HHS Public Access

Author manuscript

Addict Biol. Author manuscript; available in PMC 2020 March 01.

Published in final edited form as:

Addict Biol. 2019 March ; 24(2): 275–289. doi:10.1111/adb.12591.

Beyond Genome-wide Significance: Integrative Approaches to the Interpretation and Extension of GWAS Findings for Alcohol Use Disorder

Jessica E. Salvatore[1,2], **Shizhong Han**[3,4], **Sean P. Farris**[5], **Kristin M. Mignogna**[2], **Michael F. Miles**[6], and **Arpana Agrawal**[7]

[1]Department of Psychology, Virginia Commonwealth University, Box 842018, Richmond, VA 23284-2018, United States

[2]Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Box 980126, Richmond, VA 23298, United States

[3]Department of Psychiatry, University of Iowa, 22G General Hospital, Iowa City, Iowa 52242, United States

[4]Department of Psychiatry and Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, Maryland, 21287, United States

[5]Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, TX 78712, United States

[6]Department of Pharmacology and Toxicology, Virginia Commonwealth University, Molecular Medicine Research Building 6034, Box 980613, Richmond, VA 23298, United States

[7]Department of Psychiatry, Washington University School of Medicine, 660 S. Euclid, CB 8134, Saint Louis, MO 63110, United States

Abstract

Alcohol use disorder (AUD) is a heritable complex behavior. Due to the highly polygenic nature of AUD, identifying genetic variants that comprise this heritable variation has proved to be challenging. With the exception of functional variants in alcohol metabolizing genes (e.g., *ADH1B* and *ALDH2*), few other candidate loci have been confidently linked to AUD. Genome-wide association studies (GWAS) of AUD and other alcohol-related phenotypes have either produced few hits with genome-wide significance or have failed to replicate on further study. These issues reinforce the complex nature of the genetic underpinnings for AUD and suggest that both GWAS studies with larger samples and additional analysis approaches that better harness the nominally significant loci in existing GWAS are needed. Here, we review approaches of interest in the post-

Correspondence concerning this manuscript should be addressed to: Jessica E. Salvatore and Arpana Agrawal. JES: Box 842018, 806 West Franklin Street, Richmond, VA 23284-2018, USA. Phone: +1 (804) 828-8132; fax: +1 (804) 828-2237. jesalvatore@vcu.edu. AA: Department of Psychiatry, Washington University School of Medicine, 660 S. Euclid, CB 8134, Saint Louis, MO 63110, USA. Phone: +1 (314) 286-1778; fax: +1 (314) 286-2213. arpana@wustl.edu.

Author Contributions

All authors contributed to the drafting and critically reviewing manuscript content. All authors approved of the final, submitted version.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

GWAS era, including *in silico* functional analyses; functional partitioning of SNP heritability; aggregation of signal into genes and gene networks; and validation of identified loci, genes, and gene networks in postmortem brain tissue and across species. These integrative approaches hold promise to illuminate our understanding of the biological basis of AUD; however, we recognize that the main challenge continues to be the extremely polygenic nature of AUD, which necessitates large samples to identify multiple loci associated with AUD liability.

Keywords

alcohol use disorder; cross-species validation; functional genomics; translational genomics

Introduction

Alcohol use disorder (AUD) is a heritable ($h^2 = 50 – 60\%$) and highly polygenic behavior (Hart et al. 2015; Verhulst et al. 2015). The associations between AUD and single nucleotide polymorphisms (SNPs) in *ADH1B* (across populations) and *ALDH2* (only in certain Asian populations) represent some of the largest effect sizes in psychiatry (Edenberg et al. 2014). Despite this, only the largest gene discovery efforts have identified associations with *ADH1B* (Bierut et al. 2012; Gelernter et al. 2014). Further, it is expected that other loci contributing to heritable variation in AUD are of far more modest effect size. In fact, of the extant AUD GWAS (for a review see Hart et al. 2015; see also Mbarek et al. 2015), only three have identified any genome-wide significant ($p < 5×10^{−8}$) loci, in *ADH1B* (Gelernter et al. 2014), *ADH1C* (Frank et al. 2012), and an intergenic region on chromosome 2q35 (Treutlein et al. 2009). The largest currently published AUD GWAS includes 7,677 cases (4,938 of European ancestry) and 6,123 controls, therefore, it is not surprising that individual studies have produced no more than single genome-wide significant loci (Gelernter et al. 2014). For instance, in the case of schizophrenia, there was only one genome-wide significant locus with a similar 8,008 cases (Shi et al. 2009). However, an expansion of the sample size to nearly 37,000 cases identified more than 100 independent loci (Schizophrenia Working Group of the Psychiatric Genomics Consortium 2014). It is projected that AUD will be more polygenic than schizophrenia, thus requiring an even greater sample size to identify novel genome-wide significant loci.

The GWAS approach examines individual SNPs across the genome, agnostic to any functional relevance. Although this increases our confidence in unbiased true positive findings, GWAS could miss the contributions of biologically relevant genetic signals that do not survive the stringent correction for multiple testing. Separating the “true” genetic signal from the noise is therefore important for identifying likely causal variants, understanding their functional roles in disease pathogenesis, and better characterizing aggregate genetic risk (Nature Genetics Editorial Board 2010). Recent critiques of GWAS have also questioned the utility of widespread genome-wide polygenic signal that generally comprises total SNP-derived heritable variation. Investigators, for instance, have proposed a model that implies that in addition to “top” loci that pinpoint disease-specific etiologic pathways (e.g., major histocompatibility complex for schizophrenia), the remaining loci of smaller effect may be related to genes with no direct relevance to the disease but instead, of broader

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

relevance from a cell-specific regulatory perspective (Boyle et al. 2017). Overall, there is growing interest in the "next steps" that might follow a traditional GWAS. In this review, we aim to cover three broad areas of interest in the post-GWAS era:

a. If a genome-wide significant locus is identified, there are several computational biology approaches that can provide *in silico* support for its functional significance. We briefly outline these approaches.

b. Even when genome-wide significance is not achieved, discoveries can be made by aggregating across the effects of SNPs to assess heritability or to identify genes and networks of biological importance.

c. Ultimately, the importance of single loci, gene, or networks identified using *in silico* approaches lies in validating their role on regulating expression differences. We discuss two approaches for validating and extending findings - postmortem expression studies of brain tissue, and cross-species validation.

As this review covers many tools and types of genomic information that may not be familiar to all readers, we include a glossary in Table 1.

Functional annotation of GWAS results

Association results can be used as a starting point to understand the biology underlying a disease, either at the level of single locus or across the genome.

Single locus approaches

Many GWAS identify loci that do not have a straightforward functional relevance (i.e., not nonsynonymous; Maurano et al. 2012). In such instances, several steps may be undertaken to annotate the locus:

a. Annotation information about genic location (e.g., whether a SNP is located in the 5′ or 3′ untranslated regions; an exon; an intron; or is intergenic) demonstrates that SNPs that tag regulatory and coding regions are likely to be enriched for association in GWAS (Schork et al. 2013). Further, databases such as RegulomeDB (Boyle et al. 2012) and HaploReg (Ward et al. 2012) offer preliminary evidence of functional relevance. For instance, a score of 1(a–f) represents supporting data that the SNP is an expression quantitative trait locus (eQTL), modifies transcription factor binding or motifs, and is potentially in a region of enhanced DNase activity (i.e., a region of epigenetic regulation).

b. A simple follow-up of a potential eQTL for AUD might also involve queries in datasets, such as the Genotype Tissue Expression (GTeX), BRAINEAC, or other curated databases that match GWAS data to mRNA expression changes in a variety of brain regions (popular databases are summarized in Table 2 and detailed below). Similar databases are becoming available for comparable methylation, or meQTL, analyses (Hernandez et al. 2012). Two potential drawbacks of these repositories include the relatively small numbers of brain samples from select brain regions (and including a mixture of cell-types) derived from participants with diverse causes of death, and limited ability to examine

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

whether a SNP is an eQTL in AUD individuals. Databases that examine eQTL effects in blood are larger (e.g., Westra et al. 2013) and may also be of interest when exploring the peripheral effects of AUD; such databases can also begin to provide evidence for trans-eQTL effects. In fact, at least one recent study suggests that cross-tissue correlation for local, but not distal, regulation of gene expression might be correlated (Liu et al. 2017).

c. Studies of epigenomic elements (i.e., regulatory marks) offer additional insights into the regulatory landscape of GWAS results for complex traits. For example, genome-wide significant SNPs for complex traits tend to be located in DNA regulatory regions such as DNase I hypersensitive sites (DHSs) (Maurano et al. 2012). The Human Epigenome Browser (Zhou et al. 2011) allows comprehensive annotation of histone methylation and acetylation marks in a diversity of tissue types as well as characterization of standard conservation (e.g., PhyloP scores) metrics. There are a number of publically available resources along these lines, including the UCSC genome browser (Speir et al. 2016), ENCODE (ENCODE Project Consortium 2012), and RoadMap Epigenomics (Roadmap Epigenomics Consortium et al. 2015).

d. If a SNP is an eQTL for a gene, causality (i.e., whether the effect of the locus on AUD is due to its effect on the gene) can be inferred by tests of Mendelian Randomization (e.g., Zhu et al. 2016).

Genome-wide approaches

When a GWAS does not produce genome-wide significant loci, the power of the analysis may be questionable; still, aggregating information across the genome may produce interesting insights. For instance, methods such as Genomewide Complex Traits Analysis (GCTA) and Linkage Disequilibrium Score Regression (LDScR) allow for the computation of SNP-heritability in single ancestry samples (Yang et al. 2011; Bulik-Sullivan et al. 2015). LDScR has some advantage in that it uses summary statistics, whereas GCTA requires the raw genotypes. One study suggests that common SNPs explain 30% of the variance in AUD (Palmer et al. 2015).

