Published in final edited form as:

Hum Genet. 2012 March ; 131(3): 373–391. doi:10.1007/s00439-011-1082-x.

Ion channels and schizophrenia: a gene set-based analytic approach to GWAS data for biological hypothesis testing

Kathleen Askland,

Department of Psychiatry and Human Behavior, Butler Hospital, Brown University, 345 Blackstone Blvd, Providence, RI 02906, USA

Cynthia Read,

Department of Psychiatry and Human Behavior, Butler Hospital, Brown University, 345 Blackstone Blvd, Providence, RI 02906, USA

Chloe O'Connell, and

Brown University, Providence, RI, USA

Jason H. Moore

Departments of Genetics and Community and Family Medicine, Institute for Quantitative Biomedical Sciences, Dartmouth College, Lebanon, NH 03756, USA

Kathleen Askland: k.askland@gmail.com

Abstract

Schizophrenia is a complex genetic disorder. Gene set-based analytic (GSA) methods have been widely applied for exploratory analyses of large, high-throughput datasets, but less commonly employed for biological hypothesis testing. Our primary hypothesis is that variation in ion channel genes contribute to the genetic susceptibility to schizophrenia. We applied Exploratory Visual Analysis (EVA), one GSA application, to analyze European-American (EA) and African-American (AA) schizophrenia genome-wide association study datasets for statistical enrichment of ion channel gene sets, comparing GSA results derived under three SNP-to-gene mapping strategies: (1) GENIC; (2) 500-Kb; (3) 2.5-Mb and three complimentary SNP-to-gene statistical reduction methods: (1) minimum p value (pMIN); (2) a novel method, proportion of SNPs per Gene with p -values below a pre-defined α -threshold (PROP); and (3) the truncated product method (TPM). In the EA analyses, ion channel gene set(s) were enriched under all mapping and statistical approaches. In the AA analysis, ion channel gene set(s) were significantly enriched under pMIN for all mapping strategies and under PROP for broader mapping strategies. Less extensive enrichment in the AA sample may reflect true ethnic differences in susceptibility, sampling or case ascertainment differences, or higher dimensionality relative to sample size of the AA data. More consistent findings under broader mapping strategies may reflect enhanced power due to increased SNP inclusion, enhanced capture of effects over extended haplotypes or significant contributions from regulatory regions. While extensive pMIN findings may reflect gene size bias, the extent and significance of PROP and TPM findings suggest that common variation at ion channel genes may capture some of the heritability of schizophrenia.

Introduction

General trends in neuropsychiatric genetics

For most neuropsychiatric disorders, strong association or linkage findings have been uncommon; replication has been sparse. This, in combination with generally inconclusive results from the recent swell of GWAS work (Alaerts and Del-Favero 2009; Medina et al. 2009; Moskvina et al. 2009), has led investigators to conclude that most neuro-psychiatric disorders have complex genetic architectures. Whether this complexity is mediated by genetic interactions (expressed at the individual level) or genetic heterogeneity (expressed at the population level) is a matter of ongoing debate. Nonetheless, given this complexity, it is unlikely that further traditional genetic investigations will yield conclusive results.

Sequencing technology is rapidly becoming logistically and financially feasible for many institutions. However, the rate-limiting factor in genetic discovery under these methods will be the development of computational and statistical approaches that can extract meaning from the vast sequencing data generated. Given the 3 billion potential sites of variation across the genome, and the likely contribution of low-frequency and rare variants to disease risk, even comprehensive whole-genome sequencing of a massive target population may not produce readily obvious patterns of variation with clear causative implications.

Alternatively, we suggest that a fruitful approach to genetic discovery using data at the whole genome level, including that derived from GWAS or sequencing, will be twofold. First, it will be hypothesis driven. Second, it will entail a methodology capable of measuring the collective contribution to overall risk of disease by biologically defined sets of variants.

Schizophrenia

A growing body of research within different psychiatric disorders, including autism, obsessive-compulsive disorder (OCD), attention deficit hyperactivity disorder (ADHD), and schizophrenia, supports dimensional symptom structures. Symptoms of schizophrenia may be roughly defined by dysfunction along three, at least partially distinct, phenotypic dimensions: executive functioning (symptoms of cognitive disorganization), social responsiveness ('negative' symptoms, or asociality) and sensory-perceptual integration (classical 'positive' symptoms, including hallucinations and delusions). Replicable findings localize executive functional capacities to prefrontal cortex (Camchong et al. 2006; Huffaker et al. 2009; MacDonald et al. 2005; Nigg and Casey 2005; Perlstein et al. 2001; Russell et al. 2003), social responsiveness to medial prefrontal circuitry and cingulate (Ashwin et al. 2007; Fujiwara et al. 2007; Pavuluri et al. 2007; Singer 2007), and sensory-perceptual integration to higher-order sensory/heteromodal association cortices (Booth et al. 2002; Buchanan et al. 2004; Ho et al. 2009; Keshavan et al. 2003; Silbersweig and Stern 1998). Despite advances in our understanding of schizophrenia at the level of both phenotype and brain system, however, untangling the molecular and genetic substrates of schizophrenia has been especially challenging.

Most generally, the accumulated results of genetic studies to date strongly suggest that schizophrenia is a complex genetic disorder whose genetic architecture is likely characterized by locus, allelic and trait heterogeneity at the population level with genetic interactions and epigenetic mechanisms mediating disease expression at the individual level (see Moore et al. 2006; Moore and Williams 2005; Thornton-Wells et al. 2004 for reviews of these concepts). Additionally, evidence is mounting that disease risk in the population is, in part, mediated by inherited or de novo rare variants (Alaerts and Del-Favero 2009; Gorlov et al. 2008; McClellan et al. 2007) or copy number variations (CNVs) (Alaerts and Del-Favero 2009; Glessner et al. 2010; ISC 2008; McCarroll 2008; Need et al. 2009; Stefansson et al. 2008; Walsh et al. 2008).

Specific genetic and pathophysiologic hypotheses in schizophrenia have evolved over many decades; the most longstanding centers on dopaminergic dysfunction (Glatt et al. 2003; Miyamoto et al. 2003; Verhoeff 1999). Alternate hypotheses tend to focus on the role of other neuro-transmitter systems including GABAergic (Lang et al. 2007; Petryshen et al. 2005; Reynolds and Harte 2007), glutamatergic (Corti et al. 2011; Dekeyne and Millan 2003; Lang et al. 2007; Nudmamud-Thanoi et al. 2007; Reynolds and Harte 2007), serotonergic (Gaddum and Hameed 1954; Schildkraut 1965; Woolley and Shaw 1954) and noradrenergic (Breier et al. 1998; Farley et al. 1978; Siuta et al. 2010; Sternberg et al. 1982; Wise and Stein 1973) systems and interactions thereof (see (Lang et al. 2007) and (Meltzer and Roth 1995) for recent reviews).

Importantly, the functional specificity of each of the putatively relevant CNS neurotransmitter systems is sub-served not only by the neurotransmitter-specific proteins (i.e., receptors, transporters, metabolic enzymes) expressed therein, but also by the unique subset of ion channels expressed in each system (Beaulieu and Gainetdinov 2011; Krause et al. 2002; Lachowicz and Sibley 1997; Missale et al. 1998; Nicoll 1988; Wolfart et al. 2001). However, despite a growing body of genetic, pharmacologic, functional and brain expression analyses implicating ion channels as primary molecular mediators of numerous physiological and pathophysiological processes likely involved in schizophrenia (see references discussed below), we could identify no published reports explicitly testing an ion channel hypothesis in schizophrenia.

Hypothesis and aims

The primary working hypothesis of this study is that genetic variation in a host of ion channel genes (including voltage- and ligand-gated subtypes) contribute to susceptibility to schizophrenia across affected populations. Moreover, we suspect the genetic architecture of these disorders, and of the contributions of ion channels in particular, is characterized by extensive locus, allelic and trait heterogeneity. We hypothesize that across the entire population of individuals affected by schizophrenia, there are many distinct ion channel gene variants and that most such variants are uncommon to rare. That said, we expect that some proportion of disease risk may be mediated by variants in linkage disequilibrium with the common tagSNPs representing ion channel genes in GWAS arrays.

Because our hypothesis entails that substantial locus and allelic heterogeneity characterize the risk architecture of the ion channel gene contribution to disease risk, we submit that gene set-based analytic approaches (GSAs) are well suited to the detection of such genetic contributions in case-control data. Our primary aim, then, was to test our biological hypothesis using GSA. Secondary aims were to compare results derived using (1) three SNP-to-gene mapping approaches and (2) three analytic approaches to SNP-to-gene reduction for GSA.

Background and rationale for our hypothesis, aims and methods

Ion channels and schizophrenia

Systematic examination of the role of ion channels in mediating risk of schizophrenia has not yet been undertaken. However, pathophysiologic, etiologic and pharmacologic roles for particular ion channel genes or proteins in schizophrenia have been explored.

Most extensively studied among the ion channel candidates is the calcium-activated potassium channel, KCNN3. Localized to chromosomal band 1q21.3 (Wittekindt et al. 1998), this gene was proposed as a functional candidate for schizophrenia and bipolar disorder because of its role in mediating neuronal excitability through its regulation of the slow component of hyperpolarization (Ritsner et al. 2002). As reviewed by Ritsner et al.

(2002), several early association studies found evidence for association between schizophrenia and KCNN3 CAG repeat length (Bowen et al. 1998; Cardno et al. 1999; Chandy et al. 1998; Dror et al. 1999; Stober et al. 1998), though several follow-up analyses did not replicate the initial findings (Antonarakis et al. 1999; Austin et al. 1999; Chowdari et al. 2000; Joobar et al. 1999; Li et al. 1998; Rohrmeier et al. 1999; Stober et al. 2000; Ujike et al. 2001). Despite the lack of consistent replication, several lines of evidence provide additional support (Ritsner et al. 2002), including: a large genomewide linkage scan in 22 extended schizophrenia pedigrees which revealed highly significant linkage evidence at 1q21-q22 (Brzustowicz et al. 2000), the finding of a KCNN3 mutation in a schizophrenic patient (Bowen et al. 2001) that trafficked to the nucleus and suppressed endogenous KCNN3 in a dominant negative manner (Miller et al. 2001), and an independent finding that schizophrenic patients had significantly different allele sizes than controls (Saleem et al. 2000). The complicated trajectory of investigations of KCNN3 in schizophrenia demonstrates a relatively typical pattern in candidate gene investigations across many neuropsychiatric disorders. However, under an architecture characterized by extensive genetic heterogeneity, such a trajectory of across samples and methodologic approaches is to be expected.

More recent literature from various disciplines implicates a host of other ion channel genes in schizophrenia. Candidate gene association (Bigos et al. 2010; Green et al. 2010; Nyegaard et al. 2010; Wei and Hemmings 2006), genome-wide (Curtis et al. 2011; Tam et al. 2010), gene-wide (Moskvina et al. 2009), gene set enrichment (Glessner et al. 2010) and meta-analytic (Huffaker et al. 2009) studies have yielded evidence for association between schizophrenia and several voltage-gated ion channels (KCNH2, KCNE1, KCNE2CACNA1C, CACNA1B, CACNA1F, CACNA5). In addition, Papassotiropoulos et al. (2011) were able to replicate (in three independent samples) evidence of association of a voltage-gated sodium channel (SCN1A) polymorphism with human short-term memory performance, known to be impaired in schizophrenia. Moreover, a further fMRI investigation demonstrated SCN1A allele-dependent activation differences in brain regions typically involved in working memory processes and implicated in schizophrenia.

Numerous functional, pharmacologic and translational studies have demonstrated functional links between schizophrenia, related endophenotypes or phenotypic dimensions and physiological and/or pathophysiological functioning in particular ion channel classes, including voltage-gated potassium channels [KCNH2(Huffaker et al. 2009); KCNQ channels (Fedorenko et al. 2008; Kapfhamer et al. 2010; Sotty et al. 2009); CHIP3, KCNA1, KCNAB1(Duncan et al. 2008)], outward-rectifying potassium channels [KCNK10 (Xiao et al. 2009); KCNK2/10, TREK channels (Thummler et al. 2007)], large conductance, voltage- and calcium-sensitive potassium channels [KCNMB1(Wong et al. 2005)], and voltage-gated calcium channels (CAC-NA1C (Bigos et al. 2010)), as well as broader classes of ion channels (Butler-Munro et al. 2010). Notably, in their recent review of mRNA expression profiling studies in animal models of schizophrenia, Van Schijndel and Martens (2010) note that while the majority of the genes implicated across the 26 profiling studies they reviewed were implicated only in a single study, genes belonging to sodium and potassium voltage-gated channel families were implicated in seven. Finally, reviews have highlighted the promise of ion channels as therapeutic targets in pharmacologic development due, in particular, to their membrane localization, structural heterogeneity, highly differentiated and specialized CNS distribution, essential roles in diverse physiological processes and likely relevance to several neuropathological diseases including schizophrenia (Ashcroft 2006; Camerino et al. 2008; Hansen et al. 2008; Judge et al. 2007).

Alongside the specific evidence supporting an etiologic or pathophysiologic role for ion channels in schizophrenia, evidence from molecular genetics and biology supports the

candidacy of ion channels in mediating risk for human neuropsychiatric disorders more generally. Approximately 410 human genes encode known ion channel proteins (eEnsembl, Ensembl release 62, accessed 04/29/11). Ion channels have important roles in diverse processes including nerve excitation, cell proliferation, sensory transduction, and learning and memory (Ashcroft 2006). At least 60 ion channel genes have been associated with human disease (i.e., channelopathies); many channelopathies are genetically heterogeneous with the same phenotype being caused by mutations in different ion channel genes (locus heterogeneity) or affecting different alleles within the same gene (allelic heterogeneity). In addition, there are several mechanisms by which genetic mutation can disrupt channel function, including alteration of channel activity (e.g., disruption of ligand binding or open probability), alteration in channel synthesis or membrane targeting or, less commonly, alteration of single channel conductance. Furthermore, the form and severity of the clinical phenotype conferred by a particular channel mutation depends on the relative contribution the mutated channel makes to the electrical activity of the cells relevant to the phenotype of interest. In other words, there may be minimal disruption to electrical activity in some neurons and significant disruption in others, depending on the complement of ion channels expressed in each. That said, in some cases very subtle change in channel activity conferred by mutation can produce severe disease (Ashcroft 2006; Du et al. 2005).

