime positional candidate from the schizophrenia susceptibility locus on 1q22 (10). An abnormal expression pattern of this gene was observed in the brains of individuals with schizophrenia or bipolar disorder (63). Two replication studies, one positive and one negative, have been reported (64, 65). *CAPON* is involved in NMDA-receptor-coupled nitric oxide signaling (66).

ZDHHC8

The involvement of this gene in schizophrenia was identified in the same LD screen of the 22q11 locus that led to the discovery of the *PRODH*–schizophrenia association (5, 18). More recently, it was shown that one of the *ZDHHC8* risk alleles (at SNP rs175174), located in intron 4, affects the ratio of an intron-4-containing unspliced form (encoding a putative truncated inactive form of the protein) to the fully spliced active form (11). The relatively subtle consequent change in the level of the active protein led to ~1.5-fold increase in disease risk in two tested family samples (11). Other variants of the gene (affecting distinct aspects of its complex splicing or its expression level) could modulate the disease risk in other samples. One positive family-based study and one negative family-based study have been reported (67, 68). The effect of the gene is predicted to be much stronger in individuals with 22q11 deletions and schizophrenia, in which a 50% (or ~65% when the non-deleted allele carries, for example, the risk SNP rs175174 variant) decrease in ZDHHC8 activity levels is predicted. *ZDHHC8* encodes a transmembrane palmitoyltransferase that modifies, among other targets, postsynaptic density protein of 95 kDa (PSD-95) and could have an important role in excitatory synaptic transmission (69).

TRACE AMINE RECEPTOR 4

Trace amine receptor 4 (*TAAR6*) was identified in an LD study (12) of European-ancestry and African–American families with schizophrenia that previously showed evidence of linkage to 6q13–q26 (70). This LD study (12) focused on subregion q23.2, which contains several functional candidate genes for schizophrenia. A primary screen using 31 SNPs and a follow-up higher-density screen using 23 SNPs over a 21.6-kb region highlighted *TAAR6* (12) as being a prime positional candidate gene from the schizophrenia susceptibility locus on 6q23.2. Two negative replication studies have been reported (71, 72). However, an independent study (73) implicated *TAAR6* (which is a G-protein-coupled receptor that is widely expressed in the brain (12, 74)) in susceptibility to bipolar disorder.

EPSIN 4

Chromosome 5q33 is a region that has shown evidence of linkage to schizophrenia in four independent linkage studies. Four adjacent markers (and associated haplotypes) at the 5′ end of the epsin 4 (*EPN4*) gene, which is located in this region, showed significant evidence of LD with schizophrenia in a fine-mapping study that used 450 unrelated English, Irish, Welsh and Scottish research subjects with schizophrenia and 450 ancestrally matched supernormal controls (13). The

EPN4 gene encodes the clathrin-associated protein enthoprotin, which has a role in the transport and stability of neurotransmitter vesicles at the synapses and within neurons (75). No replication studies have been reported.

γ-AMINOBUTYRIC ACID A RECEPTOR SUBUNIT GENE CLUSTER

An early genome-wide linkage scan in Portuguese families with schizophrenia identified a risk locus on chromosome 5q31–q35 (76) – a finding supported by subsequent meta-analysis. A two-stage candidate gene association approach focused on a group of γ-aminobutyric acid (GABA)A receptor subunit genes (*GABRA1*, *GABRA6*, *GABRB2*, *GABRG2* and *GABRP*) within this linkage peak (14). In the first stage, associations were detected in a Portuguese patient sample with SNPs and haplotypes in *GABRA1*, *GABRP* and *GABRA6*. The *GABRA1* and *GABRP* findings were replicated in the second stage in an independent German family-based sample (14). These genes are plausible candidates based on prior speculation about the involvement of the GABA system in schizophrenia (77, 78).

CANDIDATE GENES LOCATED IN THE VICINITY OF LINKAGE SIGNALS IDENTIFIED THROUGH CANDIDATE GENE APPROACHES

The candidacy of the genes described in this section is based on convergent genetic and biological evidence. Although unproven, the recurrent observation of the clustering of candidate susceptibility genes might indicate that more than one gene could contribute to at least some of the linkage signals in psychiatric disorders.