Genome partitioning and enrichment methods can be used to determine which locations in the genome account for heritable variation and are enriched for disease-associated variants (Gusev et al. 2014). For example, in analyses of 11 common diseases, DHSs accounted for 79% of the heritability (Gusev et al. 2014), while coding SNPs explained only 10% of the heritability. More recently, SNP-based heritability partitioning methods for GWAS summary statistics based on LDScR in 9 complex traits and diseases demonstrated that a number of genomic categories are enriched for heritability, including regions conserved in mammals (Finucane et al. 2015). With respect to alcohol-related phenotypes, enrichment analyses indicated that SNPs associated with alcohol problems tended to be localized in genomic regions of potential regulatory significance (as indicated by DNase I Hypersensitive sites or H3K4me3 histone marks) (Edwards et al. 2015).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Aggregating GWAS data into biological units

GWAS data can be further combined into biological units using gene and network-based approaches.

Gene-based approaches

There is a high multiple testing burden in the context of a GWAS. Gene-based approaches, which aggregate across summary statistics derived from association analyses of multiple loci to derive p-values for association at the level of the gene, developed as one way to reduce multiple testing. The statistical genetics program PLINK (Purcell et al. 2007; Chang et al. 2015) combines single locus association data using a permutation procedure to calculate gene-based p-values. The versatile gene-based association study (VEGAS; Liu et al. 2010; Mishra et al. 2015) was developed in response to limitations related to PLINK's computational demands and dependence on local the local linkage disequilibrium (LD) structure. VEGAS uses a more flexible and efficient Monte Carlo-based approach based on GWAS summary statistics. However, like PLINK, VEGAS relies on single-locus association results to calculate gene-based p-values, which reduces power (Moskvina et al. 2012). More recently, Multi-marker Analysis of GenoMic Annotation (MAGMA; de Leeuw et al. 2015) was introduced as a multiple regression based method that takes into account LD. Simulations showed that MAGMA produced highly similar results to PLINK and VEGAS, but with greater power and efficiency (de Leeuw et al. 2015). It is worth noting that although the multiple testing burden is lower for gene-based tests than for single-locus tests, these aggregation techniques still rely on association statistics for single loci, or the SNP matrix for a gene (in the case of MAGMA) to calculate p-values. Thus, the power of the GWAS remains an issue for gene-based tests.

Gene-based tests can also be expanded to incorporate other types of genomic information, such as tissue-specific gene expression. Table 2, for instance, outlines a series of databases that can be queried in a tissue-specific manner regarding the effects of SNPs and genes on variations in mRNA expression and methylation change. For example, newer statistical methods such as PrediXcan (Gamazon et al. 2015) and Transcriptome-wide association studies (TWAS) (Gusev et al. 2016) allow for common variation from GWAS arrays to be linked to gene expression profiles in smaller training samples where both SNP and mRNA expression data are available. The training sample is used to link gene expression to SNP-level variation, and these "imputed" gene expression profiles are then examined for association with disease status (at the level of the gene) in a larger test GWAS sample that itself does not include expression data. A strength of this approach is that it uses information about the potential tissue-specific molecular mechanisms through which a gene has its effect (e.g., through global up- or down-regulation of gene expression) to identify associated genes. For instance, a recent TWAS of 30 complex traits (e.g., height) and diseases (e.g., Type 2 Diabetes), identified 1,789 gene-trait associations, of which 9% were more than 0.5Mb from the transcription start site of genes containing a genome-wide significant SNP. The study also identified 43 expression-based trait correlations, of which only 22 had been previously noted (Mancuso et al. 2017). However, as mentioned previously, brain expression

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

data are drawn from select regions from few subjects with heterogeneous psychiatric histories.

Gene set-based analysis leveraging functional relationships of genes

Gene set-based analysis examines a group of functionally related genes whose effects may be too small to detect individually, but that may be detected when examined jointly (Wang et al. 2010; Wang et al. 2011). This approach is premised on the idea that although many genes may be involved in complex, polygenic diseases, they should converge on underlying biological pathways and molecular networks. Gene set-based analysis has several advantages (Fridley et al. 2011), including increased power through reduced multiple testing and aggregating weak signals distributed across a gene set.

Pathway-based analysis—Pathway-based analysis aims to detect significantly enriched gene sets from predefined canonical pathways or functional annotations. Over the past decade, numerous pathway-based analytical approaches have been developed for GWAS (Jin et al. 2014). These include SETSCREEN (Moskvina et al. 2011), MAGENTA (Segre et al. 2010), INRICH (Lee et al. 2012), FORGE (Pedroso et al. 2012), and ALIGATOR (Holmans et al. 2009). However, it is not clear which one is optimal, and the power of existing methods is often dependent on the underlying genetic architecture (de Leeuw et al. 2016). Of note, the Psychiatric Genomics Consortium found that the results were highly correlated between methods in a comparison of methods applied across several psychiatric disorders (Network Pathway Analysis Subgroup of Psychiatric Genomics Consortium 2015). A second limitation of pathway-based analysis is that it is still biased by our incomplete prior knowledge of gene function in the etiology of psychiatric illness.

Despite these challenges, pathway-based analyses have identified biological pathways underlying various complex diseases (Wang et al. 2010), including AUD. Table 3 summarizes pathway-based GWAS of alcohol-related traits. For example, Juraeva et al. (2015) combined pathway-based analysis with functional follow-up studies in Drosophila and humans and found convergent evidence for the role of *XRCC5*. Although pathway-based analysis gives additional biological insights, it is important to note that these results are not consistent across studies, which may be attributable to different analytical methods, databases, and definitions of alcohol-related behaviors.

Network-based analysis—Network-based analysis searches for a group of interacting genes in a functional gene network that contribute to disease risk. Compared with pathway-based analysis, the network-based approach has the advantages of more flexibly defining gene sets, detecting genes that work across pathways, and being less biased by prior knowledge (Torkamani et al. 2009; Leiserson et al. 2013). Network analysis can also identify “hub genes” that may have critical functions as network organizing foci that may be potential therapeutic targets for AUD (Farris et al. 2012; Wolen et al. 2012). Network-based analysis of GWAS has identified novel pathways and candidate genes across diverse diseases including cancer (AlQuraishi et al. 2014), coronary artery disease (Zhao et al. 2016), Alzheimer’s disease (Song et al. 2016), and Autism spectrum disorder (Ben-David et al. 2012; Hillenmeyer et al. 2016). In an effort to apply a network-based approach to AUD, Han

et al. (2013) integrated GWAS of alcohol dependence with a PPI network (which assesses functional relationships between genes based on protein-protein interaction) and identified a network enriched for genes involved in biologically relevant processes for AUD, including cation transport, synaptic transmission, and transmission of nerve impulses.

Tissue-specific network-based analysis—Although the human PPI network is useful in identifying gene subnetworks underlying diseases, PPI networks are not tissue-specific. Since gene expression and interaction patterns may vary across tissue types, a generalized PPI network may not closely reflect the complex interactions among risk genes in tissues specific to that disease.

To overcome this limitation, tools like GLITTER (Liu et al. 2016) examine the tissue-specific functional connections among risk genes from GWAS. In an application to schizophrenia, Liu et al. (2016) found that schizophrenia risk genes tend to be more functionally connected than expected by chance in a number of brain regions (e.g., amygdala and hippocampus), but not in non-brain tissues (e.g., whole blood). Incorporating tissue-specific gene networks into GWAS analysis has benefitted gene discovery for other complex diseases. For example, using a data-driven Bayesian methodology, Greene et al. (2015) developed a comprehensive resource of 144 cell type- and tissue-specific gene networks for humans and an approach called the network-wide association study (NetWAS). NetWAS uses a machine-learning algorithm to identify potential susceptibility genes based on their network connectivity patterns. Further re-prioritization of hypertension GWAS by tissue-specific NetWAS better identified genes associated with hypertension than GWAS alone.

We are not aware of any tissue-specific network-based applications to alcohol GWAS data. One potential reason for this is limited availability of brain-specific tissue that can contrast expression-level variation between individuals with AUD and healthy controls. Peripheral tissue (e.g., liver) and cell-types involved in neuroimmune signaling, other targets for the action of alcohol, are also likely to provide interesting contrasts. However, considering the brain-centric origins of AUD, we reason that gene relationships in the brain regions related to reward, motivation and cognition may more closely reflect the functional relationships that exist among AUD risk genes than does the general PPI network. Therefore, transcriptome data specific to AUD is critical.

Validation in postmortem tissue

Genetically-informed neuroimaging studies in humans have identified associations between candidate genes and intricate neurobehavioral phenotypes (Hariri et al. 2002; Dreher et al. 2009; Whelan et al. 2012). Although neuroimaging techniques unveil certain facets of CNS structure and function, the human brain's molecular profile is only attainable through examination of postmortem tissue. Many of the characteristics of the human brain may not be conserved across species, emphasizing the inherent value of postmortem human tissue for interrogating neuropsychiatric disorders (Hynd et al. 2003; Sutherland et al. 2016). Further, high-resolution maps for gene expression of the human brain across developmental periods, combined with separate genetic and proteomic datasets, can reveal potential neurobiological pathways and circuits underlying disease (Parikshak et al. 2013; Willsey et al. 2013).

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript

Neuroimaging and neuropathology studies show that significant brain atrophy can occur from chronic and excessive alcohol abuse (Harper et al. 1985). The generalized reduction in brain volume is attributed to the loss of central nervous system (CNS) white-matter (Harper et al. 1990). Early genome-based microarray studies of the frontal cortex from postmortem alcoholic brain tissue demonstrated a coordinated decrease in multiple myelin-related genes responsible for the formation and maintenance of CNS white-matter (Lewohl et al. 2000). Microarray studies of postmortem alcoholic brain tissue also revealed dynamic changes in gene expression involving the neuroimmune system (Liu et al. 2004), which may correspond to a proliferation of the CNS immune cells' microglia that occurs in chronic alcoholics (Dennis et al. 2014). This underscores the value of human brain tissue for distinguishing systematic biological alterations involved in neuropsychiatric and neurodegenerative disorders.