The dimensional nature of human neuropsychiatric disorders necessitates that their molecular substrates have tightly regulated and specific expression patterns in order to preferentially disrupt specialized cognitive functions, while preserving basic brain functions and leaving other higher-order processes largely intact. There are several mechanisms by which the expression of ion channel proteins in the human CNS is regulated. First, there is a multitude of discrete isoforms and molecular subunit combinations within each ion channel subclass, each of which has distributional specificity that is controlled developmentally, temporally and adaptively (Abernethy and Soldatov 2002; Ader et al. 2008; Angelino and Brenner 2007; Anselmi et al. 2007; Catterall and Few 2008; Cerda et al. 2011; Chahine et al. 2008; Dai et al. 2009; Gaudet 2007; Goetz et al. 2007; Gotti et al. 2009; Hashimoto and Panchenko 2010; Jegla et al. 2009; Jensen et al. 2008; Kurokawa et al. 2009; Levitan 2006; Marionneau et al. 2011; Milstein et al. 2007; Molina et al. 2006; Olsen and Sieghart 2009; Paoletti 2011; Perrais et al. 2010; Picton and Fisher 2007; Tseng et al. 2007; Vanoye et al. 2010; Waxman et al. 2000; Yang et al. 2009). Second, most ion channels have known alternatively spliced isoforms, offering additional modulatory capacity (Chahine et al. 2008; Daniel and Ohman 2009; Honore 2008; Jenkinson 2006; Kurokawa et al. 2009; Lin et al. 2009; Mohapatra et al. 2007; Vazquez and Valverde 2006; Yan et al. 2008). Third, there are likely an infinite number of isoform- or complex-specific interactions with other CNS genes that further extend the capacity for developmental, temporal, and adaptive modulation (Chahine et al. 2008; Dib-Hajj and Waxman 2010; Fernandez-Alacid et al. 2009; Thompson and Begenisich 2006; Waxman 2007). Thus, ion channels are quite likely primary molecular mediators of the functional specificity of brain subsystems and circuitry and, therefore, are rational candidates in the search for molecular substrates of complex neuropsychiatric disorders.

From an evolutionary perspective, ion channels, relative to most other neuronally expressed proteins, demonstrate a remarkable, and relatively unique, simultaneity of phylogenetic conservation (Anderson and Greenberg 2001; Strong and Gutman 1993; Trimmer and Rhodes 2004), species differentiation (Abernethy and Soldatov 2002; Anderson and Greenberg 2001; Strong and Gutman 1993), structural diversity (Anderson and Greenberg 2001; Ashcroft 2006; Strong and Gutman 1993; Waxman 2000), and developmental and distributional specificity (Abernethy and Soldatov 2002; Anderson and Greenberg 2001; Freudenberg et al. 2007; Mechaly et al. 2005; Sailer et al. 2004; Stocker 2004; Stocker et al. 2004; Waxman 2000; Wolfart et al. 2001). Together, these qualities support a critical role

for ion channels in the functioning and functional specialization of nervous systems, from the most primitive eukaryotes to human beings. In a very compelling bioinformatics analysis of human CNS-expressed ion channels, Freudenberg et al. (2007) examined the phylogenetic attributes of human ion channels relative to other CNS-expressed genes (Freudenberg et al. 2007). First, they observed that relative to invertebrate, vertebrate genomes had an increased percentage of ion channel genes. This pattern was shared by other important CNS-expressed genes. Second, they found that, in contrast to the other CNS-expressed genes, ion channels have longer intron and protein sequences, features typical of genes with more specialized expression patterns. Third, they found that ion channels, in contrast to non-channel genes, have increased human-rodent transcription start site conservation, indicating the functional relevance of mutations affecting ion channel transcriptional regulation. Taken together, the molecular genetic and phylogenetic characteristics of ion channels strongly support their candidacy in the genetic risk of neuropsychiatric disorders.

Thus, we propose that ion channels, as a class, represent ideal etiologic candidates underlying the genetic susceptibility to schizophrenia. Moreover, given the diversity of the implicated genes within this class and the likely heterogeneous nature of the underlying genetic architecture of schizophrenia, we submit that GSA represents an optimal and currently accessible approach to test such a hypothesis.

Rationale for gene set-based approach

Gene set- and pathways-based analytic approaches have emerged over the last decade, following the advent of genome-wide expression and GWAS microarray platforms. One of the earliest GSA approaches, gene set enrichment analysis (GSEA), was introduced by Subramanian et al. (2005) for the analysis of expression microarray data. Wang et al. (2007) published a study applying a modified GSEA algorithm to analyze individual-level genotype data from a GWAS while many additional investigations have elaborated on the relative strengths of gene set-based approaches in genetic discovery (Al-Shahrour et al. 2007; Chen et al. 2009; Medina et al. 2009). As a result, GSA methods and applications designed to apply similar algorithms to marker-level summary results (e.g., marker-level p values) of GWAS have emerged [e.g., gene set-based analysis of polymorphisms (GeSBAP) (Medina et al. 2009), Exploratory visual analysis (EVA) (Reif et al. 2005, 2007), improved gene set enrichment analysis (iGSEA) (Zhang et al. 2010), GSA-SNP (Nam et al. 2010), exploratory gene association network (EGAN) (Paquette and Tokuyasu 2010)].

Most simply defined, GSA is a set of methods that examine the extent to which more significant test statistics tend to aggregate or cluster within gene groups (or sets) that share some biological characteristic(s), or modules of functionally related genes (Medina et al. 2009). Thus, GSA may employ any type of annotations to classify genes into biologically relevant gene sets, such as shared molecular function, tissue expression patterns or shared biological pathways involvement. In addition to often being more biologically informative, GSAs, like pathways-based analyses, can overcome the limitations imposed by single-marker analysis of such high-throughput data, such as their extensive multiple testing corrections requirements through gene- or gene set-based data reduction and analysis.

Rationale for hypothesis-driven investigations

Thus far, GSA methods have been largely applied for exploratory analyses of large, high-throughput datasets, including the results of GWAS. Though apparently uncommon, these methods can also be employed to conduct explicit biological hypothesis testing. Notable strengths of exploratory analyses include the potential for illuminating latent patterns in the data that were not detected on initial (marker-level) analysis and that may not have been

considered if inconsistent with extant biological perspectives. That said, hypothesis-driven analyses remain critical to scientific progress and have several advantages that we sought to exploit in our investigation. First, the scientific method entails that scientific investigations begin with a biological hypothesis that, in turn, should inform the selection of both the biological material and the methodologic approach to its analysis. Second, without an *a priori* hypothesis, interpretation of results relies on speculation rather than scientific deduction. Finally, the testing of explicit hypotheses frees the investigator from the burden of extensive hypothesis testing correction necessary under ‘hypothesis-free’ or exploratory analysis. Depending on the gene set or pathways annotations used, exploratory GSA necessarily entails the correction for hundreds to several thousand hypothesis tests.

Rationale for secondary aims: mapping strategies and GSA methods

The current debate in application of GSA methods, and the focus of our present comparative analyses, centers on two challenges: (1) the SNP-to-gene mapping strategy employed and (2) the analytic approach for SNP-to-gene statistical reduction. SNP-to-gene mapping is a pre-processing step in which the investigator must determine how the SNP-level data will be mapped to known genes in the human genome. The investigator may choose to map (and therefore include in the analysis) only those SNPs that lie within genic regions (i.e., coding sequences (CDS), exonic, intronic, 5'UTR, 3'UTR) or may opt to map all SNPs falling within some predetermined distance from the start/end of a gene's boundaries (e.g., 500 Kb upstream and downstream of a gene start and end location.) While 500 Kb up/downstream should be expected to capture most enhancer/promoter regions (Wang et al. 2007), several recent studies have suggested that measured associations at common SNPs may capture information about regional rare variants that may be as far as 2.5 Mb away from the tagSNP (Dickson et al. 2010; Wang et al. 2010). Thus, it remains unclear whether extensive up/downstream mapping approaches may capture additional information about association within extended haplotypes and, of course, whether such information will ultimately enhance the accuracy or utility of GSA. To address the question of whether SNP-to-gene mapping parameters influence GSA results, we employed three mapping strategies in each of our analyses: GENIC (SNPs within gene boundaries), 500 Kb (GENIC plus 500 Kb up/downstream) and 2.5 Mb (GENIC plus 2.5 Mb up/downstream) mapping.

The second, and perhaps more challenging, problem facing the application of GSA strategies to GWAS data is the analytic method employed for SNP-to-gene reduction or the derivation of gene-level summary statistics from the original SNP-level summary data (*p* values, in particular). Until quite recently, the most commonly employed SNP-to-gene reduction method was to use the minimum SNP-level *p* value to represent each gene. The rationale, which is still valid, is that in a gene-centric analysis one wants the ‘best representative’ statistic for each gene. The use of the minimum *p* value also enables the analysis to capture effects of genes that may contribute a single susceptibility locus to the overall risk of disease. (Any method that combines effects across SNPs within a gene will necessarily reduce the magnitude of the single, best locus contribution and this may not be warranted.)

The now well-recognized disadvantage of this approach is that genes represented by a large number of SNPs on genotyping arrays (usually, but not always, larger genes) are more likely to have lower minimum *p* values based on chance alone. Thus, the use of minimum *p* value in GSA may be more likely to produce evidence of enrichment in gene sets that disproportionately contain larger genes. Given this, it is essential to address this potential bias by employing complimentary methods that account for gene size. We believe it is worthwhile to note, however, the mere fact is that a statistical result *may* be due to chance does not mean that the finding *is* due to chance. While it is true that ion channel genes, as a class, have larger-than-average gene size (and thus, that many ion channel genes have more

than average probes on microarrays and GWAS platforms), it is also true that they represent excellent biological candidates and that this candidacy may, in part, be *due to* the size of their genes. Thus, we elected to conduct a set of GSAs to compare the results obtained using minimum p value (pMIN method) for each gene to those obtained using two additional SNP-to-gene reduction strategies that account for variation in gene size: proportion of SNPs per Gene with p value $< \alpha$ -threshold (PROP α), and the truncated product method (TPM) (see “Methods” for further description).

Finally, our choices of SNP-to-gene reduction methods were made not only to address the issue of gene size bias, but also for their statistical and biological complementarity. We believe that the GSA results obtained using each method may have unique implications for the genetic architecture of schizophrenia. If real (i.e., not a statistical artifact), gene sets implicated by pMIN analysis alone may be those in which genes have a single locus, or finite set of proximal loci, contributing to disease susceptibility. The PROP α method, by contrast, will be more robust to the identification of gene sets in which its gene members have disproportionately larger numbers of disease-associated SNPs, suggesting the potential for substantial allelic heterogeneity. Finally, the TPM method will implicate gene sets in which a disproportionate number of its genes had very strong evidence of association and/or a large number of disease-associated SNPs and would most strongly implicate those genes manifesting both characteristics. Thus, the confluence of findings across pMIN, PROP and TPM methods may suggest the relative likelihood of contributions from allelic and locus heterogeneity to risk architecture. In summary, marker-level GWAS data used in the GSAs cannot, however, address the nature and extent of genetic epistasis relevant at the individual level.

Methods

Overview of data preparation and analytic approach

From each original GWAS dataset, we created three experimental datasets based on three distinct SNP-to-gene mapping strategies (GENIC, 500 Kb, 2.5 Mb) to be examined. On each experimental dataset (e.g., EA-GENIC), we derived a gene-level p value using three distinct analytic strategies (pMIN, PROP, TPM). For ease of reference, we refer to each analysis by original GWAS dataset, experimental mapping dataset, analytic strategy and α -threshold as in EA-500 Kb-TPM01 for the European-American GWAS, 500 Kb mapping strategy, Truncated Product Method strategy for SNP-to-gene reduction and TPM $\alpha < 0.01$. We conducted separate GSAs on each set of experimental values, testing for enrichment of 11 nested ion channel activity gene sets in each (see Fig. 1).

Gene set annotations

Arguably, the most comprehensive biological classification scheme for genes based on their molecular function is the molecular function gene ontology (GO-MF). According to the AmiGO project, the Molecular Function ontology captures the elemental activities describing the actions of a gene product at the molecular level. Relative to other gene classification schemes (e.g., biological pathways), such elemental activities are most likely to be comprehensive, well-characterized and reliable. With regard to ion channel gene classes, in particular, it is important to note that several commonly used biological pathways annotations (e.g., Panther) do not include most known ion channel genes within any pathways (Askland et al. 2009). (For example, the KCNN3 gene has ‘No pathway information available’, Panther Database, accessed 04/28/11).

The molecular function ontology term, ‘Ion channel activity’ is defined as ‘catalysis of facilitated diffusion of an ion (by an energy-independent process) by passage through a

transmembrane aqueous pore or channel without evidence for a carrier-mediated mechanism.' [AmiGO, accessed 02/08/11]. There are 394 human genes that fall within this molecular function gene set. This gene set is a 6th generation term of the molecular function ontology under the following lineage (from least to most specific): molecular function (15,540 gene products), transporter activity (1,189), transmembrane transporter activity (926), substrate-specific transporter activity (855), substrate-specific channel activity (403), ion channel activity (394). The human ion channel activity set contains 4 daughter terms: anion channel activity, cation channel activity, ligand-gated ion channel activity and voltage-gated ion channel activity. There is substantial overlap among the genes included among the daughter subsets (e.g., many cation channel activity genes are also ligand- and voltage-gated channel activity genes).