CATECHOL-*O*-METHYLTRANSFERASE

The catechol-*O*-methyltransferase (*COMT*) gene is located in the 22q11 locus between the *PRODH* and *ZDHHC8* genes, and is a strong positional and functional candidate. COMT metabolizes released dopamine, and variation in COMT activity could have effects that are specific to the prefrontal cortex (PFC). This regionally selective effect of COMT might depend on the relatively low abundance and non-synaptic localization of the dopamine transporter in the PFC compared with the striatum (79). A high-activity form of the enzyme (Val158) was proposed to increase susceptibility to schizophrenia (79). Association studies using the clinical diagnosis of schizophrenia as phenotype are equivocal (80–86), although this form of the gene modulated executive function in some studies, which is affected in individuals with schizophrenia (79, 87). More-recent studies in animal models, however, indicate that low activity of this enzyme could be a risk factor for schizophrenia and that COMT might function as part of the genome buffering capacity to counteract the effect of other primary mutations that affect dopamine turnover and signaling in the frontal cortex (26). This prediction is supported by the results of a longitudinal follow-up study of children with 22q11 microdeletions. This study showed that the low-activity form of the enzyme (Met158) is a risk factor for decline in prefrontal cortical volume and cognition, and the consequent development of psychotic symptoms, during adolescence in these children (22). Overall, the potential contribution of *COMT* to schizophrenia is likely to be complex. In addition, the gene seems to have a functionally complex allelic architecture, with some alleles (Val158Met) determining the stability of the protein (88) and others determining the level of expression (86, 89).

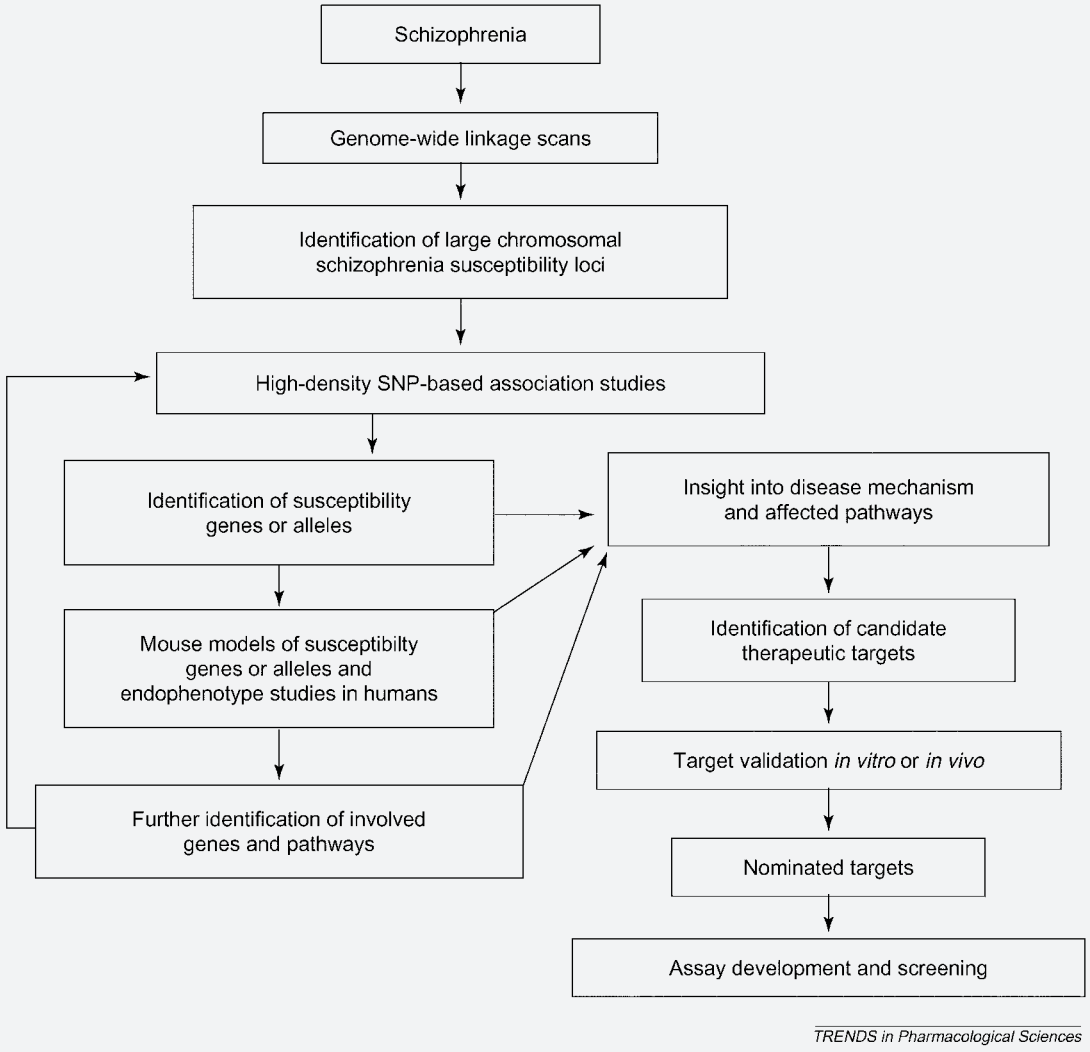
REGULATOR OF G-PROTEIN SIGNALING 4

Regulator of G-protein signaling (*RGS*)*4* was initially identified as being the only transcript (out of 7800 sampled by Mirnics *et al.* (90)) that was consistently reduced in the DLPFC of individuals with schizophrenia. The gene maps to 1q21–22, 0.7 Mb from *CAPON*. Chowdari *et al.* (91) genotyped 13 SNPs across a 300-kb segment spanning the gene in several independent datasets and found weak evidence of association with schizophrenia in each of the samples, although not in an allele-consistent manner. In most cases, association was present for a haplotype block stretching from intron 1 to several kilobase pairs upstream of the transcription start site. Independent replications have been reported but negative studies also exist (20, 92–94). Of the 19 human RGS transcripts, RGS4 shows the highest expression in the brain compared with all other tissues and is abundant in the cerebral cortex (95). RGS4 is a GTPase activator that desensitizes $G_{i/o}$ and G_q and, thereby, negatively modulates G-protein-mediated signaling by dopamine, metabotropic glutamate and muscarinic receptors (96).