Technologies for the study of gene expression in the brain

Advancement of sequencing technologies and bioinformatics provides a global view of disease architecture. Reciprocal interactions of genetic predisposition, epigenetic modifiers, and direct effects of drugs of abuse regulate widespread patterns of CNS gene expression. RNA sequencing (RNA-Seq) along with the combination of chromatin immunoprecipitation with sequencing (ChIP-Seq) of postmortem hippocampus for chronic cocaine and alcohol abuse shows that these substances of abuse affect the expression of several unique and overlapping candidate genes (Zhou et al. 2011). Transcriptional regulation of gene expression conferred by histone H3 lysine 4 trimethylation (H3K4me3) for either cocaine or alcohol abuse is unrelated between the two biological platforms overall; however, particular networks involving H3K4me3 variation and the transcriptome may be jointly linked to each substance of abuse (Farris et al. 2015). Gene network-based analysis of the transcriptome from adult postmortem brain regions has determined discrete networks associated with CNS disease (including alcohol abuse and dependence), delineating gene sets with convergent GWAS signals (Voineagu et al. 2011; Farris et al. 2015). Occupancy of H3K4me3 overlaps disease or trait-associated SNPs within select cell types (Trynka et al. 2013), suggesting coinciding histone marks and risk SNPs may functionally regulate gene expression within specific cellular populations. These examples illustrate how integrating human genetic variation and epigenomic and transcriptomic adaptations across multiple brain regions and CNS cell-types may delineate disease-related mechanisms.

Alternative splicing

Genetic and epigenetic factors participate in the regulation of alternative splicing of human genes to increase diversity of the human proteome (Luco et al. 2010; Pickrell et al. 2010). Approximately 95% of human genes undergo alternative splicing (Pan et al. 2008), with a greater degree of splicing variation occurring between human tissues than individuals (Wang et al. 2008; Mele et al. 2015). The human brain contains the largest number of alternative splicing events (Yeo et al. 2004). Alternative splicing of the human transcriptome is evolutionarily distinct from other species (Barbosa-Morais et al. 2012), capable of affecting signaling networks through modification of phosphorylation sites (Merkin et al. 2012). Acute and chronic alcohol abuse influence diverse signaling cascades responsible for modulating receptor function, transcriptional regulation, and long-term behavior (Ron et al.

2013), suggesting addiction is manifested by a broad spectrum of human-specific molecular interaction networks involving multiple genetic and non-genetic alternative splicing events. The production of alternatively spliced transcripts, and subsequent proteins, may vary among individual cells of the same cell type (Shalek et al. 2013), affecting drug-response of single cells (Cohen et al. 2008). Given the extensive circuitry and cellular heterogeneity of the mammalian brain, regulation of alternative splicing within single cells may be a prominent component in CNS plasticity and habitual human behavior.

Single cell sequencing

Although still emerging, single-cell sequencing is starting to classify major mammalian CNS cell-subtypes according to their unique transcriptional landscape (Zeisel et al. 2015). The underlying transcriptome determines the electrochemical properties and responsiveness of neuronal subclasses (Usoskin et al. 2015; Fuzik et al. 2016; Tasic et al. 2016). Experience-dependent plasticity transpires within individual neuronal nuclei of mice, demonstrating the large repertoire of genes associated with neuronal activation (Lacar et al. 2016). Enrichment of gene expression signatures of mouse CNS cellular populations have assisted in the unbiased classification of cell-types from healthy adult human brain tissue (Darmanis et al. 2015). Single-cell sequencing of human neuronal nuclei from postmortem human brain tissue has shown active transcription may introduce somatic mutations that are associated with individual neuronal lineage (Lodato et al. 2015). The effect of nucleotide variation on alternative splicing of transcribed RNAs and their relationship to phenotypes has yet to be established; however, such information may improve with sequencing-based methodologies (Dey et al. 2015; Macaulay et al. 2015). Assembling a complete sequencing-based denomination of individual cells spanning differing anatomical regions will provide a well-characterized molecular map of the brain. The information from these sequencing experiments will provide a foundation for articulating polygenic effects related to evolutionary divergence in brain function and human disorders. GWAS of psychiatric disease are only beginning to draw upon these neuroinformatics resources (Network Pathway Analysis Subgroup of Psychiatric Genomics Consortium 2015).

Cross-species validation of genes and gene networks

Studies of gene expression in brain tissue from AUD-affected individuals (Mayfield et al. 2002; Liu et al. 2004; Farris et al. 2015; Mamdani et al. 2015) are limited due to small sample sizes and the inability to: control alcohol access; discretely measure alcohol consumption; control the time between last drink and tissue collection; manipulate gene expression; and collect tissue from living subjects. To circumvent some of these problems, studies in model organisms have successfully identified ethanol regulated gene expression and ethanol-responsive gene networks (Kerns et al. 2005; Farris et al. 2012; Wolen et al. 2012; Farris et al. 2013; Smith et al. 2016). Integrated analyses of genomic data across human and animal model studies have also identified candidate genes involved in alcohol dependence (Zhao et al. 2012). Additionally, animal models allow experimental manipulation of gene expression or function that is not possible in humans, permitting direct interrogation of a gene's role in ethanol-related behavior. In this section, we discuss several approaches for integrating human GWAS data with gene expression and genetic studies

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

across species, including set-based enrichment, co-analysis, and direct testing of signals emerging from GWAS. Such analyses provide tests of the functional relevance of GWAS loci and related genes and networks under the specific influence of alcohol exposure.

Set-based over-representation analysis

Derivation of gene expression modules or networks from basal expression patterns or after acute or chronic ethanol exposure has been done predominantly with brain tissue from rodent models. Tools such as traditional cluster analysis or weighted gene correlation network analysis (WGCNA) have derived correlated expression patterns responding to ethanol or correlated with ethanol behaviors (Kerns et al. 2005; Wolen et al. 2012; Farris et al. 2013; Smith et al. 2016). However, ranking the overall importance of such networks is oftentimes difficult even following bioinformatics analysis for over-represented biological functions. The superimposition of human GWAS signals (derived from the pathway or network methods discussed above) onto such model organism expression networks is thus an attractive approach for leveraging nominal GWAS signals and prioritizing derived expression networks.

Co-analysis of model organism expression networks and human GWAS

A second integrative method, co-analysis, is exemplified by dense module searching for GWAS (dmGWAS). This algorithm utilizes information from GWAS p-values to query PPI databases for modules of genes with smaller GWAS p-values (Jia et al. 2011). This method can be used for cross-species integration by incorporating model organism gene expression data and human GWAS and PPI data. Unlike validation methods like overrepresentation, Edge-Weighted dmGWAS (EW-dmGWAS) incorporates both expression values and GWAS signals into the module-building process, and weights their respective contributions. The resulting PPI modules consist of genes with smaller GWAS p-values and correlated expression level differences. Alternatively, the background network scaffold could be derived from additional expression correlation network studies, thus avoiding the limitations of employing PPI data that might underrepresent gene-gene interactions from brain.

Direct interrogation of GWAS candidates via model organism studies

Validation studies on candidates for Mendelian disorders, such as cystic fibrosis, have made extensive use of gene targeting approaches in rodents or other model organisms. However, for AUD, very few loci are genome-wide significant, and thus gene targeting model organism studies can be risky. Bioinformatics approaches (Bubier et al. 2016) or integration of GWAS and model organism gene expression data (Zhao et al. 2012), such as those outlined above, can sometimes provide a limited gene list or network more amenable for gene-targeting studies. This approach can be aided through use of high throughput genetic models for ethanol behaviors, such as the invertebrate species *Drosophila* and *C. elegans*. Follow-up gene-targeting studies on a more limited list of promising loci can then be done, for example, in mouse models. The latter approach is increasingly possible over a relatively short timeframe given the availability of several resources: large repositories of viral vectors for over-expression, knock-down or deletion of target genes in select brain regions; large repositories of targeted mouse models (or their embryonic stem cells) encompassing nearly

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

all protein coding genes across the mouse genome; and new methods for rapid generation of targeted genes, such as CRISPR/Cas9.

Cross-species integrated analysis recently validated the *Clic/Clic4* gene in ethanol behaviors though viral vector or gene targeting studies across mouse, *Drosophila* and *C. elegans* models (Bhandari et al. 2012). In all three models, modulation of *Clic4* or the invertebrate homologs produced significant changes in sensitivity to ethanol. A recent striking example of direct cross-species validation of GWAS data demonstrated that multiple members of the SWI/SNF chromatin remodeling complex altered behavioral responses to ethanol in *C. elegans* and *SMARCA2*, a human SWI/SNF member, which showed suggestive association ($P = 7.29 \times 10^{-06}$) with alcohol dependence in a human GWAS study (Mathies et al. 2015).

Discussion

The methods highlighted in this review show that a GWAS is but the first step towards understanding the biological basis of AUD. We show that integrating GWAS summary statistics with other sources of information have yielded novel insights into the etiology of AUD. Gene networks and disease-related modules have been identified using the interface of GWAS and curated brain expression and PPI data (Han et al. 2013), as well as in transcriptome and epigenomic signatures in postmortem brain tissue from individuals with AUD (e.g., Farris et al. 2013). Animal models have allowed for the identification of new genes that are responsive to ethanol and have also shown the value in the identification of novel genetic pathways when combining GWAS and model organism expression data (e.g., Zhao et al. 2012). Furthermore, model organisms that can be genetically manipulated in their ethanol responsiveness have begun to provide excellent validation of genetic signals from GWAS (Mathies et al. 2015; Schumann et al. 2016).