Our primary hypothesis is that, over the entire affected population, a diverse set of ion channel gene variants contributes to the genetic susceptibility to schizophrenia. However, due to random sampling effects, any particular case-control population may, by chance, carry disproportionate disease-associated variation in a specific ion channel subset. Thus, if under this condition, we test only for the most general ion channel activity set, we could miss strong enrichment that might be expected, by chance, within a relevant subset. Thus, we elected to simultaneously test for enrichment of the most general set, ion channel activity, as well as 10 specific ion channel activity subsets. Though any one of the subsets are potentially relevant, we selected the 10 most basic subsets in terms of their molecular function because of strong reliability of the functional definitions as well as their reasonable size: potassium channel activity (131 genes), sodium channel activity (32), calcium channel activity (81), chloride channel activity (73), calcium release channel activity (9), voltage-gated ion channel activity (192), voltage-gated potassium channel activity (98), voltage-gated sodium channel activity (15), voltage-gated calcium channel activity (27), and voltage-gated chloride channel activity (16).

SNP-to-gene mapping

The original GWAS datasets, obtained via dbGaP permission, used in the current analysis contained 729,454 (EA) and 845,814 (AA) SNP-level summary statistics (a SNP-level Odds ratio and corresponding *p* value). Our analytic approach is gene-centric, in that it is measuring the extent to which particular sets of genes, based on shared molecular functions, are enriched for more significant test statistics. As such, the approach entails deriving gene-level summary statistics for all genes represented in the original SNP-level datasets. To do this, each SNP was first mapped to its nearest gene, using the Affymetrix 6.0 Annotation dataset, Release 30 (Release date November 15, 2009). Affymetrix annotations provide gene mapping information (including gene symbol, distance from gene, SNP-Gene relational information) for 98.8% of genotyped EA and 98.7% of genotyped AA SNPs. When more than one gene mapped to a single SNP, a single gene was assigned using the following prioritization method: CDS > UTR > exon > intron; and for intergenic SNPs, the gene with the closest up/downstream distance was assigned. The resulting, fully mapped SNP EA and AA datasets were then subject to further reduction into separate mapping sets, as below.

Based on prior biological rationale, we employed three mapping strategies to derive three mapping datasets from each primary (EA and AA) GWAS dataset. The first strategy retained only those SNPs that mapped within the boundaries of a gene, including CDS, exonic, intronic, 5'UTR, 3'UTR (we refer to this as the GENIC dataset). The GENIC mapping strategy is considered the most conservative as it does not include SNPs that may lie within up/downstream promoter/enhancer regions. The second strategy retained only SNPs residing within and up to 500 Kb up- or downstream of a gene's boundaries (500 Kb dataset). The 500 Kb approach, consistent with that employed by Wang et al. (2007), should capture most effects mediated (or represented) by SNPs within the promoter/enhancer

elements in addition to the genic SNPs. The third and broadest strategy retained all SNPs within 2.5 Mb of its nearest gene. This approach was included in order to investigate the potential for effects of long-range haplotypes that may be captured by very distant SNPs (Dickson et al. 2010; Wang et al. 2010).

Analytic approaches to SNP-to-gene reduction for GSA

To test our biological hypothesis, we applied three analytic approaches to derive gene-level summary statistics from the SNP-level GWAS data for each mapping set. The first, and thus far most commonly employed method, was to use the minimum p value to represent each gene (pMIN). For each of the three mapping datasets, the lowest SNP-level p value was selected to represent each gene in the GSA. Since pMIN does not control for the number of mapped SNPs, we also employed two additional analytic approaches, both of which control for the number of SNPs mapped to each gene in their derivation.

In the second approach, $\text{PROP}\alpha$, we used a novel procedure to derive a summary statistic (p value) to each gene based on the gene's proportion of mapped SNPs with p values less than specified α -thresholds. For each mapping set, we used EVA to calculate a proportion of SNPs/gene with $p < \alpha$ and derived a corresponding Fisher's exact probability of finding such a proportion by chance given the size of the gene. The resulting p value was then used as the experimental statistic for each gene to conduct the GSA in EVA. As PROP is a novel strategy, we employed three different α -thresholds (standard = 0.05, conservative = 0.01 and very conservative = 0.001) to assess sensitivity.

Our third approach was the truncated product method (TPM), originally developed for combining p values by Zaykin et al. (2002) (Zaykin et al. 2002) and recently employed by Moskvina et al. (2009) (Moskvina et al. 2009) in their analysis of bipolar and schizophrenia GWAS data. This method is intermediate between Fisher's product method (Province 2001) and Sidak's correction (Abdi 2007; Sidak 1967). For each gene, the test statistic is calculated as the product of all p values $< \alpha$. The null distribution for this statistic depends on the total number of SNPs for that gene, and is calculated with a formula described by Zaykin et al. (Zaykin et al. 2002). The resulting p value is then used as the experimental statistic in the GSA. Following the example of Zaykin et al., for genes containing over 1,000 SNPs we used a Monte Carlo algorithm to approximate the null distribution. This was done to avoid arithmetic overflow. For genes with no SNPs that had p value $< \alpha$, Sidak's correction (Sidak 1967) was applied. For TPM, we employed two α -thresholds for comparability to other published and future work (0.05 and 0.01). All TPM calculations were performed in R and the resulting p values were exported and uploaded into EVA to conduct the GSAs.

GSA by exploratory visual analysis (EVA)

To conduct our GSA, we employed the Exploratory Visual Analysis (EVA) application (Reif et al. 2005; Reif et al. 2007). EVA enables the use of either Fisher's Exact or permutation-based significance testing among gene sets. For each experimental dataset (i.e., EA-GENIC, EA-500 Kb, EA-2.5 Mb, AA-GENIC, AA-500 Kb, AA-2.5 Mb), the gene-level p values derived under each analytic method (i.e., pMIN, $\text{PROP}\alpha$ and $\text{TPM}\alpha$) were used to perform all GSAs in EVA using an enrichment threshold of $p < 0.05$. Thus, for each analysis, EVA calculates the proportion of genes in each GO-MF-defined gene set whose experimental p value is < 0.05 then derives a corresponding Fisher's exact probability of finding such a proportion by chance given the size of the gene set.

EVA parameter settings

In most gene set-based approaches, the investigator must choose a threshold criteria that allows calculation of the enrichment statistic from a comparison of the 'high' to the 'low'

scores in the experiment of interest. For example, when using the pMIN method, the investigator must choose a pMIN cutoff value for the calculation of the enrichment statistic. This choice is essentially an arbitrary one, so convention is often followed. We chose a more conservative pMIN cutoff of $p = 0.01$ because minimum p values do not follow a uniform distribution and an expectedly large proportion of genes had pMIN < 0.05 . In the case of the PROP and TPM methods, the derived gene-level p values follow a standard uniform distribution. Thus, for our primary analyses, we choose a standard threshold of $p = 0.05$ for those experiments.

Multiple hypothesis testing correction

In the analysis of each experimental dataset, we simultaneously tested 11 ion channel hypotheses. Under the gene ontology structure employed (GO-MF), these gene sets (and therefore, the respective hypotheses) are not independent. In fact, ion channel activity is a parent term under which the remaining ten ion channel subsets are subsumed. Thus, we employed an a priori threshold of 0.025 for our primary analyses and also applied the extremely stringent Bonferroni correction, which assumes complete independence (Bonferroni- $p < 4.55\text{E-}03$).

Results

Results of our primary European-American (EA) and African-American (AA) GSAs are shown in Tables 1 and 2, respectively. All tested ion channel gene sets reaching nominal ($p < 0.05$) or a priori significance ($p < 0.025$) levels, under our primary EVA enrichment threshold of $p = 0.05$, are shown in the tables.

Minimum p value (pMIN) method

Multiple ion channel gene sets were significantly enriched under all three mapping strategies using the pMIN approach in both the EA and AA analyses. Several sets retained significance under conservative multiple testing correction across all mapping strategies, including the parent set, ion channel activity.

Proportional (PROP) method

Under all mapping strategies and all α -thresholds (0.05, 0.01, 0.001), all EA PROP α GSAs produced statistically significant enrichment ($p < 0.025$) of at least one tested ion channel gene set. The most robust enrichment was detected under the 500 Kb and 2.5 Mb mapping strategies. Of particular note in the EA PROP α analyses is the fact that for both the 500 Kb and 2.5 Mb datasets, the significance of the implicated ion channel sets increased with decreasing (i.e., more conservative) α -thresholds. Thus, in the 500 Kb set for example, employing an α -threshold of 0.001 (i.e., PROP001) to calculate the proportion of subthreshold SNPs for each gene produced more statistically significant gene set enrichment for the implicated sets than did the use of $\alpha = 0.01$ (PROP01) and $\alpha = 0.05$ (PROP05). As shown in Table 1, several of the implicated gene sets also retained significance after extremely conservative Bonferroni corrections across one or more mapping/analytic approaches including: ion channel activity, potassium channel activity and calcium-release channel activity.

By contrast, the AA GSAs produced more circumscribed findings. The potassium channel activity (KCN) set was significantly enriched under the two broadest mapping strategies (500 Kb and 2.5 Mb) and for PROP05 analyses. Of note, the KCN findings in the AA 500 Kb-PROP05 ($p = 9.33\text{E-}04$) and 2.5 Mb-PROP05 ($p = 2.92\text{E-}03$) GSAs also retained significance after Bonferroni correction.

Truncated product method (TPM)

Under all mapping strategies and both α -thresholds (0.05, 0.01), each EA TPM analysis produced statistically significant enrichment ($p < 0.025$) of at least one tested ion channel gene set. Similar to the EA PROP analyses, we found more significant enrichment (except for the calcium-release channel gene set) under the more conservative α -threshold (TPM01). The ion channel and potassium channel activity gene sets were enriched only in the conservative TPM01 analyses. The most robust enrichment was detected under the 500 Kb-TPM01 analysis, wherein both ion channel activity ($p = 3.72E-03$) and calcium-release channel activity ($p = 4.13E-03$) retained significance after Bonferroni correction.

No tested ion channel gene set reached a priori significance threshold in any AA TPM analysis. In the AA 500 Kb-TPM05 analysis, the potassium channel activity gene set did reach nominal significance ($p = 0.04228$), consistent with the significance of this gene set in the AA 500 Kb-pMIN and -PROP05 analyses and its nominal significance in AA 500 Kb-PROP01. In addition, when the gene set enrichment criterion was relaxed to $p < 0.075$ in a post-hoc TPM01 analyses, two tested ion channel gene sets each reached nominal significance in the 2.5 Mb and 500 Kb mapping sets: voltage-gated sodium channel (2.5 Mb: $p = 0.02911$; 500 Kb: $p = 0.02826$), and calcium channel (2.5 Mb: $p = 0.04125$; 500 Kb: $p = 0.03902$) activity.

Discussion

Differential implications for pMIN, PROP and TPM

Overall, the pMIN approach found significant enrichment of several ion channel gene sets in all three mapping strategies for EA and AA datasets. The extent of the findings under pMIN, relative to the other methods, does support the possibility that pMIN may be biased by gene size. However, we would again emphasize that while large genes are more likely to have disproportionately low minimum p values by chance, such statistical probabilities cannot address the potential that such differences in distribution of more significant minimum test statistics may have a biological basis. Until more definitive investigations, such as deep resequencing and functional analyses, have been completed, the results produced under pMIN methods may yet provide valid biological insights for future investigations.

The fact that the PROP and TPM analytic methods, each of which do account for gene size, found significant enrichment for several tested ion channel gene sets across all three mapping strategies in the European-American sample provides very strong statistical evidence that variation at common polymorphic markers within these genes may explain some proportion of the heritability of schizophrenia in these populations. Interestingly, however, the fact that our GSAs, like most, utilize measures of association at common polymorphic markers does not necessarily suggest that the relevant underlying functional variants are, themselves, common in the population. As alluded to in the introduction, recent investigations have demonstrated the plausibility, perhaps likelihood, that tagSNPs may be capturing effects of rare variants over extended genomic haplotypes. While it remains unclear exactly what type of functional variants (i.e., common, low-frequency or rare) may be creating the effects measurable in GSAs, our findings support the pathophysiological relevance of this class of genes in mediating susceptibility to schizophrenia. Thus, we would submit that, in combination with previous findings implicating specific ion channel genes, our findings suggest that ion channel genes, as a class, are ideal candidates for deep resequencing. In addition, our finding of strong GSA evidence across all three methods for the EA sample suggests that both locus and allelic heterogeneity are likely to factor strongly into the genetic architecture of schizophrenia, and the contribution of variation in ion channels in particular. For the AA sample, significant findings were limited to the pMIN and

PROP05 analyses, which may suggest a greater role for allelic heterogeneity within this population (see “Comparison of EA and AA results”, below, for further discussion of EA-AA discrepancies).

Results generated under genic versus broader mapping strategies

A closer examination of some specific results may be revealing. Under the most stringent thresholds (i.e., PROP001 and TPM01) in the EA analyses, the 500 Kb and 2.5 Mb mapping strategies yielded very similar results (see Table 1). Ion channel activity and the potassium and calcium-release channel activity subsets each were strongly implicated, though their rank orders varied somewhat across mapping and analytic strategies. By contrast, under the GENIC mapping strategy, the TPM01 analysis found significant enrichment for the general ion channel activity set only, while the PROP001 found significant enrichment only in the calcium-release channel set. The more limited findings under the GENIC, as opposed to the broader, mapping sets may reflect true discrepancies in the extent of disease-related genomic variation captured by intra- versus proximal intergenic SNPs. In other words, these results could suggest that the disease susceptibility contributed by these genes is primarily via regulatory alterations (e.g., regulation of temporal or regional expression patterns), rather than via structural or splicing alterations. An alternative interpretation of the discrepant findings between GENIC and broader mapping strategies in the EA sample is that the GENIC results are simply less robust because they were derived from the analysis of a much more limited set of SNPs (i.e., 278424 EA-Genic SNPs vs. 675986 EA-500 Kb SNPs vs. 720477 EA-2.5 Mb SNPs), or because intergenic SNPs are likely to harbor more false positives.