CALCINEURIN γ CATALYTIC SUBUNIT

Forebrain-specific calcineurin-knockout mice were reported to have a spectrum of behavioral abnormalities related to altered behaviors observed in

Figure 1. Flow diagram of the schizophrenia genetics research process and its potential application to drug discovery. In this scheme, the identification of susceptibility genes by SNP-based association studies, coupled with the generation and characterization of relevant mouse models and endophenotype studies in humans, forms a comprehensive system with which to identify the genes and molecular pathways involved in schizophrenia pathogenesis. This knowledge base provides a framework for mechanism-based drug-discovery efforts.



schizophrenia patients (97). Follow-up studies identified calcineurin γ catalytic subunit (*PPP3CC*) as a potential schizophrenia susceptibility gene (98) and led to the proposal that alterations in calcineurin signaling contribute to schizophrenia pathogenesis. In support of this proposal, the gene is downregulated in the hippocampus of individuals with schizophrenia (99). The genetic association was not replicated, however, in a sample of Ashkenazi Jewish nuclear families (20). *PPP3CC* is located at 8p21.3, 10 Mb from *NRG1* but adjacent to previously described linkage signals (98). Calcineurin is a multifunctional calcium-dependent serine/threonine phosphatase that is centrally involved in many aspects of synaptic plasticity. It has particular roles in glutamate and dopamine signaling and their interactions, including the regulation of DARPP32, a molecular node of convergence between dopamine receptor 1 and NMDA receptor signaling pathways (97).

STATISTICAL SUPPORT AND GENERALIZATION OF GENETIC FINDINGS

The statistical burden of proof is lower for genes identified through systematic follow-up of linkage

signals compared with genes picked in an essentially random fashion, irrespective of their location relative to linkage signals (for details, see Ref. (100)). Nevertheless, support for at least some of the findings described (e.g. *PRODH*, *DTNBP1*, *NRG1*, *G72*, *DISC1* and *COMT*) seems to be strong, based on a combination of criteria: the degree of statistical significance, the reproducibility of the associations in independent family samples, the identification of independent rare risk alleles and the consistent findings from animal model studies and endophenotype-based studies in humans.

However, it is too early to draw firm conclusions about the generalization of these findings among different samples and populations based on the published replication studies, primarily because of issues regarding the extent of coverage of the implicated loci, the structure of the samples used in replication studies and publication bias. One important issue of concern regards the structure of the tested replication samples. It is becoming increasingly clear that, when allele frequencies differ notably among subpopulations that are not represented equally among cases and controls (population stratification), unreliable results can be obtained (4). The possibility, therefore, that replication studies using case–control samples represent false positives (or negatives) must be considered seriously. This is a problem that is relevant to all common complex disorders but it is likely to be more pronounced in genetic studies of psychiatric disorders, which are confounded by a larger degree of phenotypic heterogeneity. In addition, several of the employed 'replication' samples have been used repeatedly in genetic association studies, making the issue of multiple-testing corrections extremely relevant. These are important considerations given some striking inconsistencies among the variant alleles and haplotypes implicated in replication studies for at least some of the genes. It should be noted, however, that such inconsistencies are sometimes observed even in more-reliable family-based samples and can be explained in some instances by the presence of distinct variations that affect different functional elements within the gene that have emerged independently on a more recent ancestral background.

Future directions of genetic research

Clearly, there are still several 'orphan' linkage loci that await the identification of positional candidate genes, a task that will be facilitated by the sequencing of the human genome. It is likely that additional genes will also be identified through the genome-wide association studies that are starting to be implemented. As more genes are identified, a goal of future research will be to understand the functional implications and interactions of the susceptibility genes and their variants in the context of schizophrenia. Genetic studies of endophenotypes (79) (provided they are designed to avoid all of the pitfalls described earlier that are associated with genetic studies of the clinical syndrome), in addition to biological data from animal model studies, promise to advance the understanding of the disease pathophysiology in the coming years.

The question of true biological interaction among susceptibility genes is also extremely important in the field of complex psychiatric genetics, and might ultimately be better answered primarily by using a combination of molecular-based and animal-model-based approaches, as suggested by recent studies (26,60). Understanding the interactions between individual susceptibility elements could eventually aid the specificity of diagnosis and lead to the design of custom therapies with fewer side-effects and more-positive long-term disease outcomes for patients with specific genetic predispositions (Figure 1).

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