These are, by no means, the only post-GWAS approaches that exist. In fact, the landscape of methodology is continuously evolving as computationally burdensome approaches are optimized (e.g., several newer approaches that rely on Bayesian inference are now readily accessible) and more resources, such as those listed in Table 2, become available. Our review documents the importance of additional alcohol-specific resources, such as further studies that integrate well-powered GWAS with expression data from postmortem brains of individuals with rich characterization of AUD history, as is being done with schizophrenia (e.g., Birnbaum et al. 2017). With AUD research, we are also uniquely poised to harness resources from studies of model organisms.

The principal challenge that we are confronted with is the lack of a well- or even adequately powered GWAS of AUD. Even though the approaches outlined here more efficiently harness all existing GWAS data, reliability of the results from these GWAS hinge on their sample size. One of the largest efforts that is currently under way is being led by the Psychiatric Genomics Consortium's Substance Use Disorders group and includes ≈15,000 cases with DSM-IV alcohol dependence and >37, 000 controls that are largely alcohol exposed (Agrawal et al. 2016). A GWAS of Alcohol Use Disorder Identification Test (AUDIT) scores was completed on >20,000 subjects from 23andMe, but failed to identify genomewide significant loci (Sanchez-Roige et al. bioRxiv preprint). There are likely to be other large-

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

scale studies that focus on related aspects of normative and heavy drinking. For instance, the UKBioBank effort identified 12 genome-wide significant loci for alcohol consumption (Clarke et al. 2017), and similar efforts in the US GERA cohort found corroborating evidence for genes involved in alcohol metabolism (*ALDH2* in East Asians and *ADH1B* in non-Hispanic Whites and Hispanic/Latinos (Jorgenson et al. 2017)). The SNP-based genetic correlation between alcohol intake measures and AUD is likely significant but not perfect (Dick et al. 2011). However, gene- and network-based analyses, such as those outlined in this review, may allow for systems-based approaches to integrate across alcohol phenotypes and even other substance use disorders to identify commonality and specificity in biological pathways.

Conclusion

We propose a shift from cataloging statistical genetic associations to using post-GWAS tools to make biological sense of them. Incorporating the strategies outlined here should help prioritize individual gene targets amenable to functional and mechanistic validation across species, which can create opportunities to better characterize polygenic risk for AUD, test the prognostic utility of these loci and scores, and identify therapeutic starting points.

Acknowledgments

This study was supported by National Institutes of Health grants R01AA020634 and P50AA022537 (MM); R01AA022994 (SH); K02DA032573 and U01MH109532 (AA); and K01AA024152 (JES). We thank Abbas Parsian at NIAAA for organizing a symposium at the Research Society for Alcoholism (RSoA) 2016 meeting that inspired this work and for facilitating the development of this review.

References

Adkins DE, Clark SL, Copeland WE, Kennedy M, Conway K, Angold A, Maes H, Liu Y, Kumar G, Erkanli A, Patkar AA, Silberg J, Brown TH, Fergusson DM, Horwood LJ, Eaves L, van den Oord EJ, Sullivan PF, Costello EJ. 2015; Genome-Wide Meta-Analysis of Longitudinal Alcohol Consumption Across Youth and Early Adulthood. Twin Res Hum Genet. 18:335–347. [PubMed: 26081443]

Agrawal A, Edenberg HJ, Gelernter J. 2016; Meta-Analyses of Genome-Wide Association Data Hold New Promise for Addiction Genetics. J Stud Alcohol Drugs. 77:676–680. [PubMed: 27588522]

AlQuraishi M, Koytiger G, Jenney A, MacBeath G, Sorger PK. 2014; A multiscale statistical mechanical framework integrates biophysical and genomic data to assemble cancer networks. Nat Genet. 46:1363–1371. [PubMed: 25362484]

Barbosa-Morais NL, Irimia M, Pan Q, Xiong HY, Gueroussov S, Lee LJ, Slobodeniuc V, Kutter C, Watt S, Colak R, Kim T, Misquitta-Ali CM, Wilson MD, Kim PM, Odom DT, Frey BJ, Blencowe BJ. 2012; The evolutionary landscape of alternative splicing in vertebrate species. Science. 338:1587–1593. [PubMed: 23258890]

Ben-David E, Shifman S. 2012; Networks of neuronal genes affected by common and rare variants in autism spectrum disorders. PLoS Genet. 8:e1002556. [PubMed: 22412387]

Bhandari P, Hill JS, Farris SP, Costin B, Martin I, Chan CL, Alaimo JT, Bettinger JC, Davies AG, Miles MF, Grotewiel M. 2012; Chloride intracellular channels modulate acute ethanol behaviors in Drosophila, Caenorhabditis elegans and mice. Genes Brain Behav. 11:387–397. [PubMed: 22239914]

Biernacka JM, Geske J, Jenkins GD, Colby C, Rider DN, Karpyak VM, Choi DS, Fridley BL. 2013; Genome-wide gene-set analysis for identification of pathways associated with alcohol dependence. Int J Neuropsychopharmacol. 16:271–278. [PubMed: 22717047]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Bierut LJ, Goate AM, Breslau N, Johnson EO, Bertelsen S, Fox L, Agrawal A, Bucholz KK, Grucza R, Hesselbrock V, Kramer J, Kuperman S, Nurnberger J, Porjesz B, Saccone NL, Schuckit M, Tischfield J, Wang JC, Foroud T, Rice JP, Edenberg HJ. 2012; ADH1B is associated with alcohol dependence and alcohol consumption in populations of European and African ancestry. Mol Psychiatry. 17:445–450. [PubMed: 21968928]

Birnbaum R, Jaffe AE, Chen Q, Shin JH, Kleinman JE, Hyde TM, Weinberger DR. BrainSeq Consortium. 2017Investigating the neuroimmunogenic architecture of schizophrenia. Mol Psychiatry.

Boyle AP, Hong EL, Hariharan M, Cheng Y, Schaub MA, Kasowski M, Karczewski KJ, Park J, Hitz BC, Weng S, Cherry JM, Snyder M. 2012; Annotation of functional variation in personal genomes using RegulomeDB. Genome Res. 22:1790–1797. [PubMed: 22955989]

Boyle EA, Li YI, Pritchard JK. 2017; An Expanded View of Complex Traits: From Polygenic to Omnigenic. Cell. 169:1177–1186. [PubMed: 28622505]

Bubier JA, Wilcox TD, Jay JJ, Langston MA, Baker EJ, Chesler EJ. 2016; Cross-species integrative functional genomics in GeneWeaver reveals a role for Pafah1b1 in altered response to alcohol. Front Behav Neurosci. 10:1. [PubMed: 26834590]

Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J, Patterson N, Daly MJ, Price AL, Neale BM. Schizophrenia Working Group of the Psychiatric Genomics Consortium. 2015; LD Score regression distinguishes confounding from polygenicity in genome-wide association studies. Nat Genet. 47:291–295. [PubMed: 25642630]

Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. 2015; Second-generation PLINK: rising to the challenge of larger and richer datasets. Gigascience. 4:7. [PubMed: 25722852]

Clarke T-K, Adams MJ, Davies G, Howard DM, Hall LS, Padmanabhan S, Murray AD, Smith BH, Campbell A, Hayward C, Porteous DJ, Deary IJ, McIntosh AM. 2017Genome-wide association study of alcohol consumption and genetic overlap with other health-related traits in UK Biobank (N=112,117). Mol Psychiatr.

Cohen AA, Geva-Zatorsky N, Eden E, Frenkel-Morgenstern M, Issaeva I, Sigal A, Milo R, Cohen-Saidon C, Liron Y, Kam Z, Cohen L, Danon T, Perzov N, Alon U. 2008; Dynamic proteomics of individual cancer cells in response to a drug. Science. 322:1511–1516. [PubMed: 19023046]

Darmanis S, Sloan SA, Zhang Y, Enge M, Caneda C, Shuer LM, Hayden Gephart MG, Barres BA, Quake SR. 2015; A survey of human brain transcriptome diversity at the single cell level. Proc Natl Acad Sci USA. 112:7285–7290. [PubMed: 26060301]

de Leeuw CA, Mooij JM, Heskes T, Posthuma D. 2015; MAGMA: generalized gene-set analysis of GWAS data. PLoS Comput Biol. 11:e1004219. [PubMed: 25885710]

de Leeuw CA, Neale BM, Heskes T, Posthuma D. 2016; The statistical properties of gene-set analysis. Nat Rev Genet. 17:353–364. [PubMed: 27070863]

Dennis CV, Sheahan PJ, Graeber MB, Sheedy DL, Kril JJ, Sutherland GT. 2014; Microglial proliferation in the brain of chronic alcoholics with hepatic encephalopathy. Metab Brain Dis. 29:1027–1039. [PubMed: 24346482]

Dey SS, Kester L, Spanjaard B, Bienko M, van Oudenaarden A. 2015; Integrated genome and transcriptome sequencing of the same cell. Nat Biotechnol. 33:285–289. [PubMed: 25599178]

Dick DM, Meyers JL, Rose RJ, Kaprio J, Kendler KS. 2011; Measures of current alcohol consumption and problems: two independent twin studies suggest a complex genetic architecture. Alcohol Clin Exp Res. 35:2152–2161. [PubMed: 21689117]

Dreher JC, Kohn P, Kolachana B, Weinberger DR, Berman KF. 2009; Variation in dopamine genes influences responsivity of the human reward system. Proc Natl Acad Sci USA. 106:617–622. [PubMed: 19104049]

Edenberg HJ, Foroud T. 2014; Genetics of alcoholism. Handbook of clinical neurology. 125:561–571. [PubMed: 25307596]

Edwards AC, Aliev F, Wolen AR, Salvatore JE, Gardner CO, McMahon G, Evans DM, Macleod J, Hickman M, Dick DM, Kendler KS. 2015; Genomic influences on alcohol problems in a population-based sample of young adults. Addiction. 110:461–470. [PubMed: 25439982]