Comparison of EA and AA results

Comparing the particular significant results yielded in the EA and AA analyses, we note that there are far fewer significantly enriched ion channel gene sets in the AA analyses beyond the AA-pMIN analyses. One interpretation of the less substantial AA findings is that there are true ethnic differences in the susceptibility genes for schizophrenia and that ion channels are not prominent molecular substrates of schizophrenia phenotypes in AA populations. This is certainly possible given their rather distinct evolutionary histories and genomic architectures (Tishkoff et al. 2009; Tishkoff and Williams 2002), relative to EA populations. There are also several alternative explanations for the ethnic discrepancies in our findings. First, it may simply be a random sampling effect. Namely, due to the extensive genetic heterogeneity of schizophrenia, it's possible that the particular sample of 1,241 AA cases represented in the GWAS analyzed did not harbor a substantial amount of disease-associated variation in these genes (but that they do exist in the larger affected population). A second alternative is that case ascertainment strategies inadvertently selected for different phenotypic characteristics in the two ethnic groups. In fact, it has been shown through previous epidemiologic work that there are differences in diagnostic rates and patterns between European and African-American samples in schizophrenia (Neighbors et al. 2003; Trierweiler et al. 2000; Trierweiler et al. 2006). Third, the AA results may have been less robust because of higher dimensionality relative to sample size, as compared to the EA sample. More specifically, the EA GWAS genotyped at 729,454 SNPs in 1,404 cases and 1,442 controls while the AA GWAS genotyped 835,143 SNPs in 1,241 cases and 979 controls. This could have resulted in reduced power to detect gene set enrichment in the AA relative to the EA datasets. This may be particularly true if, as the AA-pMIN and highly significant AA-PROP05 results suggest, there is a substantial contribution to disease risk of variation in the KCN gene set. As potassium channels are the largest specific subclass of ion channels, the issues of higher dimensionality are especially pertinent. Finally, given the known reductions in LD in populations of African ancestry, it is possible (perhaps likely) that the AA SNP panel employed is insufficient to capture the extent of genomic variation

present in African-American genomes. Thus, although the AA panel is comprised of a greater number of SNPs relative to the EA panel, it captures proportionally less than does the EA panel.

Comparison of genes conferring enrichment in the potassium channel activity gene set: EA versus AA

Our findings of significant to highly significant enrichment in the potassium channel (KCN) activity set in both populations (EA-500 Kb-pMIN, EA-2.5 Mb-pMIN, EA-500 Kb-PROP01/PROP001, EA-500 Kb-TPM01, EA-2.5 Mb-PROP01/PROP001, EA-2.5 Mb-TPM01; AA-Genic-pMIN, AA-500 Kb-pMIN, AA-2.5 Mb-pMIN, AA-500 Kb-PROP05, AA-2.5 Mb-PROP05) are noteworthy for several reasons. First, aside from the early work in the KCNN3 gene, the smattering of independent findings for other potassium channel genes described in the “Introduction”, and one recent study investigating the mechanism of lithium in bipolar disorder (Butler-Munro et al. 2010), the general class of potassium channel genes has been explored much less extensively than other ion channel classes in neuropsychiatric disorders. Following the significant bipolar GWAS findings for CACNA1C (Sklar et al. 2008), much attention [e.g., follow-up GWAS meta-analyses (Ferreira et al. 2008)] has been paid to the potential role of this gene and related voltage-gated calcium channels and calcium-channel interactants [e.g., ANK3 allelic heterogeneity study (Schulze et al. 2009)] in both bipolar disorder and schizophrenia. In addition, a role for sodium channel genes has long been speculated due to their role as pharmacologic targets of many of the standard, and most effective, mood stabilizing agents used in the treatment of bipolar disorder. Thus, it is interesting that it was the more neglected, yet largest and most functionally diverse, ion channel subset that produced the most consistent findings of all subsets tested.

Finally, one of our most interesting observations derives from closer examination of the specific genes conferring enrichment to the KCN gene set across the samples and analytic methods. As shown in Table 3, across the 500 Kb analyses in which the KCN gene sets reached at least nominal significance (EA-pMIN, EA-PROP01, EA-PROP001, EA-TPM01, AA-pMIN, AA-PROP05, AA-PROP01, AA-TPM05), the KCN gene set enrichment is conferred by a total of 45 KCN genes. Of those 23 genes (51%) contribute to enrichment of the KCN gene set only in AA analysis(es), 8 (18%) contribute only in EA analysis(es) and 14 (31%) contribute in both EA and AA analyses. Thus, the majority of genes (69%) mediating the enrichment of this gene set across samples and methods are unique to the results of only one ethnic sample. In addition, from the list of genes mediating enrichment of the KCN gene set, we can observe that only a very small number (~4, <10%, based on our literature review) have been previously implicated in genetic or other investigations (Borsotto et al. 2007; Duncan et al. 2008; Tam et al. 2010; Xiao et al. 2009). Interestingly, three of the four contributed to enrichment in GSA(s) of one ethnic group only (see Table 3).

To summarize, our review of previous literature found significant independent findings supporting various ion channel candidates in genetic, pharmacologic and functional neuroscience investigations and minimal replication of specific genes across diverse genetic investigations while the present analyses found strong replication of KCN gene set enrichment across methods and ethnic samples. Thus, we submit that this confluence of results provides especially compelling evidence, not only for a role of ion channel genes in mediating risk of schizophrenia, but also for an architecture characterized by substantial genetic heterogeneity. By extension, these findings suggests that the particular KCN genes that have been idiosyncratically subject to independent investigation may represent only a small proportion of the KCN genes likely to be relevant to the genetic architecture of schizophrenia.

Future directions

Though more computationally demanding, some emergent GSA applications enable the incorporation of information on LD structure into the SNP-to-gene mapping procedures. Such approaches represent an alternative to the more conventional use of arbitrary distances based on gene boundaries and may offer some advantages in cross-sample comparisons.

Findings from ours and future GSAs in schizophrenia may be most useful to direct targeted resequencing efforts. While whole genome and whole exome sequencing are becoming more feasible, the capacity to store, manipulate and analyze such large scale data are major rate-limiting steps in their use for genetic discovery. In addition to being computationally more manageable, the use of targeted resequencing around biologically rational candidates will be more immediately interpretable. In addition, the development of gene set based analytic approaches to whole-genome sequencing data will enable both exploratory and hypothesis-driven analysis on a larger scale.

Ultimately, the confirmation of disease-related functional variants in ion channel genes will have profound implications for biomarker and pharmacologic development. As common markers and rare functional variants are identified across the population of affected individuals, catalogs can be developed from which biomarker arrays can be constructed for diagnosis and risk profiling. The ultimate application of such genetic information will be in their use for personalized treatment plans. Though still far short of their potential diversity and scale, existing ion channel modulating drugs comprise an extremely successful and highly profitable drug class (Xie et al. 2004). This pharmaceutically tractable class of molecules holds the potential for highly specialized molecular targeting. Building on currently available targeting mechanisms, further developments may enable more specialized ion channel class (e.g., calcium channel), subclass (e.g., inwardly rectifying potassium channels), protein domain (e.g., ionic pore) or conformation state (e.g., open, closed or inactivated) targeting. Finally, the evolution in gene-based therapies may offer the possibility of altering expression or functioning of specific ion channels.

Acknowledgments

The project described was supported by Award Number K08MH085810 from the National Institute of Mental Health. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institute of Mental Health or the National Institutes of Health. The authors would like to thank Brady Tang, A.M., for his assistance with implementing the TPM algorithm. We would also like to thank Ben Greenberg, MD, PhD, for his editorial contributions.

References

- Abdi, H. The Bonferroni and Šidák corrections for multiple comparisons. In: Salkind, N., editor. Encyclopedia of measurement and statistics. Sage; Thousand Oaks, CA: 2007. p. 1-9.
- Abernethy DR, Soldatov NM. Structure-functional diversity of human L-type Ca²⁺ channel: perspectives for new pharmacological targets. J Pharmacol Exp Ther. 2002; 300:724–728. [PubMed: 11861774]
- Ader C, Schneider R, Hornig S, Velisetty P, Wilson EM, Lange A, Giller K, Ohmert I, Martin-Eauclaire MF, Trauner D, Becker S, Pongs O, Baldus M. A structural link between inactivation and block of a K⁺ channel. Nat Struct Mol Biol. 2008; 15:605–612. [PubMed: 18488040]
- Alaerts M, Del-Favero J. Searching genetic risk factors for schizophrenia and bipolar disorder: learn from the past and back to the future. Hum Mutat. 2009; 30:1139–1152. [PubMed: 19626716]
- Al-Shahrour F, Arbiza L, Dopazo H, Huerta-Cepas J, Minguez P, Montaner D, Dopazo J. From genes to functional classes in the study of biological systems. BMC Bioinform. 2007; 8:114.
- Anderson PA, Greenberg RM. Phylogeny of ion channels: clues to structure and function. Comp Biochem Physiol B Biochem Mol Biol. 2001; 129:17–28. [PubMed: 11337248]

- Angelino E, Brenner MP. Excitability constraints on voltage-gated sodium channels. *PLoS Comput Biol*. 2007; 3:1751–1760. [PubMed: 17892320]
- Anselmi C, Carloni P, Torre V. Origin of functional diversity among tetrameric voltage-gated channels. *Proteins*. 2007; 66:136–146. [PubMed: 17044069]
- Antonarakis SE, Blouin JL, Lasseter VK, Gehrig C, Radhakrishna U, Nestadt G, Housman DE, Kazazian HH, Kalman K, Gutman G, Fantino E, Chandy KG, Gargus JJ, Pulver AE. Lack of linkage or association between schizophrenia and the polymorphic trinucleotide repeat within the KCNN3 gene on chromosome 1q21. *Am J Med Genet*. 1999; 88:348–351. [PubMed: 10402501]
- Ashcroft FM. From molecule to malady. *Nature*. 2006; 440:440–447. [PubMed: 16554803]
- Ashwin E, Baron-Cohen S, Wheelwright S, O'Riordan M, Bullmore ET. Differential activation of the amygdala and the 'social brain' during fearful face-processing in Asperger syndrome. *Neuropsychologia*. 2007; 45:2–14. [PubMed: 16806312]
- Askland K, Read C, Moore J. Pathways-based analyses of whole-genome association study data in bipolar disorder reveal genes mediating ion channel activity and synaptic neurotransmission. *Hum Genet*. 2009; 125:63–79. [PubMed: 19052778]
- Austin CP, Holder DJ, Ma L, Mixson LA, Caskey CT. Mapping of hKCa3 to chromosome 1q21 and investigation of linkage of CAG repeat polymorphism to schizophrenia. *Mol Psychiatry*. 1999; 4:261–266. [PubMed: 10395216]
- Beaulieu JM, Gainetdinov RR. The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev*. 2011; 63:182–217. [PubMed: 21303898]
- Bigos KL, Mattay VS, Callicott JH, Straub RE, Vakkalanka R, Kolachana B, Hyde TM, Lipska BK, Kleinman JE, Weinberger DR. Genetic variation in CACNA1C affects brain circuitries related to mental illness. *Arch Gen Psychiatry*. 2010; 67:939–945. [PubMed: 20819988]
- Booth JR, Burman DD, Meyer JR, Gitelman DR, Parrish TB, Mesulam MM. Functional anatomy of intra- and cross-modal lexical tasks. *Neuroimage*. 2002; 16:7–22. [PubMed: 11969313]
- Borsotto M, Cavarec L, Bouillot M, Romey G, Macciardi F, Delaye A, Nasroune M, Bastucci M, Sambucy JL, Luan JJ, Charpagne A, Jouet V, Leger R, Lazdunski M, Cohen D, Chumakov I. PP2A-Bgamma subunit and KCNQ2 K⁺ channels in bipolar disorder. *Pharmacogenomics J*. 2007; 7:123–132. [PubMed: 16733521]
- Bowen T, Guy CA, Craddock N, Cardno AG, Williams NM, Spurlock G, Murphy KC, Jones LA, Gray M, Sanders RD, McCarthy G, Chandy KG, Fantino E, Kalman K, Gutman GA, Gargus JJ, Williams J, McGuffin P, Owen MJ, O'Donovan MC. Further support for an association between a polymorphic CAG repeat in the hKCa3 gene and schizophrenia. *Mol Psychiatry*. 1998; 3:266–269. [PubMed: 9672903]
- Bowen T, Williams N, Norton N, Spurlock G, Wittekindt OH, Morris-Rosendahl DJ, Williams H, Brzustowicz L, Hoogendoorn B, Zammit S, Jones G, Sanders RD, Jones LA, McCarthy G, Jones S, Bassett A, Cardno AG, Owen MJ, O'Donovan MC. Mutation screening of the KCNN3 gene reveals a rare frameshift mutation. *Mol Psychiatry*. 2001; 6:259–260. [PubMed: 11326292]
- Breier A, Elman I, Goldstein DS. Norepinephrine and schizophrenia: a new hypothesis for antipsychotic drug action. *Adv Pharmacol*. 1998; 42:785–788. [PubMed: 9328015]
- Brzustowicz LM, Hodgkinson KA, Chow EW, Honer WG, Bassett AS. Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21–q22. *Science*. 2000; 288:678–682. [PubMed: 10784452]
- Buchanan RW, Francis A, Arango C, Miller K, Lefkowitz DM, McMahon RP, Barta PE, Pearlson GD. Morphometric assessment of the heteromodal association cortex in schizophrenia. *Am J Psychiatry*. 2004; 161:322–331. [PubMed: 14754782]
- Butler-Munro C, Coddington EJ, Shirley CH, Heyward PM. Lithium modulates cortical excitability in vitro. *Brain Res*. 2010; 1352:50–60. [PubMed: 20637740]
- Camchong J, Dyckman KA, Chapman CE, Yanasak NE, McDowell JE. Basal ganglia-thalamocortical circuitry disruptions in schizophrenia during delayed response tasks. *Biol Psychiatry*. 2006; 60:235–241. [PubMed: 16458267]
- Camerino DC, Desaphy JF, Tricarico D, Pierno S, Liantonio A. Therapeutic approaches to ion channel diseases. *Adv Genet*. 2008; 64:81–145. [PubMed: 19161833]