ENCODE Project Consortium. 2012; An integrated encyclopedia of DNA elements in the human genome. Nature. 489:57–74. [PubMed: 22955616]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Farris SP, Arasappan D, Hunicke-Smith S, Harris RA, Mayfield RD. 2015; Transcriptome organization for chronic alcohol abuse in human brain. Mol Psychiatry. 20:1438–1447. [PubMed: 25450227]

Farris SP, Harris RA, Ponomarev I. 2015; Epigenetic modulation of brain gene networks for cocaine and alcohol abuse. Front Neurosci. 9:176. [PubMed: 26041984]

Farris SP, Miles MF. 2012; Ethanol modulation of gene networks: implications for alcoholism. Neurobiol Dis. 45:115–121. [PubMed: 21536129]

Farris SP, Miles MF. 2013; Fyn-dependent gene networks in acute ethanol sensitivity. PLoS One. 8:e82435. [PubMed: 24312422]

Finucane HK, Bulik-Sullivan B, Gusev A, Trynka G, Reshef Y, Loh PR, Anttila V, Xu H, Zang C, Farh K, Ripke S, Day FR, ReproGen C, Purcell S, Stahl E, Lindstrom S, Perry JR, Okada Y, Raychaudhuri S, Daly MJ, Patterson N, Neale BM, Price AL. Schizophrenia Working Group of the Psychiatric Genomics Consortium, Raci Consortium. 2015; Partitioning heritability by functional annotation using genome-wide association summary statistics. Nat Genet. 47:1228–1235. [PubMed: 26414678]

Frank J, Cichon S, Treutlein J, Ridinger M, Mattheisen M, Hoffmann P, Herms S, Wodarz N, Soyka M, Zill P, Maier W, Mossner R, Gaebel W, Dahmen N, Scherbaum N, Schmal C, Steffens M, Lucae S, Ising M, Muller-Myhsok B, Nothen MM, Mann K, Kiefer F, Rietschel M. 2012; Genome-wide significant association between alcohol dependence and a variant in the ADH gene cluster. Addict Biol. 17:171–180. [PubMed: 22004471]

Fridley BL, Biernacka JM. 2011; Gene set analysis of SNP data: benefits, challenges, and future directions. Eur J Hum Genet. 19:837–843. [PubMed: 21487444]

Fuzik J, Zeisel A, Mate Z, Calvigioni D, Yanagawa Y, Szabo G, Linnarsson S, Harkany T. 2016; Integration of electrophysiological recordings with single-cell RNA-seq data identifies neuronal subtypes. Nat Biotechnol. 34:175–183. [PubMed: 26689544]

Gamazon ER, Wheeler HE, Shah KP, Mozaffari SV, Aquino-Michaels K, Carroll RJ, Eyler AE, Denny JC, Nicolae DL, Cox NJ, Im HK. Consortium GT. 2015; A gene-based association method for mapping traits using reference transcriptome data. Nat Genet. 47:1091–1098. [PubMed: 26258848]

Gelernter J, Kranzler HR, Sherva R, Almasy L, Koesterer R, Smith AH, Anton R, Preuss UW, Ridinger M, Rujescu D, Wodarz N, Zill P, Zhao H, Farrer LA. 2014; Genome-wide association study of alcohol dependence:significant findings in African- and European-Americans including novel risk loci. Mol Psychiatry. 19:41–49. [PubMed: 24166409]

Greene CS, Krishnan A, Wong AK, Ricciotti E, Zelaya RA, Himmelstein DS, Zhang R, Hartmann BM, Zaslavsky E, Sealfon SC, Chasman DI, Fitzgerald GA, Dolinski K, Grosser T, Troyanskaya OG. 2015; Understanding multicellular function and disease with human tissue-specific networks. Nat Genet. 47:569–576. [PubMed: 25915600]

Gusev A, Ko A, Shi H, Bhatia G, Chung W, Penninx BW, Jansen R, de Geus EJ, Boomsma DI, Wright FA, Sullivan PF, Nikkola E, Alvarez M, Civelek M, Lusis AJ, Lehtimaki T, Raitoharju E, Kahonen M, Seppala I, Raitakari OT, Kuusisto J, Laakso M, Price AL, Pajukanta P, Pasaniuc B. 2016; Integrative approaches for large-scale transcriptome-wide association studies. Nat Genet. 48:245–252. [PubMed: 26854917]

Gusev A, Lee SH, Trynka G, Finucane H, Vilhjalmsson BJ, Xu H, Zang C, Ripke S, Bulik-Sullivan B, Stahl E, Kahler AK, Hultman CM, Purcell SM, McCarroll SA, Daly M, Pasaniuc B, Sullivan PF, Neale BM, Wray NR, Raychaudhuri S, Price AL. Schizophrenia Working Group of the Psychiatric Genomics C, Consortium S-S, Schizophrenia Working Group of the Psychiatric Genomics C Consortium S-S. 2014; Partitioning heritability of regulatory and cell-type-specific variants across 11 common diseases. Am J Hum Genet. 95:535–552. [PubMed: 25439723]

Han S, Yang BZ, Kranzler HR, Liu X, Zhao H, Farrer LA, Boerwinkle E, Potash JB, Gelernter J. 2013; Integrating GWASs and human protein interaction networks identifies a gene subnetwork underlying alcohol dependence. Am J Hum Genet. 93:1027–1034. [PubMed: 24268660]

Hariri AR, Mattay VS, Tessitore A, Kolachana B, Fera F, Goldman D, Egan MF, Weinberger DR. 2002; Serotonin transporter genetic variation and the response of the human amygdala. Science. 297:400–403. [PubMed: 12130784]

Harper C, Kril J. 1985; Brain atrophy in chronic alcoholic patients: a quantitative pathological study. J Neurol Neurosurg Psychiatry. 48:211–217. [PubMed: 3981189]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Harper CG, Kril JJ. 1990; Neuropathology of alcoholism. Alcohol Alcohol. 25:207–216. [PubMed: 2198036]

Hart AB, Kranzler HR. 2015; Alcohol dependence genetics: lessons learned from genome-wide association studies (GWAS) and post-GWAS analyses. Alcohol Clin Exp Res. 39:1312–1327. [PubMed: 26110981]

Hernandez DG, Nalls MA, Moore M, Chong S, Dillman A, Trabzuni D, Gibbs JR, Ryten M, Arepalli S, Weale ME, Zonderman AB, Troncoso J, O'Brien R, Walker R, Smith C, Bandinelli S, Traynor BJ, Hardy J, Singleton AB, Cookson MR. 2012; Integration of GWAS SNPs and tissue specific expression profiling reveal discrete eQTLs for human traits in blood and brain. Neurobiol Dis. 47:20–28. [PubMed: 22433082]

Hillenmeyer S, Davis LK, Gamazon ER, Cook EH, Cox NJ, Altman RB. 2016; STAMS: STRING-assisted module search for genome wide association studies and application to autism. Bioinformatics. 32:3815–3822. [PubMed: 27542772]

Holmans P, Green EK, Pahwa JS, Ferreira MA, Purcell SM, Sklar P, Owen MJ, O'Donovan MC, Craddock N. Wellcome Trust Case-Control C. 2009; Gene ontology analysis of GWA study data sets provides insights into the biology of bipolar disorder. Am J Hum Genet. 85:13–24. [PubMed: 19539887]

Hynd MR, Lewohl JM, Scott HL, Dodd PR. 2003; Biochemical and molecular studies using human autopsy brain tissue. J Neurochem. 85:543–562. [PubMed: 12694381]

Jia P, Zheng S, Long J, Zheng W, Zhao Z. 2011; dmGWAS: dense module searching for genome-wide association studies in protein-protein interaction networks. Bioinformatics. 27:95–102. [PubMed: 21045073]

Jin L, Zuo XY, Su WY, Zhao XL, Yuan MQ, Han LZ, Zhao X, Chen YD, Rao SQ. 2014; Pathway-based analysis tools for complex diseases: a review. Genomics Proteomics Bioinformatics. 12:210–220. [PubMed: 25462153]

Jorgenson E, Thai KK, Hoffmann TJ, Sakoda LC, Kvale MN, Banda Y, Schaefer C, Risch N, Mertens J, Weisner C, Choquet H. 2017; Genetic contributors to variation in alcohol consumption vary by race/ethnicity in a large multi-ethnic genome-wide association study. Mol Psychiatry. 22:1359–1367. [PubMed: 28485404]

Juraeva D, Treutlein J, Scholz H, Frank J, Degenhardt F, Cichon S, Ridinger M, Mattheisen M, Witt SH, Lang M, Sommer WH, Hoffmann P, Herms S, Wodarz N, Soyka M, Zill P, Maier W, Junger E, Gaebel W, Dahmen N, Scherbaum N, Schmal C, Steffens M, Lucae S, Ising M, Smolka MN, Zimmermann US, Muller-Myhsok B, Nothen MM, Mann K, Kiefer F, Spanagel R, Brors B, Rietschel M. 2015; XRCC5 as a risk gene for alcohol dependence: evidence from a genome-wide gene-set-based analysis and follow-up studies in Drosophila and humans. Neuropsychopharmacol. 40:361–371.

Kendler KS, Kalsi G, Holmans PA, Sanders AR, Aggen SH, Dick DM, Aliev F, Shi J, Levinson DF, Gejman PV. 2011; Genomewide association analysis of symptoms of alcohol dependence in the molecular genetics of schizophrenia (MGS2) control sample. Alcohol Clin Exp Res. 35:963–975. [PubMed: 21314694]

Kerns RT, Ravindranathan A, Hassan S, Cage MP, York T, Sikela JM, Williams RW, Miles MF. 2005; Ethanol-responsive brain region expression networks: implications for behavioral responses to acute ethanol in DBA/2J versus C57BL/6J mice. J Neurosci. 25:2255–2266. [PubMed: 15745951]

Kos MZ, Yan J, Dick DM, Agrawal A, Bucholz KK, Rice JP, Johnson EO, Schuckit M, Kuperman S, Kramer J, Goate AM, Tischfield JA, Foroud T, Nurnberger J Jr, Hesselbrock V, Porjesz B, Bierut LJ, Edenberg HJ, Almasy L. 2013; Common biological networks underlie genetic risk for alcoholism in African- and European-American populations. Genes Brain Behav. 12:532–542. [PubMed: 23607416]

Lacar B, Linker SB, Jaeger BN, Krishnaswami S, Barron J, Kelder M, Parylak S, Paquola A, Venepally P, Novotny M, O'Connor C, Fitzpatrick C, Erwin J, Hsu JY, Husband D, McConnell MJ, Lasken R, Gage FH. 2016; Nuclear RNA-seq of single neurons reveals molecular signatures of activation. Nat Commun. 7:11022. [PubMed: 27090946]

Lee PH, O'Dushlaine C, Thomas B, Purcell SM. 2012; INRICH: interval-based enrichment analysis for genome-wide association studies. Bioinformatics. 28:1797–1799. [PubMed: 22513993]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Leiserson MD, Eldridge JV, Ramachandran S, Raphael BJ. 2013; Network analysis of GWAS data. Curr Opin Genet Dev. 23:602–610. [PubMed: 24287332]

Lewohl JM, Wang L, Miles MF, Zhang L, Dodd PR, Harris RA. 2000; Gene expression in human alcoholism: microarray analysis of frontal cortex. Alcohol Clin Exp Res. 24:1873–1882. [PubMed: 11141048]

Liu J, Lewohl JM, Dodd PR, Randall PK, Harris RA, Mayfield RD. 2004; Gene expression profiling of individual cases reveals consistent transcriptional changes in alcoholic human brain. J Neurochem. 90:1050–1058. [PubMed: 15312160]

Liu JZ, McRae AF, Nyholt DR, Medland SE, Wray NR, Brown KM, Hayward NK, Montgomery GW, Visscher PM, Martin NG, Macgregor S. Investigators A. 2010; A versatile gene-based test for genome-wide association studies. Am J Hum Genet. 87:139–145. [PubMed: 20598278]

Liu X, Finucane HK, Gusev A, Bhatia G, Gazal S, O'Connor L, Bulik-Sullivan B, Wright FA, Sullivan PF, Neale BM, Price AL. 2017; Functional architectures of local and distal regulation of gene expression in multiple human tissues. Am J Hum Genet. 100:605–616. [PubMed: 28343628]

Liu X, Yu P, Cheng C, Potash JB, Han S. 2016; GLITTER: a web-based application for gene link inspection through tissue-specific coexpression. Sci Rep. 6:33460. [PubMed: 27623690]

Lodato MA, Woodworth MB, Lee S, Evrony GD, Mehta BK, Karger A, Lee S, Chittenden TW, D'Gama AM, Cai X, Luquette LJ, Lee E, Park PJ, Walsh CA. 2015; Somatic mutation in single human neurons tracks developmental and transcriptional history. Science. 350:94–98. [PubMed: 26430121]

Luco RF, Pan Q, Tominaga K, Blencowe BJ, Pereira-Smith OM, Misteli T. 2010; Regulation of alternative splicing by histone modifications. Science. 327:996–1000. [PubMed: 20133523]

Macaulay IC, Haerty W, Kumar P, Li YI, Hu TX, Teng MJ, Goolam M, Saurat N, Coupland P, Shirley LM, Smith M, Van der Aa N, Banerjee R, Ellis PD, Quail MA, Swerdlow HP, Zernicka-Goetz M, Livesey FJ, Ponting CP, Voet T. 2015; G&T-seq: parallel sequencing of single-cell genomes and transcriptomes. Nat Methods. 12:519–522. [PubMed: 25915121]

Mamdani M, Williamson V, McMichael GO, Blevins T, Aliev F, Adkins A, Hack L, Bigdeli T, van der Vaart AD, Web BT, Bacanu SA, Kalsi G, Kendler KS, Miles MF, Dick D, Riley BP, Dumur C, Vladimirov VI. Consortium C. 2015; Integrating mRNA and miRNA weighted gene co-expression networks with eQTLs in the nucleus accumbens of subjects with alcohol dependence. PLoS One. 10:e0137671. [PubMed: 26381263]

Mancuso N, Shi H, Goddard P, Kichaev G, Gusev A, Pasaniuc B. 2017; Integrating gene expression with summary association statistics to identify genes associated with 30 complex traits. Am J Hum Genet. 100:473–487. [PubMed: 28238358]

Mathies LD, Blackwell GG, Austin MK, Edwards AC, Riley BP, Davies AG, Bettinger JC. 2015; SWI/SNF chromatin remodeling regulates alcohol response behaviors in Caenorhabditis elegans and is associated with alcohol dependence in humans. Proc Natl Acad Sci USA. 112:3032–3037. [PubMed: 25713357]

Maurano MT, Humbert R, Rynes E, Thurman RE, Haugen E, Wang H, Reynolds AP, Sandstrom R, Qu H, Brody J, Shafer A, Neri F, Lee K, Kutyavin T, Stehling-Sun S, Johnson AK, Canfield TK, Giste E, Diegel M, Bates D, Hansen RS, Neph S, Sabo PJ, Heimfeld S, Raubitschek A, Ziegler S, Cotsapas C, Sotoodehnia N, Glass I, Sunyaev SR, Kaul R, Stamatoyannopoulos JA. 2012; Systematic localization of common disease-associated variation in regulatory DNA. Science. 337:1190–1195. [PubMed: 22955828]

Mayfield RD, Lewohl JM, Dodd PR, Herlihy A, Liu J, Harris RA. 2002; Patterns of gene expression are altered in the frontal and motor cortices of human alcoholics. J Neurochem. 81:802–813. [PubMed: 12065639]

Mbarek H, Milaneschi Y, Fedko IO, Hottenga JJ, de Moor MH, Jansen R, Gelernter J, Sherva R, Willemsen G, Boomsma DI, Penninx BW, Vink JM. 2015; The genetics of alcohol dependence: Twin and SNP-based heritability, and genome-wide association study based on AUDIT scores. Am J Med Genet B Neuropsychiatr Genet. 168:739–748. [PubMed: 26365420]

Mele M, Ferreira PG, Reverter F, DeLuca DS, Monlong J, Sammeth M, Young TR, Goldmann JM, Pervouchine DD, Sullivan TJ, Johnson R, Segre AV, Djebali S, Niarchou A, Wright FA, Lappalainen T, Calvo M, Getz G, Dermitzakis ET, Ardlie KG, Guigo R. Consortium GT. 2015;

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Human genomics. The human transcriptome across tissues and individuals. Science. 348:660–665. [PubMed: 25954002]

Merkin J, Russell C, Chen P, Burge CB. 2012; Evolutionary dynamics of gene and isoform regulation in Mammalian tissues. Science. 338:1593–1599. [PubMed: 23258891]

Mishra A, Macgregor S. 2015; VEGAS2: software for more flexible gene-based testing. Twin Res Hum Genet. 18:86–91. [PubMed: 25518859]

Moskvina V, O'Dushlaine C, Purcell S, Craddock N, Holmans P, O'Donovan MC. 2011; Evaluation of an approximation method for assessment of overall significance of multiple-dependent tests in a genomewide association study. Genet Epidemiol. 35:861–866. [PubMed: 22006681]

Moskvina V, Schmidt KM, Vedernikov A, Owen MJ, Craddock N, Holmans P, O'Donovan MC. 2012; Permutation-based approaches do not adequately allow for linkage disequilibrium in gene-wide multi-locus association analysis. Eur J Hum Genet. 20:890–896. [PubMed: 22317971]

Nature Genetics Editorial Board. 2010; On beyond GWAS. Nat Genet. 42:551. [PubMed: 20581872]

Network Pathway Analysis Subgroup of Psychiatric Genomics Consortium. 2015; Psychiatric genome-wide association study analyses implicate neuronal, immune and histone pathways. Nat Neurosci. 18:199–209. [PubMed: 25599223]

Palmer RH, Brick L, Nugent NR, Bidwell LC, McGeary JE, Knopik VS, Keller MC. 2015; Examining the role of common genetic variants on alcohol, tobacco, cannabis and illicit drug dependence: genetics of vulnerability to drug dependence. Addiction. 110:530–537. [PubMed: 25424661]

Pan Q, Shai O, Lee LJ, Frey BJ, Blencowe BJ. 2008; Deep surveying of alternative splicing complexity in the human transcriptome by high-throughput sequencing. Nat Genet. 40:1413–1415. [PubMed: 18978789]

Parikshak NN, Luo R, Zhang A, Won H, Lowe JK, Chandran V, Horvath S, Geschwind DH. 2013; Integrative functional genomic analyses implicate specific molecular pathways and circuits in autism. Cell. 155:1008–1021. [PubMed: 24267887]

Pedroso I, Lourdusamy A, Rietschel M, Nothen MM, Cichon S, McGuffin P, Al-Chalabi A, Barnes MR, Breen G. 2012; Common genetic variants and gene-expression changes associated with bipolar disorder are over-represented in brain signaling pathway genes. Biol Psychiatry. 72:311–317. [PubMed: 22502986]

Pickrell JK, Marioni JC, Pai AA, Degner JF, Engelhardt BE, Nkadori E, Veyrieras JB, Stephens M, Gilad Y, Pritchard JK. 2010; Understanding mechanisms underlying human gene expression variation with RNA sequencing. Nature. 464:768–772. [PubMed: 20220758]

Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira M, Bender D, Maller J, Sklar P, de Bakker P, Daly M, Sham P. 2007; PLINK: a tool set for whole-genome association and population-based linkage analyses. Am J Hum Genet. 81:559–575. [PubMed: 17701901]