- Cardno AG, Bowen T, Guy CA, Jones LA, McCarthy G, Williams NM, Murphy KC, Spurlock G, Gray M, Sanders RD, Craddock N, McGuffin P, Owen MJ, O'Donovan MC. CAG repeat length in the hKCa3 gene and symptom dimensions in schizophrenia. *Biol Psychiatry*. 1999; 45:1592–1596. [PubMed: 10376120]
- Catterall WA, Few AP. Calcium channel regulation and presynaptic plasticity. *Neuron*. 2008; 59:882–901. [PubMed: 18817729]
- Cerda O, Baek JH, Trimmer JS. Mining recent brain proteomic databases for ion channel phosphosite nuggets. *J Gen Physiol*. 2011; 137:3–16. [PubMed: 21149544]
- Chahine M, Chatelier A, Babich O, Krupp JJ. Voltage-gated sodium channels in neurological disorders. *CNS Neurol Disord Drug Targets*. 2008; 7:144–158. [PubMed: 18537643]
- Chandy KG, Fantino E, Wittekindt O, Kalman K, Tong LL, Ho TH, Gutman GA, Crocq MA, Ganguli R, Nimgaonkar V, Morris-Rosendahl DJ, Gargus JJ. Isolation of a novel potassium channel gene hSKCa3 containing a polymorphic CAG repeat: a candidate for schizophrenia and bipolar disorder? *Mol Psychiatry*. 1998; 3:32–37. [PubMed: 9491810]
- Chen L, Zhang L, Zhao Y, Xu L, Shang Y, Wang Q, Li W, Wang H, Li X. Prioritizing risk pathways: a novel association approach to searching for disease pathways fusing SNPs and pathways. *Bioinformatics*. 2009; 25:237–242. [PubMed: 19029127]
- Chowdari KV, Wood J, Ganguli R, Gottesman II, Nimgaonkar VL. Lack of association between schizophrenia and a CAG repeat polymorphism of the hSKCa3 gene in a north eastern US sample. *Mol Psychiatry*. 2000; 5:237–238. [PubMed: 10889524]
- Corti C, Xuereb JH, Crepaldi L, Corsi M, Michielin F, Ferraguti F. Altered levels of glutamatergic receptors and Na(+)/K(+) ATPase- α 1 in the prefrontal cortex of subjects with schizophrenia. *Schizophr Res*. 2011; 128:7–14. [PubMed: 21353485]
- Curtis D, Vine AE, McQuillin A, Bass NJ, Pereira A, Kandaswamy R, Lawrence J, Anjorin A, Choudhury K, Datta SR, Puri V, Krasucki R, Pimm J, Thirumalai S, Quedsted D, Gurling HM. Case-case genome-wide association analysis shows markers differentially associated with schizophrenia and bipolar disorder and implicates calcium channel genes. *Psychiatr Genet*. 2011; 21:1–4. [PubMed: 21057379]
- Dai S, Hall DD, Hell JW. Supramolecular assemblies and localized regulation of voltage-gated ion channels. *Physiol Rev*. 2009; 89:411–452. [PubMed: 19342611]
- Daniel C, Ohman M. RNA editing and its impact on GABAA receptor function. *Biochem Soc Trans*. 2009; 37:1399–1403. [PubMed: 19909284]
- Dekeyne A, Millan MJ. Discriminative stimulus properties of antidepressant agents: a review. *Behav Pharmacol*. 2003; 14:391–407. [PubMed: 14501253]
- Dib-Hajj SD, Waxman SG. Isoform-specific and pan-channel partners regulate trafficking and plasma membrane stability; and alter sodium channel gating properties. *Neurosci Lett*. 2010; 486:84–91. [PubMed: 20817075]
- Dickson SP, Wang K, Krantz I, Hakonarson H, Goldstein DB. Rare variants create synthetic genome-wide associations. *PLoS Biol*. 2010; 8:e1000294. [PubMed: 20126254]
- Dror V, Shamir E, Ghanshani S, Kimhi R, Swartz M, Barak Y, Weizman R, Avivi L, Litmanovitch T, Fantino E, Kalman K, Jones EG, Chandy KG, Gargus JJ, Gutman GA, Navon R. hKCa3/KCNN3 potassium channel gene: association of longer CAG repeats with schizophrenia in Israeli Ashkenazi Jews, expression in human tissues and localization to chromosome 1q21. *Mol Psychiatry*. 1999; 4:254–260. [PubMed: 10395215]
- Du W, Bautista JF, Yang H, Diez-Sampedro A, You SA, Wang L, Kotagal P, Luders HO, Shi J, Cui J, Richerson GB, Wang QK. Calcium-sensitive potassium channelopathy in human epilepsy and paroxysmal movement disorder. *Nat Genet*. 2005; 37:733–738. [PubMed: 15937479]
- Duncan CE, Chetcuti AF, SchoWeld PR. Coregulation of genes in the mouse brain following treatment with clozapine, haloperidol, or olanzapine implicates altered potassium channel subunit expression in the mechanism of antipsychotic drug action. *Psychiatr Genet*. 2008; 18:226–239. [PubMed: 18797397]
- Farley IJ, Price KS, McCullough E, Deck JH, Hordynski W, Hornykiewicz O. Norepinephrine in chronic paranoid schizophrenia: above-normal levels in limbic forebrain. *Science*. 1978; 200:456–458. [PubMed: 644310]

- Fedorenko O, Strutz-Seebohm N, Henrion U, Ureche ON, Lang F, Seebohm G, Lang UE. A schizophrenia-linked mutation in PIP5K2A fails to activate neuronal M channels. *Psychopharmacology (Berl)*. 2008; 199:47–54. [PubMed: 18545987]
- Fernandez-Alacid L, Aguado C, Ciruela F, Martin R, Colon J, Cabanero MJ, Gassmann M, Watanabe M, Shigemoto R, Wickman K, Bettler B, Sanchez-Prieto J, Lujan R. Subcellular compartment-specific molecular diversity of pre- and post-synaptic GABA-activated GIRK channels in Purkinje cells. *J Neurochem*. 2009; 110:1363–1376. [PubMed: 19558451]
- Ferreira MA, O'Donovan MC, Meng YA, Jones IR, Ruderfer DM, Jones L, Fan J, Kirov G, Perlis RH, Green EK, Smoller JW, Grozeva D, Stone J, Nikolov I, Chambert K, Hamshere ML, Nimgaonkar VL, Moskvina V, Thase ME, Caesar S, Sachs GS, Franklin J, Gordon-Smith K, Ardlie KG, Gabriel SB, Fraser C, Blumenstiel B, Defelice M, Breen G, Gill M, Morris DW, Elkin A, Muir WJ, McGhee KA, Williamson R, MacIntyre DJ, MacLean AW, St CD, Robinson M, Van Beck M, Pereira AC, Kandaswamy R, McQuillin A, Collier DA, Bass NJ, Young AH, Lawrence J, Ferrier IN, Anjorin A, Farmer A, Curtis D, Scolnick EM, McGuYn P, Daly MJ, Corvin AP, Holmans PA, Blackwood DH, Gurling HM, Owen MJ, Purcell SM, Sklar P, Craddock N. Collaborative genome-wide association analysis supports a role for ANK3 and CACNA1C in bipolar disorder. *Nat Genet*. 2008; 40:1056–1058. [PubMed: 18711365]
- Freudenberg J, Fu YH, Ptacek LJ. Bioinformatic analysis of human CNS-expressed ion channels as candidates for episodic nervous system disorders. *Neurogenetics*. 2007; 8:159–168. [PubMed: 17333079]
- Fujiwara H, Hirao K, Namiki C, Yamada M, Shimizu M, Fukuyama H, Hayashi T, Murai T. Anterior cingulate pathology and social cognition in schizophrenia: a study of gray matter, white matter and sulcal morphometry. *Neuroimage*. 2007; 36:1236–1245. [PubMed: 17524666]
- Gaddum JH, Hameed KA. Drugs which antagonize 5-hydroxytryptamine. *Br J Pharmacol Chemother*. 1954; 9:240–248. [PubMed: 13172437]
- Gaudet, R. Structural insights into the function of TRP channels. In: Liedtke, WB.; Heller, S., editors. *Frontiers in Neuroscience*. CRC Press; Boca Raton, FL: 2007.
- Glatt SJ, Faraone SV, Tsuang MT. Meta-analysis identifies an association between the dopamine D2 receptor gene and schizophrenia. *Mol Psychiatry*. 2003; 8:911–915. [PubMed: 14593428]
- Glessner JT, Reilly MP, Kim CE, Takahashi N, Albano A, Hou C, Bradfield JP, Zhang H, Sleiman PM, Flory JH, Imielinski M, Frackelton EC, Chiavacci R, Thomas KA, Garriss M, Otieno FG, Davidson M, Weiser M, Reichenberg A, Davis KL, Friedman JI, Cappola TP, Margulies KB, Rader DJ, Grant SF, Buxbaum JD, Gur RE, Hakonarson H. Strong synaptic transmission impact by copy number variations in schizophrenia. *Proc Natl Acad Sci USA*. 2010; 107:10584–10589. [PubMed: 20489179]
- Goetz T, Arslan A, Wisden W, Wulff P. GABA(A) receptors: structure and function in the basal ganglia. *Prog Brain Res*. 2007; 160:21–41. [PubMed: 17499107]
- Gorlov IP, Gorlova OY, Sunyaev SR, Spitz MR, Amos CI. Shifting paradigm of association studies: value of rare single-nucleotide polymorphisms. *Am J Hum Genet*. 2008; 82:100–112. [PubMed: 18179889]
- Gotti C, Clementi F, Fornari A, Gaimarri A, Guiducci S, Manfredi I, Moretti M, Pedrazzi P, Pucci L, Zoli M. Structural and functional diversity of native brain neuronal nicotinic receptors. *Biochem Pharmacol*. 2009; 78:703–711. [PubMed: 19481063]
- Green EK, Grozeva D, Jones I, Jones L, Kirov G, Caesar S, Gordon-Smith K, Fraser C, Forty L, Russell E, Hamshere ML, Moskvina V, Nikolov I, Farmer A, McGuffin P, Holmans PA, Owen MJ, O'Donovan MC, Craddock N. The bipolar disorder risk allele at CACNA1C also confers risk of recurrent major depression and of schizophrenia. *Mol Psychiatry*. 2010; 15:1016–1022. [PubMed: 19621016]
- Hansen HH, Waroux O, Seutin V, Jentsch TJ, Aznar S, Mikkelsen JD. Kv7 channels: interaction with dopaminergic and serotonergic neurotransmission in the CNS. *J Physiol*. 2008; 586:1823–1832. [PubMed: 18174210]
- Hashimoto K, Panchenko AR. Mechanisms of protein oligomerization, the critical role of insertions and deletions in maintaining different oligomeric states. *Proc Natl Acad Sci USA*. 2010; 107:20352–20357. [PubMed: 21048085]

- Ho TC, Brown S, Serences JT. Domain general mechanisms of perceptual decision making in human cortex. *J Neurosci.* 2009; 29:8675–8687. [PubMed: 19587274]
- Honore E. Alternative translation initiation further increases the molecular and functional diversity of ion channels. *J Physiol.* 2008; 586:5605–5606. [PubMed: 19043120]
- Huffaker SJ, Chen J, Nicodemus KK, Sambataro F, Yang F, Mattay V, Lipska BK, Hyde TM, Song J, Rujescu D, Giegling I, Mayilyan K, Proust MJ, Soghoyan A, Caforio G, Callicott JH, Bertolino A, Meyer-Lindenberg A, Chang J, Ji Y, Egan MF, Goldberg TE, Kleinman JE, Lu B, Weinberger DR. A primate-specific, brain isoform of KCNH2 affects cortical physiology, cognition, neuronal repolarization and risk of schizophrenia. *Nat Med.* 2009; 15:509–518. [PubMed: 19412172]
- ISC ISC. Rare chromosomal deletions and duplications increase risk of schizophrenia. *Nature.* 2008; 455:237–241. [PubMed: 18668038]
- Jegla TJ, Zmasek CM, Batalov S, Nayak SK. Evolution of the human ion channel set. *Comb Chem High Throughput Screen.* 2009; 12:2–23. [PubMed: 19149488]
- Jenkinson DH. Potassium channels—multiplicity and challenges. *Br J Pharmacol.* 2006; 147(Suppl 1):S63–S71. [PubMed: 16402122]
- Jensen AA, Davies PA, Brauner-Osborne H, Krzywkowski K. 3B but which 3B and that's just one of the questions: the heterogeneity of human 5-HT3 receptors. *Trends Pharmacol Sci.* 2008; 29:437–444. [PubMed: 18597859]
- Joobar R, Benkelfat C, Brisebois K, Toulouse A, Lafreniere RG, Turecki G, Lal S, Bloom D, Labelle A, Lalonde P, Fortin D, Alda M, Palmour R, Rouleau GA. Lack of association between the hSKCa3 channel gene CAG polymorphism and schizophrenia. *Am J Med Genet.* 1999; 88:154–157. [PubMed: 10206235]
- Judge SI, Smith PJ, Stewart PE, Bever CT Jr. Potassium channel blockers and openers as CNS neurologic therapeutic agents. *Recent Pat CNS Drug Discov.* 2007; 2:200–228. [PubMed: 18221232]
- Kapfhamer D, Berger KH, Hopf FW, Seif T, Kharazia V, Bonci A, Heberlein U. Protein Phosphatase 2a and glycogen synthase kinase 3 signaling modulate prepulse inhibition of the acoustic startle response by altering cortical M-Type potassium channel activity. *J Neurosci.* 2010; 30:8830–8840. [PubMed: 20592205]
- Keshavan MS, Sanders RD, Sweeney JA, Diwadkar VA, Goldstein G, Pettegrew JW, Schooler NR. Diagnostic specificity and neuroanatomical validity of neurological abnormalities in first-episode psychoses. *Am J Psychiatry.* 2003; 160:1298–1304. [PubMed: 12832245]
- Krause M, Offermanns S, Stocker M, Pedarzani P. Functional specificity of G alpha q and G alpha 11 in the cholinergic and glutamatergic modulation of potassium currents and excitability in hippocampal neurons. *J Neurosci.* 2002; 22:666–673. [PubMed: 11826096]
- Kurokawa J, Bankston JR, Kaihara A, Chen L, Furukawa T, Kass RS. KCNE variants reveal a critical role of the beta subunit carboxyl terminus in PKA-dependent regulation of the IKs potassium channel. *Channels (Austin).* 2009; 3:16–24. [PubMed: 19077539]
- Lachowicz JE, Sibley DR. Molecular characteristics of mammalian dopamine receptors. *Pharmacol Toxicol.* 1997; 81:105–113. [PubMed: 9335067]
- Lang UE, Puls I, Muller DJ, Strutz-Seebohm N, Gallinat J. Molecular mechanisms of schizophrenia. *Cell Physiol Biochem.* 2007; 20:687–702. [PubMed: 17982252]
- Leviton IB. Signaling protein complexes associated with neuronal ion channels. *Nat Neurosci.* 2006; 9:305–310. [PubMed: 16498425]
- Li T, Hu X, Chandy KG, Fantino E, Kalman K, Gutman G, Gargus JJ, Freeman B, Murray RM, Dawson E, Liu X, Bruinvels AT, Sham PC, Collier DA. Transmission disequilibrium analysis of a triplet repeat within the hKCa3 gene using family trios with schizophrenia. *Biochem Biophys Res Commun.* 1998; 251:662–665. [PubMed: 9792831]
- Lin WH, Wright DE, Muraro NI, Baines RA. Alternative splicing in the voltage-gated sodium channel DmNav regulates activation, inactivation, and persistent current. *J Neurophysiol.* 2009; 102:1994–2006. [PubMed: 19625535]
- MacDonald AW 3rd, Carter CS, Kerns JG, Ursu S, Barch DM, Holmes AJ, Stenger VA, Cohen JD. Specificity of prefrontal dysfunction and context processing deficits to schizophrenia in never-

- medicated patients with first-episode psychosis. *Am J Psychiatry*. 2005; 162:475–484. [PubMed: 15741464]
- Marionneau C, Townsend RR, Nerbonne JM. Proteomic analysis highlights the molecular complexities of native Kv4 channel macromolecular complexes. *Semin Cell Dev Biol*. 2011; 22:145–152. [PubMed: 20959143]
- McCarroll SA. Extending genome-wide association studies to copy-number variation. *Hum Mol Genet*. 2008; 17:R135–R142. [PubMed: 18852202]
- McClellan JM, Susser E, King MC. Schizophrenia: a common disease caused by multiple rare alleles. *Br J Psychiatry*. 2007; 190:194–199. [PubMed: 17329737]
- Mechaly I, Scamps F, Chabbert C, Sans A, Valmier J. Molecular diversity of voltage-gated sodium channel alpha subunits expressed in neuronal and non-neuronal excitable cells. *Neuroscience*. 2005; 130:389–396. [PubMed: 15664695]
- Medina I, Montaner D, Bonifaci N, Pujana MA, Carbonell J, Tarraga J, Al-Shahrour F, Dopazo J. Gene set-based analysis of polymorphisms: finding pathways or biological processes associated to traits in genome-wide association studies. *Nucleic Acids Res*. 2009; 37:W340–W344. [PubMed: 19502494]
- Meltzer, HY.; Roth, B. The role of serotonin in schizophrenia. In: Bloom, FE.; Kupfer, DJ., editors. *Psychopharmacology: the fourth generation of progress*. Raven Press; New York: 1995. <http://www.acnp.org/g4/GN40100011/Default.htm>
- Miller MJ, Rauer H, Tomita H, Gargus JJ, Gutman GA, Cahalan MD, Chandy KG. Nuclear localization and dominant-negative suppression by a mutant SKCa3 N-terminal channel fragment identified in a patient with schizophrenia. *J Biol Chem*. 2001; 276:27753–27756. [PubMed: 11395478]
- Milstein AD, Zhou W, Karimzadegan S, Brecht DS, Nicoll RA. TARP subtypes differentially and dose-dependently control synaptic AMPA receptor gating. *Neuron*. 2007; 55:905–918. [PubMed: 17880894]
- Missale C, Nash SR, Robinson SW, Jaber M, Caron MG. Dopamine receptors: from structure to function. *Physiol Rev*. 1998; 78:189–225. [PubMed: 9457173]
- Miyamoto S, LaMantia AS, Duncan GE, Sullivan P, Gilmore JH, Lieberman JA. Recent advances in the neurobiology of schizophrenia. *Mol Interv*. 2003; 3:27–39. [PubMed: 14993436]
- Mohapatra DP, Park KS, Trimmer JS. Dynamic regulation of the voltage-gated Kv2.1 potassium channel by multisite phosphorylation. *Biochem Soc Trans*. 2007; 35:1064–1068. [PubMed: 17956280]
- Molina ML, Barrera FN, Fernandez AM, Poveda JA, Renart ML, Encinar JA, Riquelme G, Gonzalez-Ros JM. Clustering and coupled gating modulate the activity in KcsA, a potassium channel model. *J Biol Chem*. 2006; 281:18837–18848. [PubMed: 16670090]
- Moore JH, Williams SM. Traversing the conceptual divide between biological and statistical epistasis: systems biology and a more modern synthesis. *Bioessays*. 2005; 27:637–646. [PubMed: 15892116]
- Moore JH, Gilbert JC, Tsai CT, Chiang FT, Holden T, Barney N, White BC. A flexible computational framework for detecting, characterizing, and interpreting statistical patterns of epistasis in genetic studies of human disease susceptibility. *J Theor Biol*. 2006; 241:252–261. [PubMed: 16457852]
- Moskvina V, Craddock N, Holmans P, Nikolov I, Pahwa JS, Green E, Owen MJ, O'Donovan MC. Gene-wide analyses of genome-wide association data sets: evidence for multiple common risk alleles for schizophrenia and bipolar disorder and for overlap in genetic risk. *Mol Psychiatry*. 2009; 14:252–260. [PubMed: 19065143]
- Nam D, Kim J, Kim SY, Kim S. GSA-SNP: a general approach for gene set analysis of polymorphisms. *Nucleic Acids Res*. 2010; 38:W749–W754. [PubMed: 20501604]
- Need AC, Ge D, Weale ME, Maia J, Feng S, Heinzen EL, Shianna KV, Yoon W, Kasperaviciute D, Gennarelli M, Strittmatter WJ, Bonvicini C, Rossi G, Jayatilake K, Cola PA, McEvoy JP, Keefe RS, Fisher EM, St Jean PL, Giegling I, Hartmann AM, Moller HJ, Ruppert A, Fraser G, Crombie C, Middleton LT, St Clair D, Roses AD, Muglia P, Francks C, Rujescu D, Meltzer HY, Goldstein DB. A genome-wide investigation of SNPs and CNVs in schizophrenia. *PLoS Genet*. 2009; 5:e1000373. [PubMed: 19197363]

- Neighbors HW, Trierweiler SJ, Ford BC, Muroff JR. Racial differences in DSM diagnosis using a semi-structured instrument: the importance of clinical judgment in the diagnosis of African Americans. *J Health Soc Behav.* 2003; 44:237–256. [PubMed: 14582306]
- Nicoll RA. The coupling of neurotransmitter receptors to ion channels in the brain. *Science.* 1988; 241:545–551. [PubMed: 2456612]
- Nigg JT, Casey BJ. An integrative theory of attention-deficit/hyperactivity disorder based on the cognitive and affective neurosciences. *Dev Psychopathol.* 2005; 17:785–806. [PubMed: 16262992]
- Nudmamud-Thanoi S, Piyabhan P, Harte MK, Cahir M, Reynolds GP. Deficits of neuronal glutamatergic markers in the caudate nucleus in schizophrenia. *J Neural Transm Suppl.* 2007; 72:281–285. [PubMed: 17982904]
- Nyegaard M, Demontis D, Foldager L, Hedemand A, Flint TJ, Sorensen KM, Andersen PS, Nordentoft M, Werge T, Pedersen CB, Hougaard DM, Mortensen PB, Mors O, Borglum AD. CACNA1C (rs1006737) is associated with schizophrenia. *Mol Psychiatry.* 2010; 15:119–121. [PubMed: 20098439]
- Olsen RW, Sieghart W. GABA A receptors: subtypes provide diversity of function and pharmacology. *Neuropharmacology.* 2009; 56:141–148. [PubMed: 18760291]
- Paoletti P. Molecular basis of NMDA receptor functional diversity. *Eur J Neurosci.* 2011; 33(8):1351–1365. [PubMed: 21395862]
- Papassotiropoulos A, Henke K, Stefanova E, Aerni A, Muller A, Demougin P, Vogler C, Sigmund JC, Gschwind L, Huynh KD, Coluccia D, Mondadori CR, Hanggi J, Buchmann A, Kostic V, Novakovic I, van den Bussche H, Kaduszkiewicz H, Weyerer S, Bickel H, Riedel-Heller S, Pentzek M, Wiese B, Dichgans M, Wagner M, Jessen F, Maier W, de Quervain DJ. A genome-wide survey of human short-term memory. *Mol Psychiatry.* 2011; 16:184–192. [PubMed: 20038948]
- Paquette J, Tokuyasu T. EGAN: exploratory gene association networks. *Bioinformatics.* 2010; 26:285–286. [PubMed: 19933825]
- Pavuluri MN, O'Connor MM, Harral E, Sweeney JA. Affective neural circuitry during facial emotion processing in pediatric bipolar disorder. *Biol Psychiatry.* 2007; 62:158–167. [PubMed: 17097071]
- Perlstein WM, Carter CS, Noll DC, Cohen JD. Relation of prefrontal cortex dysfunction to working memory and symptoms in schizophrenia. *Am J Psychiatry.* 2001; 158:1105–1113. [PubMed: 11431233]
- Perrais D, Veran J, Mulle C. Gating and permeation of kainate receptors: differences unveiled. *Trends Pharmacol Sci.* 2010; 31:516–522. [PubMed: 20850188]
- Petryshen TL, Middleton FA, Tahl AR, Rockwell GN, Purcell S, Aldinger KA, Kirby A, Morley CP, McGann L, Gentile KL, Waggoner SG, Medeiros HM, Carvalho C, Macedo A, Albus M, Maier W, Trixler M, Eichhammer P, Schwab SG, Wildenauer DB, Azevedo MH, Pato MT, Pato CN, Daly MJ, Sklar P. Genetic investigation of chromosome 5q GABAA receptor subunit genes in schizophrenia. *Mol Psychiatry.* 2005; 10:1074–1088. 1057. [PubMed: 16172613]
- Picton AJ, Fisher JL. Effect of the alpha subunit subtype on the macroscopic kinetic properties of recombinant GABA(A) receptors. *Brain Res.* 2007; 1165:40–49. [PubMed: 17658489]
- Province MA. The significance of not finding a gene. *Am J Hum Genet.* 2001; 69:660–663. [PubMed: 11481587]
- Reif DM, Dudek SM, Shaffer CM, Wang J, Moore JH. Exploratory visual analysis of pharmacogenomic results. *Pac Symp Biocomput.* 2005:296–307. [PubMed: 15759635]
- Reif DM, Israel MA, Moore JH. Exploratory visual analysis of statistical results from microarray experiments comparing high and low grade glioma. *Cancer Inform.* 2007; 5:19–24. [PubMed: 19390666]
- Reynolds GP, Harte MK. The neuronal pathology of schizophrenia: molecules and mechanisms. *Biochem Soc Trans.* 2007; 35:433–436. [PubMed: 17371293]
- Ritsner M, Modai I, Ziv H, Amir S, Halperin T, Weizman A, Navon R. An association of CAG repeats at the KCNN3 locus with symptom dimensions of schizophrenia. *Biol Psychiatry.* 2002; 51:788–794. [PubMed: 12007452]