Roadmap Epigenomics Consortium. Kundaje A, Meuleman W, Ernst J, Bilenky M, Yen A, Heravi-Moussavi A, Kheradpour P, Zhang Z, Wang J, Ziller MJ, Amin V, Whitaker JW, Schultz MD, Ward LD, Sarkar A, Quon G, Sandstrom RS, Eaton ML, Wu YC, Pfenning AR, Wang X, Claussnitzer M, Liu Y, Coarfa C, Harris RA, Shoresh N, Epstein CB, Gjoneska E, Leung D, Xie W, Hawkins RD, Lister R, Hong C, Gascard P, Mungall AJ, Moore R, Chuah E, Tam A, Canfield TK, Hansen RS, Kaul R, Sabo PJ, Bansal MS, Carles A, Dixon JR, Farh KH, Feizi S, Karlic R, Kim AR, Kulkarni A, Li D, Lowdon R, Elliott G, Mercer TR, Neph SJ, Onuchic V, Polak P, Rajagopal N, Ray P, Sallari RC, Siebenthall KT, Sinnott-Armstrong NA, Stevens M, Thurman RE, Wu J, Zhang B, Zhou X, Beaudet AE, Boyer LA, De Jager PL, Farnham PJ, Fisher SJ, Haussler D, Jones SJ, Li W, Marra MA, McManus MT, Sunyaev S, Thomson JA, Tlsty TD, Tsai LH, Wang W, Waterland RA, Zhang MQ, Chadwick LH, Bernstein BE, Costello JF, Ecker JR, Hirst M, Meissner A, Milosavljevic A, Ren B, Stamatoyannopoulos JA, Wang T, Kellis M. 2015; Integrative analysis of 111 reference human epigenomes. Nature. 518:317–330. [PubMed: 25693563]

Ron D, Messing RO. 2013; Signaling pathways mediating alcohol effects. Curr Top Behav Neurosci. 13:87–126. [PubMed: 21877259]

Sanchez-Roige S, Fontanillas P, Elson SL, Gray JC, de Wit H, Davis LK, MacKillop J, Palmer AA. (bioRxiv preprint) Genome-wide association study of Alcohol Use Disorder Identification Test (AUDIT) scores in 20,328 research participants of European ancestry.

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Schizophrenia Working Group of the Psychiatric Genomics Consortium. 2014; Biological insights from 108 schizophrenia-associated genetic loci. Nature. 511:421–427. [PubMed: 25056061]

Schork AJ, Thompson WK, Pham P, Torkamani A, Roddey JC, Sullivan PF, Kelsoe JR, O'Donovan MC, Furberg H, Schork NJ, Andreassen OA, Dale AM. Tobacco, Genetics C, Bipolar Disorder Psychiatric Genomics C, Schizophrenia Psychiatric Genomics C. 2013; All SNPs are not created equal: genome-wide association studies reveal a consistent pattern of enrichment among functionally annotated SNPs. PLoS Genet. 9:e1003449. [PubMed: 23637621]

Schumann G, Liu C, O'Reilly P, Gao H, Song P, Xu B, Ruggeri B, Amin N, Jia T, Preis S, Segura Lepe M, Akira S, Barbieri C, Baumeister S, Cauchi S, Clarke TK, Enroth S, Fischer K, Hallfors J, Harris SE, Hieber S, Hofer E, Hottenga JJ, Johansson A, Joshi PK, Kaartinen N, Laitinen J, Lemaitre R, Loukola A, Luan J, Lyytikainen LP, Mangino M, Manichaikul A, Mbarek H, Milaneschi Y, Moayyeri A, Mukamal K, Nelson C, Nettleton J, Partinen E, Rawal R, Robino A, Rose L, Sala C, Satoh T, Schmidt R, Schraut K, Scott R, Smith AV, Starr JM, Teumer A, Trompet S, Uitterlinden AG, Venturini C, Vergnaud AC, Verweij N, Vitart V, Vuckovic D, Wedenoja J, Yengo L, Yu B, Zhang W, Zhao JH, Boomsma DI, Chambers J, Chasman DI, Daniela T, de Geus E, Deary I, Eriksson JG, Esko T, Eulenburg V, Franco OH, Froguel P, Gieger C, Grabe HJ, Gudnason V, Gyllensten U, Harris TB, Hartikainen AL, Heath AC, Hocking L, Hofman A, Huth C, Jarvelin MR, Jukema JW, Kaprio J, Kooner JS, Kutalik Z, Lahti J, Langenberg C, Lehtimaki T, Liu Y, Madden PA, Martin N, Morrison A, Penninx B, Pirastu N, Psaty B, Raitakari O, Ridker P, Rose R, Rotter JI, Samani NJ, Schmidt H, Spector TD, Stott D, Strachan D, Tzoulaki I, van der Harst P, van Duijn CM, Marques-Vidal P, Vollenweider P, Wareham NJ, Whitfield JB, Wilson J, Wolffenbuttel B, Bakalkin G, Evangelou E, Liu Y, Rice KM, Desrivieres S, Kliewer SA, Mangelsdorf DJ, Muller CP, Levy D, Elliott P. 2016; KLB is associated with alcohol drinking, and its gene product beta-Klotho is necessary for FGF21 regulation of alcohol preference. Proc Natl Acad Sci USA. 113:14372–14377. [PubMed: 27911795]

Segre AV, Groop L, Mootha VK, Daly MJ, Altshuler D. Consortium D, investigators M. 2010Common inherited variation in mitochondrial genes is not enriched for associations with type 2 diabetes or related glycemic traits. PLoS Genet. :6.

Shalek AK, Satija R, Adiconis X, Gertner RS, Gaublomme JT, Raychowdhury R, Schwartz S, Yosef N, Malboeuf C, Lu D, Trombetta JJ, Gennert D, Gnirke A, Goren A, Hacohen N, Levin JZ, Park H, Regev A. 2013; Single-cell transcriptomics reveals bimodality in expression and splicing in immune cells. Nature. 498:236–240. [PubMed: 23685454]

Shi J, Levinson DF, Duan J, Sanders AR, Zheng Y, Pe'er I, Dudbridge F, Holmans PA, Whittemore AS, Mowry BJ, Olincy A, Amin F, Cloninger CR, Silverman JM, Buccola NG, Byerley WF, Black DW, Crowe RR, Oksenberg JR, Mirel DB, Kendler KS, Freedman R, Gejman PV. 2009; Common variants on chromosome 6p22.1 are associated with schizophrenia. Nature. 460:753–757. [PubMed: 19571809]

Smith ML, Lopez MF, Archer KJ, Wolen AR, Becker HC, Miles MF. 2016; Time-course analysis of brain regional expression network responses to chronic intermittent ethanol and withdrawal: implications for mechanisms underlying excessive ethanol consumption. PLoS One. 11:e0146257. [PubMed: 26730594]

Song A, Yan J, Kim S, Risacher SL, Wong AK, Saykin AJ, Shen L, Greene CS. Alzheimer's Disease Neuroimaging I. 2016; Network-based analysis of genetic variants associated with hippocampal volume in Alzheimer's disease: a study of ADNI cohorts. BioData Min. 9:3. [PubMed: 26788126]

Speir ML, Zweig AS, Rosenbloom KR, Raney BJ, Paten B, Nejad P, Lee BT, Learned K, Karolchik D, Hinrichs AS, Heitner S, Harte RA, Haeussler M, Guruvadoo L, Fujita PA, Eisenhart C, Diekhans M, Clawson H, Casper J, Barber GP, Haussler D, Kuhn RM, Kent WJ. 2016; The UCSC Genome Browser database: 2016 update. Nucleic Acids Res. 44:D717–725. [PubMed: 26590259]

Sutherland GT, Sheedy D, Stevens J, McCrossin T, Smith CC, van Roijen M, Kril JJ. 2016; The NSW brain tissue resource centre: Banking for alcohol and major neuropsychiatric disorders research. Alcohol. 52:33–39. [PubMed: 27139235]

Tasic B, Menon V, Nguyen TN, Kim TK, Jarsky T, Yao Z, Levi B, Gray LT, Sorensen SA, Dolbeare T, Bertagnolli D, Goldy J, Shapovalova N, Parry S, Lee C, Smith K, Bernard A, Madisen L, Sunkin SM, Hawrylycz M, Koch C, Zeng H. 2016; Adult mouse cortical cell taxonomy revealed by single cell transcriptomics. Nat Neurosci. 19:335–346. [PubMed: 26727548]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Torkamani A, Schork NJ. 2009; Pathway and network analysis with high-density allelic association data. Methods Mol Biol. 563:289–301. [PubMed: 19597792]

Treutlein J, Cichon S, Ridinger M, Wodarz N, Soyka M, Zill P, Maier W, Moessner R, Gaebel W, Dahmen N, Fehr C, Scherbaum N, Steffens M, Ludwig KU, Frank J, Wichmann HE, Schreiber S, Dragano N, Sommer WH, Leonardi-Essmann F, Lourdusamy A, Gebicke-Haerter P, Wienker TF, Sullivan PF, Nothen MM, Kiefer F, Spanagel R, Mann K, Rietschel M. 2009; Genome-wide association study of alcohol dependence. Arch Gen Psychiat. 66:773–784. [PubMed: 19581569]

Trynka G, Sandor C, Han B, Xu H, Stranger BE, Liu XS, Raychaudhuri S. 2013; Chromatin marks identify critical cell types for fine mapping complex trait variants. Nat Genet. 45:124–130. [PubMed: 23263488]

Usoskin D, Furlan A, Islam S, Abdo H, Lonnerberg P, Lou D, Hjerling-Leffler J, Haeggstrom J, Kharchenko O, Kharchenko PV, Linnarsson S, Ernfors P. 2015; Unbiased classification of sensory neuron types by large-scale single-cell RNA sequencing. Nat Neurosci. 18:145–153. [PubMed: 25420068]