- Rohrmeier T, Putzhammer A, Schoeler A, Sartor H, Dallinger P, Nothen MM, Propping P, Knapp M, Albus M, Borrmann M, Knothe K, Kreiner R, Franzek E, Lichtermann D, Rietschel M, Maier W, Klein HE, Eichhammer P. hSKCa3: no association of the polymorphic CAG repeat with bipolar affective disorder and schizophrenia. *Psychiatr Genet.* 1999; 9:169–175. [PubMed: 10697822]
- Russell A, Cortese B, Lorch E, Ivey J, Banerjee SP, Moore GJ, Rosenberg DR. Localized functional neurochemical marker abnormalities in dorsolateral prefrontal cortex in pediatric obsessive-compulsive disorder. *J Child Adolesc Psychopharmacol.* 2003; 13(Suppl 1):S31–S38. [PubMed: 12880498]
- Sailer CA, Kaufmann WA, Marksteiner J, Knaus HG. Comparative immunohistochemical distribution of three small-conductance Ca²⁺-activated potassium channel subunits, SK1, SK2, and SK3 in mouse brain. *Mol Cell Neurosci.* 2004; 26:458–469. [PubMed: 15234350]
- Saleem Q, Sreevidya VS, Sudhir J, Savithri JV, Gowda Y, BR C, Benegal V, Majumder PP, Anand A, Brahmachari SK, Jain S. Association analysis of CAG repeats at the KCNN3 locus in Indian patients with bipolar disorder and schizophrenia. *Am J Med Genet.* 2000; 96:744–748. [PubMed: 11121173]
- Schildkraut JJ. The catecholamine hypothesis of affective disorders: a review of supporting evidence. *Am J Psychiatry.* 1965; 122:509–522. [PubMed: 5319766]
- Schulze TG, Detera-Wadleigh SD, Akula N, Gupta A, Kassem L, Steele J, Pearl J, Strohmaier J, Breuer R, Schwarz M, Propping P, Nothen MM, Cichon S, Schumacher J, Rietschel M, McMahon FJ. Two variants in Ankyrin 3 (ANK3) are independent genetic risk factors for bipolar disorder. *Mol Psychiatry.* 2009; 14:487–491. [PubMed: 19088739]
- Sidak Z. Rectangular confidence region for the means of multivariate normal distributions. *J Am Stat Assoc.* 1967; 62:626–633.
- Silbersweig DA, Stern E. Towards a functional neuroanatomy of conscious perception and its modulation by volition: implications of human auditory neuroimaging studies. *Philos Trans R Soc Lond B Biol Sci.* 1998; 353:1883–1888. [PubMed: 9854260]
- Singer T. The neuronal basis of empathy and fairness. *Novartis Found Symp.* 2007; 278:20–30. discussion 30–40, 89–96, 216–221. [PubMed: 17214308]
- Siuta MA, Robertson SD, Kocalis H, Saunders C, Gresch PJ, Khatri V, Shiota C, Kennedy JP, Lindsley CW, Daws LC, Polley DB, Veenstra-Vanderweele J, Stanwood GD, Magnuson MA, Niswender KD, Galli A. Dysregulation of the norepinephrine transporter sustains cortical hypodopaminergia and schizophrenia-like behaviors in neuronal rictor null mice. *PLoS Biol.* 2010; 8:e1000393. [PubMed: 20543991]
- Sklar P, Smoller JW, Fan J, Ferreira MA, Perlis RH, Chambert K, Nimgaonkar VL, McQueen MB, Faraone SV, Kirby A, de Bakker PI, Ogdie MN, Thase ME, Sachs GS, Todd-Brown K, Gabriel SB, Sougnez C, Gates C, Blumenstiel B, Defelice M, Ardlie KG, Franklin J, Muir WJ, McGhee KA, MacIntyre DJ, McLean A, VanBeck M, McQuillin A, Bass NJ, Robinson M, Lawrence J, Anjorin A, Curtis D, Scolnick EM, Daly MJ, Blackwood DH, Gurling HM, Purcell SM. Whole-genome association study of bipolar disorder. *Mol Psychiatry.* 2008; 13:558–569. [PubMed: 18317468]
- Sotty F, Damgaard T, Montezinho LP, Mork A, Olsen CK, Bundgaard C, Husum H. Antipsychotic-like effect of retigabine [*N*-(2-Amino-4-(fluorobenzylamino)-phenyl)carbamic acid ester], a KCNQ potassium channel opener, via modulation of mesolimbic dopaminergic neurotransmission. *J Pharmacol Exp Ther.* 2009; 328:951–962. [PubMed: 19098162]
- Stefansson H, Rujescu D, Cichon S, Pietilainen OP, Ingason A, Steinberg S, Fossdal R, Sigurdsson E, Sigmundsson T, Buizer-Voskamp JE, Hansen T, Jakobsen KD, Muglia P, Francks C, Matthews PM, Gylfason A, Halldorsson BV, Gudbjartsson D, Thorgeirsson TE, Sigurdsson A, Jonasdottir A, Bjornsson A, Mattiasdottir S, Blondal T, Haraldsson M, Magnusdottir BB, Giegling I, Moller HJ, Hartmann A, Shianna KV, Ge D, Need AC, Crombie C, Fraser G, Walker N, Lonnqvist J, Suvisaari J, Tuulio-Henriksson A, Paunio T, Touloupoulou T, Bramon E, Di Forti M, Murray R, Ruggeri M, Vassos E, Tosato S, Walshe M, Li T, Vasilescu C, Muhleisen TW, Wang AG, Ullum H, Djurovic S, Melle I, Olesen J, Kiemeny LA, Franke B, Sabatti C, Freimer NB, Gulcher JR, Thorsteinsdottir U, Kong A, Andreassen OA, Ophoff RA, Georgi A, Rietschel M, Werge T, Petursson H, Goldstein DB, Nothen MM, Peltonen L, Collier DA, St Clair D, Stefansson K.

- Large recurrent microdeletions associated with schizophrenia. *Nature*. 2008; 455:232–236. [PubMed: 18668039]
- Sternberg DE, Charney DS, Heninger GR, Leckman JF, Hafstad KM, Landis DH. Impaired presynaptic regulation of norepinephrine in schizophrenia. Effects of clonidine in schizophrenic patients and normal controls. *Arch Gen Psychiatry*. 1982; 39:285–289. [PubMed: 6279049]
- Stober G, Jatzke S, Meyer J, Okladnova O, Knapp M, Beckmann H, Lesch KP. Short CAG repeats within the hSKCa3 gene associated with schizophrenia: results of a family-based study. *Neuroreport*. 1998; 9:3595–3599. [PubMed: 9858366]
- Stober G, Meyer J, Nanda I, Wienker TF, Saar K, Jatzke S, Schmid M, Lesch KP, Beckmann H. hKCN3 which maps to chromosome 1q21 is not the causative gene in periodic catatonia, a familial subtype of schizophrenia. *Eur Arch Psychiatry Clin Neurosci*. 2000; 250:163–168. [PubMed: 11009068]
- Stocker M. Ca²⁺-activated K⁺ channels: molecular determinants and function of the SK family. *Nature Rev Neurosci*. 2004; 5:758–770. [PubMed: 15378036]
- Stocker M, Hirzel K, D'hoedt D, Pedarzani P. Matching molecules to function: neuronal Ca²⁺-activated K⁺ channels and afterhyperpolarizations. *Toxicon*. 2004; 43:933–949. [PubMed: 15208027]
- Strong M, Gutman GA. Missing link in ion channels. *Nature*. 1993; 362:26. [PubMed: 7680430]
- Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. *Proc Natl Acad Sci USA*. 2005; 102:15545–15550. [PubMed: 16199517]
- Tam GW, van de Lagemaat LN, Redon R, Strathdee KE, Croning MD, Malloy MP, Muir WJ, Pickard BS, Deary IJ, Blackwood DH, Carter NP, Grant SG. Confirmed rare copy number variants implicate novel genes in schizophrenia. *Biochem Soc Trans*. 2010; 38:445–451. [PubMed: 20298200]
- Thompson J, Begenisich T. Membrane-delimited inhibition of maxi-K channel activity by the intermediate conductance Ca²⁺-activated K channel. *J Gen Physiol*. 2006; 127:159–169. [PubMed: 16418402]
- Thornton-Wells TA, Moore JH, Haines JL. Genetics, statistics and human disease: analytical retooling for complexity. *Trends Genet*. 2004; 20:640–647. [PubMed: 15522460]
- Thummler S, Duprat F, Lazdunski M. Antipsychotics inhibit TREK but not TRAAK channels. *Biochem Biophys Res Commun*. 2007; 354:284–289. [PubMed: 17222806]
- Tishkoff SA, Williams SM. Genetic analysis of African populations: human evolution and complex disease. *Nat Rev Genet*. 2002; 3:611–621. [PubMed: 12154384]
- Tishkoff SA, Reed FA, Friedlaender FR, Ehret C, Ranciaro A, Froment A, Hirbo JB, Awomoyi AA, Bodo JM, Doumbo O, Ibrahim M, Juma AT, Kotze MJ, Lema G, Moore JH, Mortensen H, Nyambo TB, Omar SA, Powell K, Pretorius GS, Smith MW, Thera MA, Wambebe C, Weber JL, Williams SM. The genetic structure and history of Africans and African Americans. *Science*. 2009; 324:1035–1044. [PubMed: 19407144]
- Trierweiler SJ, Neighbors HW, Munday C, Thompson EE, Binion VJ, Gomez JP. Clinician attributions associated with the diagnosis of schizophrenia in African American and non-African American patients. *J Consult Clin Psychol*. 2000; 68:171–175. [PubMed: 10710852]
- Trierweiler SJ, Neighbors HW, Munday C, Thompson EE, Jackson JS, Binion VJ. Differences in patterns of symptom attribution in diagnosing schizophrenia between African American and non-African American clinicians. *Am J Orthopsychiatry*. 2006; 76:154–160. [PubMed: 16719633]
- Trimmer JS, Rhodes KJ. Localization of voltage-gated ion channels in mammalian brain. *Annu Rev Physiol*. 2004; 66:477–519. [PubMed: 14977411]
- Tseng TT, McMahon AM, Johnson VT, Mangubat EZ, Zahm RJ, Pacold ME, Jakobsson E. Sodium channel auxiliary subunits. *J Mol Microbiol Biotechnol*. 2007; 12:249–262. [PubMed: 17587873]
- Ujike H, Yamamoto A, Tanaka Y, Takehisa Y, Takaki M, Taked T, Kodama M, Kuroda S. Association study of CAG repeats in the KCNN3 gene in Japanese patients with schizophrenia, schizoaffective disorder and bipolar disorder. *Psychiatry Res*. 2001; 101:203–207. [PubMed: 11311923]

- Van Schijndel JE, Martens JM. Gene expression profiling in rodent models for schizophrenia. *Current Neuropharmacol.* 2010; 8:382–393.
- Vanoye CG, Welch RC, Tian C, Sanders CR, George AL Jr. KCNQ1/KCNE1 assembly, co-translation not required. *Channels (Austin).* 2010; 4:108–114. [PubMed: 20139709]
- Vazquez E, Valverde MA. A review of TRP channels splicing. *Semin Cell Dev Biol.* 2006; 17:607–617. [PubMed: 17174125]
- Verhoeff NP. Radiotracer imaging of dopaminergic transmission in neuropsychiatric disorders. *Psychopharmacology (Berl).* 1999; 147:217–249. [PubMed: 10639681]
- Walsh T, McClellan JM, McCarthy SE, Addington AM, Pierce SB, Cooper GM, Nord AS, Kusenda M, Malhotra D, Bhandari A, Stray SM, Rippey CF, Roccanova P, Makarov V, Lakshmi B, Findling RL, Sikich L, Stromberg T, Merriman B, Gogtay N, Butler P, Eckstrand K, Noory L, Gochman P, Long R, Chen Z, Davis S, Baker C, Eichler EE, Meltzer PS, Nelson SF, Singleton AB, Lee MK, Rapoport JL, King MC, Sebat J. Rare structural variants disrupt multiple genes in neurodevelopmental pathways in schizophrenia. *Science.* 2008; 320:539–543. [PubMed: 18369103]
- Wang K, Li M, Bucan M. Pathway-based approaches for analysis of genomewide association studies. *Am J Hum Genet.* 2007; 81(6):1278–1283. [PubMed: 17966091]
- Wang K, Dickson SP, Stolle CA, Krantz ID, Goldstein DB, Hakonarson H. Interpretation of association signals and identification of causal variants from genome-wide association studies. *Am J Hum Genet.* 2010; 86:730–742. [PubMed: 20434130]
- Waxman SG. The neuron as a dynamic electrogenic machine: modulation of sodium-channel expression as a basis for functional plasticity in neurons. *Philos Trans R Soc Lond B Biol Sci.* 2000; 355:199–213. [PubMed: 10724456]
- Waxman SG. Channel, neuronal and clinical function in sodium channelopathies: from genotype to phenotype. *Nat Neurosci.* 2007; 10:405–409. [PubMed: 17387329]
- Waxman SG, Dib-Hajj S, Cummins TR, Black JA. Sodium channels and their genes: dynamic expression in the normal neocortical system, dysregulation in disease states(1). *Brain Res.* 2000; 886:5–14. [PubMed: 11119683]
- Wei J, Hemmings GP. A further study of a possible locus for schizophrenia on the X chromosome. *Biochem Biophys Res Commun.* 2006; 344:1241–1245. [PubMed: 16650384]
- Wise CD, Stein L. Dopamine-beta-hydroxylase deficits in the brains of schizophrenic patients. *Science.* 1973; 181:344–347. [PubMed: 4719906]
- Wittekindt O, Jauch A, Burgert E, Scharer L, Holtgreve-Grez H, Yvert G, Imbert G, Zimmer J, Hoehe MR, Macher JP, Chiaroni P, van Calcar D, Crocq MA, Morris-Rosendahl DJ. The human small conductance calcium-regulated potassium channel gene (hSKCa3) contains two CAG repeats in exon 1, is on chromosome 1q21.3, and shows a possible association with schizophrenia. *Neurogenetics.* 1998; 1:259–265. [PubMed: 10732800]
- Wolfart J, Neuheff H, Franz O, Roeper J. Differential expression of the small-conductance, calcium-activated potassium channel SK3 is critical for pacemaker control in dopaminergic midbrain neurons. *J Neurosci.* 2001; 21:3443–3456. [PubMed: 11331374]
- Wong AH, Lipska BK, Likhodi O, Boffa E, Weinberger DR, Kennedy JL, Van Tol HH. Cortical gene expression in the neonatal ventral-hippocampal lesion rat model. *Schizophr Res.* 2005; 77:261–270. [PubMed: 15890497]
- Woolley DW, Shaw E. A biochemical and pharmacological suggestion about certain mental disorders. *Proc Natl Acad Sci USA.* 1954; 40:228–231. [PubMed: 16589461]
- Xiao Z, Deng PY, Rojanathammanee L, Yang C, Grisanti L, Permpoonputtana K, Weinshenker D, Doze VA, Porter JE, Lei S. Noradrenergic depression of neuronal excitability in the entorhinal cortex via activation of TREK-2K⁺ channels. *J Biol Chem.* 2009; 284:10980–10991. [PubMed: 19244246]
- Xie, M.; Holmqvist, MH.; Hsia, AY. Ion channel drug discovery expands into new disease areas; 2004. p. 31-33.<http://www.pdfio.com/k-647071.html#>
- Yan J, Olsen JV, Park KS, Li W, Bildl W, Schulte U, Aldrich RW, Fakler B, Trimmer JS. Profiling the phospho-status of the BKCa channel alpha subunit in rat brain reveals unexpected patterns and complexity. *Mol Cell Proteomics.* 2008; 7:2188–2198. [PubMed: 18573811]

- Yang KC, Jin GZ, Wu J. Mysterious alpha6-containing nAChRs: function, pharmacology, and pathophysiology. *Acta Pharmacol Sin.* 2009; 30:740–751. [PubMed: 19498417]
- Zaykin DV, Zhivotovsky LA, Westfall PH, Weir BS. Truncated product method for combining *P*-values. *Genet Epidemiol.* 2002; 22:170–185. [PubMed: 11788962]
- Zhang K, Cui S, Chang S, Zhang L, Wang J. i-GSEA4GWAS: a web server for identification of pathways/gene sets associated with traits by applying an improved gene set enrichment analysis to genome-wide association study. *Nucleic Acids Res.* 2010; 38:W90–W95. [PubMed: 20435672]

Fig. 1. Flow Diagram Illustrating SNP-to-gene mapping and analytic processing steps for gene set analyses. Flow diagram depicting the preprocessing procedures used to transform the original EA and AA GWAS data prior to performing the gene set analyses. The *center column* (Methods) depicts the methods used to transform the original and mapping datasets into the final EA and AA GSA datasets used in EVA to generate final GSA results. The *far left* (EA Data) and *right* (AA Data) *columns* each depict the starting (original GWAS), intermediate (Mapping) and final (GSA) datasets resulting from each methodological step

Table 1
GSA results (*p* values) for all tested ion channel gene sets exceeding nominal significance in European-American sample

Analytic parameters			Significant GSA results for tested gene sets						
Statistical reduction method	Mapping strategy	EVA enrichment cut-off	Ion channel activity	Voltage-gated ion channel activity	Potassium channel activity	Calcium channel activity	Chloride channel activity	Voltage-gated chloride channel activity	Calcium-release channel activity
pMIN	Genic	0.01	8.12E-05**	0.0412	NS	9.67E-05**	5.91E-03*	NS	4.69E-04**
pMIN	500 Kb	0.01	8.55E-06**	5.54E-03*	8.87E-03*	5.05E-04**	NS	NS	1.60E-04**
pMIN	2.5 Mb	0.01	1.55E-05**	7.12E-03*	0.0106*	6.35E-04**	NS	NS	1.73E-04**
PROP001	Genic	0.05	NS	NS	NS	NS	NS	NS	9.20E-04**
PROP001	500 Kb	0.05	2.86E-03**	NS	1.01E-03**	NS	NS	NS	3.29E-05**
PROP001	2.5 Mb	0.05	8.62E-03*	NS	6.08E-03*	NS	NS	NS	3.41E-05**
PROP01	Genic	0.05	0.02945	NS	NS	0.0309	NS	NS	0.0113*
PROP01	500 Kb	0.05	0.03288	NS	0.0160*	NS	NS	NS	1.26E-03**
PROP01	2.5 Mb	0.05	0.03212	NS	0.0158*	NS	NS	NS	1.25E-03**
PROP05	Genic	0.05	NS	NS	NS	NS	NS	NS	0.0462
PROP05	500 Kb	0.05	NS	NS	NS	0.0225*	NS	NS	8.88E-03*
PROP05	2.5 Mb	0.05	NS	NS	NS	0.0220*	NS	NS	8.82E-03*
TPM01	Genic	0.05	0.01926*	NS	NS	NS	0.0265	0.0393	0.0319
TPM01	500 Kb	0.05	3.76 E-03**	0.0462	5.40E-03*	NS	NS	NS	4.13E-04**
TPM01	2.5 Mb	0.05	7.91E-03*	NS	0.0152*	NS	NS	NS	4.30E-04**
TPM05	Genic	0.05	NS	NS	NS	NS	NS	0.0407	5.68E-04**
TPM05	500 Kb	0.05	NS	NS	NS	0.0428	NS	NS	1.45E-03**
TPM05	2.5 Mb	0.05	NS	NS	NS	0.0448	NS	NS	1.48E-03**

There were no significant GSA findings for sodium channel, voltage-gated potassium, voltage-gated calcium or voltage-gated sodium channel gene sets

pMIN (minimum *p* value method), PROP05, PROP01, PROP001 (proportion method using $\alpha = 0.05$, 0.01 and 0.001, respectively), TPM05, TPM01 (truncated product method using $\alpha = 0.05$ and 0.01, respectively)

* Indicates gene set reached a priori significance threshold of <0.025

** Indicates gene set significant after Bonferroni correction

Table 2
GSA results (*p* values) for all tested ion channel gene sets exceeding nominal significance in African-American sample

Analytic parameters			Significant GSA results for tested gene sets							
Statistical reduction method	Mapping strategy	EVA enrichment cut-off	Ion channel activity	Voltage-gated ion channel activity	Potassium channel activity	Calcium channel activity	Chloride channel activity	Voltage-gated potassium channel activity	Voltage-gated calcium channel activity	Calcium-release channel activity
pMIN	Genic	0.01	2.00E-08**	1.68E-05**	5.19E-04**	1.19E-03**	8.63E-04**	7.59E-03*	1.50E-03**	0.0155*
pMIN	500 Kb	0.01	4.13E-07**	1.90E-04**	1.44E-05**	8.39E-04**	0.0234*	5.78E-03*	NS	NS
pMIN	2.5 Mb	0.01	3.53E-07**	2.42E-04**	1.73E-05**	4.00E-04**	0.0258	6.59E-03*	NS	NS
PROP001	Genic	0.05	NS	NS	NS	NS	NS	NS	NS	NS
PROP001	500 Kb	0.05	NS	NS	NS	NS	NS	NS	NS	NS
PROP001	2.5 Mb	0.05	NS	NS	NS	NS	NS	NS	NS	NS
PROP01	Genic	0.05	NS	NS	NS	NS	NS	NS	NS	NS
PROP01	500 Kb	0.05	NS	NS	0.0499	NS	NS	NS	NS	NS
PROP01	2.5 Mb	0.05	NS	NS	NS	NS	NS	NS	NS	NS
PROP05	Genic	0.05	NS	NS	NS	NS	NS	NS	NS	NS
PROP05	500 Kb	0.05	NS	NS	9.33E-04**	NS	NS	0.0366	NS	NS
PROP05	2.5 Mb	0.05	NS	NS	2.92E-03**	NS	NS	NS	NS	NS
TPM01	Genic	0.05	NS	NS	NS	NS	NS	NS	NS	NS
TPM01	500 Kb	0.05	NS	NS	NS	NS	NS	NS	NS	NS
TPM01	2.5 Mb	0.05	NS	NS	NS	NS	NS	NS	NS	NS
TPM05	Genic	0.05	NS	NS	NS	NS	NS	NS	NS	NS
TPM05	500 Kb	0.05	NS	NS	0.0423	NS	NS	NS	NS	NS
TPM05	2.5 Mb	0.05	NS	NS	NS	NS	NS	NS	NS	NS

There were no significant GSA findings for sodium channel, voltage-gated chloride channel or voltage-gated sodium channel gene sets

pMIN (minimum *p* value method), PROP05, PROP01, PROP001 (proportion method using $\alpha = 0.05$, 0.01 and 0.001, respectively), TPM05, TPM01 (truncated product method using $\alpha = 0.05$ and 0.01, respectively)

* Indicates gene set reached a priori significance threshold of <0.025

** Indicates gene set significant after Bonferroni correction

Table 3

Comparison of specific potassium channel genes contributing to enrichment of the potassium channel activity gene set in the 500 Kb analyses across samples and statistical reduction methods

GENE	Gene size (bp)	EA-pMIN	EA-PROP01	EA-PROP001	EA-TPM01	AA-pMIN	AA-PROP05	AA-PROP01	AA-TPM05	SIG analysis count	SIG sample(S)
KCNQ1	614,039	8.06E-03	4.54E-02	NS	2.82E-02	1.59E-03	6.90E-03	3.62E-02	1.25E-03	7	EA & AA
KCNA4	6,722	3.89E-04	NS	9.11E-04	3.95E-03	2.25E-03	2.42E-02	NS	3.15E-02	6	EA & AA
KCND2	476,600	2.05E-03	7.12E-03	NS	1.27E-03	2.02E-03	2.92E-02	NS	2.47E-02	6	EA & AA
KCNMA1	767,531	7.77E-04	5.43E-03	3.49E-03	1.22E-04	1.15E-03	NS	NS	NS	5	EA & AA
KCNT2	382,586	8.28E-05	NS	1.17E-02	6.54E-03	1.84E-03	NS	1.31E-03	NS	5	EA & AA
TMEM38B	80,619	3.44E-05	4.52E-03	2.54E-02	1.47E-04	5.51E-04	NS	NS	NS	5	EA & AA
KCND3	411,800	2.01E-03	2.02E-03	NS	4.82E-04	7.04E-03	NS	NS	NS	4	EA & AA
KCNE2*	7,117	NS	NS	NS	NS	2.67E-04	3.39E-03	1.88E-03	1.30E-04	4	AA
KCNH7	467,716	NS	NS	NS	NS	2.21E-03	9.05E-04	4.27E-02	8.15E-04	4	AA
KCNJ15	45,084	NS	NS	NS	NS	1.80E-03	3.40E-03	7.14E-03	1.90E-04	4	AA
KCNK10*	142,011	6.60E-03	NS	NS	NS	8.24E-04	2.63E-03	NS	8.47E-04	4	EA & AA
KCNK13	124,087	8.68E-04	4.13E-04	NS	4.03E-05	4.17E-03	NS	NS	NS	4	EA & AA
KCNK3	54,237	2.63E-04	6.29E-03	2.84E-02	2.35E-04	NS	NS	NS	NS	4	EA
TMEM38A	27,894	NS	NS	NS	NS	1.35E-03	4.38E-02	1.19E-02	3.67E-03	4	AA
KCNA2	2,569	8.40E-03	NS	NS	NS	NS	1.53E-02	NS	1.92E-02	3	EA & AA
KCNB1	110,676	NS	NS	NS	NS	8.82E-03	3.29E-02	NS	3.49E-02	3	AA
KCNC2	169,615	NS	NS	NS	NS	8.44E-04	2.68E-03	NS	7.78E-04	3	AA
KCNH6	25,936	1.88E-03	2.38E-03	NS	6.08E-04	NS	NS	NS	NS	3	EA
KCNK3	40,900	5.49E-04	NS	2.60E-02	2.02E-02	NS	NS	NS	NS	3	EA
KCNQ2*	68,723	3.30E-03	1.35E-02	NS	5.97E-03	NS	NS	NS	NS	3	EA
PKD2	70,109	NS	NS	NS	NS	1.30E-03	1.83E-02	NS	5.40E-03	3	AA
HCN2	27,266	NS	NS	NS	NS	NS	2.60E-02	NS	1.37E-02	2	AA
KCNB2	533,168	2.37E-03	NS	NS	NS	9.42E-03	NS	NS	NS	2	EA & AA
KCNC1	36,665	3.63E-03	NS	NS	4.13E-02	NS	NS	NS	NS	2	EA & AA
KCNG3	52,080	NS	NS	NS	NS	2.91E-03	NS	3.63E-02	NS	2	AA
KCNIP1	450,177	2.48E-03	NS	NS	NS	2.08E-03	NS	NS	NS	2	EA & AA
KCNIP4	1,610,967	5.78E-03	NS	NS	NS	5.88E-03	NS	NS	NS	2	EA & AA

GENE	Gene size (bp)	EA-pMIN	EA-PROP01	EA-PROP001	EA-TPM01	AA-pMIN	AA-PROP05	AA-PROP01	AA-TPM05	SIG analysis count	SIG sample(S)
KCNK17	15,459	NS	NS	NS	NS	NS	2.10E-02	NS	4.20E-02	2	AA
KCNQ5	630,781	NS	NS	NS	NS	3.81E-03	4.38E-02	NS	NS	2	AA
ABCC8	84,002	9.22E-03	NS	NS	NS	NS	NS	NS	NS	1	EA
KCNA1*	8,348	NS	NS	NS	NS	4.49E-03	NS	NS	NS	1	AA
KCNA3	3,346	NS	NS	NS	NS	1.13E-03	NS	NS	NS	1	AA
KCNA5	2,764	NS	NS	NS	NS	3.07E-03	NS	NS	NS	1	AA
KCNE4	3,493	NS	NS	NS	NS	5.30E-03	NS	NS	NS	1	AA
KCNF1	2,288	NS	NS	NS	NS	3.03E-03	NS	NS	NS	1	AA
KCNK1	19,482	4.39E-03	NS	NS	NS	NS	NS	NS	NS	1	EA
KCNK2	36,148	7.96E-03	NS	NS	NS	NS	NS	NS	NS	1	EA
KCNH1	450,902	NS	NS	NS	NS	9.45E-03	NS	NS	NS	1	AA
KCNH5	394,639	NS	NS	NS	NS	2.20E-03	NS	NS	NS	1	AA
KCNH8	412,117	NS	NS	NS	NS	1.31E-03	NS	NS	NS	1	AA
KCNK5	40,504	NS	NS	NS	NS	2.58E-03	NS	NS	NS	1	AA
KCNK9	159,677	7.10E-03	NS	NS	NS	NS	NS	NS	NS	1	EA
KCNQ3*	422,993	NS	NS	NS	NS	4.52E-03	NS	NS	NS	1	AA
KCNS2	3,775	NS	NS	NS	NS	2.24E-03	NS	NS	NS	1	AA
KCNU1	270,959	NS	NS	NS	NS	2.28E-03	NS	NS	NS	1	AA

* Of note, the genes indicated by an asterix are those that have previous evidence of association or functional significance in schizophrenia. See text for relevant references