Verhulst B, Neale MC, Kendler KS. 2015; The heritability of alcohol use disorders: a meta-analysis of twin and adoption studies. Psychol Med. 45:1061–1072. [PubMed: 25171596]

Voineagu I, Wang X, Johnston P, Lowe JK, Tian Y, Horvath S, Mill J, Cantor RM, Blencowe BJ, Geschwind DH. 2011; Transcriptomic analysis of autistic brain reveals convergent molecular pathology. Nature. 474:380–384. [PubMed: 21614001]

Wang ET, Sandberg R, Luo S, Khrebtukova I, Zhang L, Mayr C, Kingsmore SF, Schroth GP, Burge CB. 2008; Alternative isoform regulation in human tissue transcriptomes. Nature. 456:470–476. [PubMed: 18978772]

Wang K, Li M, Hakonarson H. 2010; Analysing biological pathways in genome-wide association studies. Nat Rev Genet. 11:843–854. [PubMed: 21085203]

Wang L, Jia P, Wolfinger RD, Chen X, Zhao Z. 2011; Gene set analysis of genome-wide association studies: Methodological issues and perspectives. Genomics. 98:1–8. [PubMed: 21565265]

Ward LD, Kellis M. 2012; HaploReg: a resource for exploring chromatin states, conservation, and regulatory motif alterations within sets of genetically linked variants. Nucleic Acids Res. 40:D930–934. [PubMed: 22064851]

Westra HJ, Peters MJ, Esko T, Yaghootkar H, Schurmann C, Kettunen J, Christiansen MW, Fairfax BP, Schramm K, Powell JE, Zhernakova A, Zhernakova DV, Veldink JH, Van den Berg LH, Karjalainen J, Withoff S, Uitterlinden AG, Hofman A, Rivadeneira F, Hoen PAC, Reinmaa E, Fischer K, Nelis M, Milani L, Melzer D, Ferrucci L, Singleton AB, Hernandez DG, Nalls MA, Homuth G, Nauck M, Radke D, Volker U, Perola M, Salomaa V, Brody J, Suchy-Dicey A, Gharib SA, Enquobahrie DA, Lumley T, Montgomery GW, Makino S, Prokisch H, Herder C, Roden M, Grallert H, Meitinger T, Strauch K, Li Y, Jansen RC, Visscher PM, Knight JC, Psaty BM, Ripatti S, Teumer A, Frayling TM, Metspalu A, van Meurs JBJ, Franke L. 2013; Systematic identification of trans eQTLs as putative drivers of known disease associations. Nat Genet. 45:1238–1243. [PubMed: 24013639]

Whelan R, Conrod PJ, Poline JB, Lourdusamy A, Banaschewski T, Barker GJ, Bellgrove MA, Buchel C, Byrne M, Cummins TD, Fauth-Buhler M, Flor H, Gallinat J, Heinz A, Ittermann B, Mann K, Martinot JL, Lalor EC, Lathrop M, Loth E, Nees F, Paus T, Rietschel M, Smolka MN, Spanagel R, Stephens DN, Struve M, Thyreau B, Vollstaedt-Klein S, Robbins TW, Schumann G, Garavan H. Consortium I. 2012; Adolescent impulsivity phenotypes characterized by distinct brain networks. Nat Neurosci. 15:920–925. [PubMed: 22544311]

Willsey AJ, Sanders SJ, Li M, Dong S, Tebbenkamp AT, Muhle RA, Reilly SK, Lin L, Fertuzinhos S, Miller JA, Murtha MT, Bichsel C, Niu W, Cotney J, Ercan-Sencicek AG, Gockley J, Gupta AR, Han W, He X, Hoffman EJ, Klei L, Lei J, Liu W, Liu L, Lu C, Xu X, Zhu Y, Mane SM, Lein ES, Wei L, Noonan JP, Roeder K, Devlin B, Sestan N, State MW. 2013; Coexpression networks implicate human midfetal deep cortical projection neurons in the pathogenesis of autism. Cell. 155:997–1007. [PubMed: 24267886]

Wolen AR, Phillips CA, Langston MA, Putman AH, Vorster PJ, Bruce NA, York TP, Williams RW, Miles MF. 2012; Genetic dissection of acute ethanol responsive gene networks in prefrontal cortex: functional and mechanistic implications. PLoS One. 7:e33575. [PubMed: 22511924]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Yang J, Lee SH, Goddard ME, Visscher PM. 2011; GCTA: A tool for genome-wide complex trait analysis. Am J Hum Genet. 88:76–82. [PubMed: 21167468]

Yeo G, Holste D, Kreiman G, Burge CB. 2004; Variation in alternative splicing across human tissues. Genome Biol. 5:R74. [PubMed: 15461793]

Zeisel A, Munoz-Manchado AB, Codeluppi S, Lonnerberg P, La Manno G, Jureus A, Marques S, Munguba H, He L, Betsholtz C, Rolny C, Castelo-Branco G, Hjerling-Leffler J, Linnarsson S. 2015; Brain structure. Cell types in the mouse cortex and hippocampus revealed by single-cell RNA-seq. Science. 347:1138–1142. [PubMed: 25700174]

Zhao Y, Chen J, Freudenberg JM, Meng Q, Rajpal DK, Yang X. 2016; Network-based identification and prioritization of key regulators of coronary artery disease loci. Arterioscler Thromb Vasc Biol. 36:928–941. [PubMed: 26966275]

Zhao Z, Guo AY, van den Oord EJ, Aliev F, Jia P, Edenberg HJ, Riley BP, Dick DM, Bettinger JC, Davies AG, Grotewiel MS, Schuckit MA, Agrawal A, Kramer J, Nurnberger JI Jr, Kendler KS, Webb BT, Miles MF. 2012; Multi-species data integration and gene ranking enrich significant results in an alcoholism genome-wide association study. BMC Genomics. 13(Suppl 8):S16.

Zhou X, Maricque B, Xie M, Li D, Sundaram V, Martin EA, Koebbe BC, Nielsen C, Hirst M, Farnham P, Kuhn RM, Zhu J, Smirnov I, Kent WJ, Haussler D, Madden PA, Costello JF, Wang T. 2011; The Human Epigenome Browser at Washington University. Nat Methods. 8:989–990. [PubMed: 22127213]

Zhou Z, Yuan Q, Mash DC, Goldman D. 2011; Substance-specific and shared transcription and epigenetic changes in the human hippocampus chronically exposed to cocaine and alcohol. Proc Natl Acad Sci USA. 108:6626–6631. [PubMed: 21464311]

Zhu Z, Zhang F, Hu H, Bakshi A, Robinson MR, Powell JE, Montgomery GW, Goddard ME, Wray NR, Visscher PM, Yang J. 2016; Integration of summary data from GWAS and eQTL studies predicts complex trait gene targets. Nat Genet. 48:481–487. [PubMed: 27019110]

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 1

Glossary of terms

Term	Definition/description
AUD	Alcohol Use Disorder
ChIP-Seq	Chromatin immunoprecipitation with sequencing
CNS	Central nervous system
DHSs	DNase I hypersensitive sites
DNase	Deoxyribonuclease
eQTL	Expression quantitative trait locus
GCTA	Genomewide Complex Trait Analysis (Yang et al. 2011)
GWAS	Genome-wide association study
H3K4me3	Trimethylation of lysine 4 on the histone H3 protein subunit regulatory mark
LD	Linkage disequilibrium
LDScR	Linkage Disequilibrium Score Regression (Bulik-Sullivan et al., 2015)
MAGMA	Multi-marker Analysis of GenoMic Annotation (de Leeuw et al. 2015)
meQTL	Methylation quantitative trait locus
mRNA	Messenger RNA
NetWAS	Network-wide association study
PLINK	Statistical genetics software program (Purcell et al. 2007; Chang et al. 2015)
PPI	Protein-protein interaction
RNA-Seq	RNA sequencing
SNP	Single-nucleotide polymorphism
TWAS	Transcriptome-wide association studies (Gusev et al. 2016)
VEGAS	Versatile gene-based association study (Liu et al. 2010; Mishra et al. 2015)

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 2

Resources for expression-related validation of single nucleotide polymorphisms (eQTL) and gene sets in tissue from the central nervous system

Source	Brain Tissue type	dbGaP or other accession points	URL for online queries
GTEx	Anterior cingulate cortex (n = 72), caudate (n = 100), cerebellar hemisphere (n = 89), cerebellum (n = 103), cortex (n = 96), frontal cortex (n = 92), hippocampus (n = 81), hypothalamus (n = 81), nucleus accumbens (n = 92), putamen (n = 82)	phs000424.v6.p1	http://www.gtexportal.org/home/
BRAINEAC	Cerebellum, frontal cortex, hippocampus, medulla, occipital cortex, putamen, substantia nigra, thalamus, intralobular white matter, temporal cortex (n = 134)	-	http://www.braineac.org/
CommonMind	Dorsolateral prefrontal cortex (n = 291 control, 275 SCZ, 47 BIP)	https://www.nimhgenetics.org/available_data/commonmind/ https://www.synapse.org/#!Synapse:syn2759792/wiki/197295	
Braincloud	Prefrontal cortex (14 weeks to 80 years, n = 269)	phs000417.v2.p1	http://braincloud.jhmi.edu/downloads.htm
Meta-analysis of eQTL data	Cortical samples from 5 studies (n = 424)	Significant SNP – gene pair associations in supplement of http://www.nature.com/tp/journal/v4/n10/full/tp201496a.html	
PsychENCODE	dorsolateral prefrontal cortex, anterior cingulate cortex, inferior temporal cortex, hippocampus, amygdala, caudate nucleus, nucleus accumbens and cerebellar cortex (n = 1,000 including control and disease-related)	https://www.synapse.org//#!Synapse:syn4921369/wiki/390660	
DNA Methylation and expression in human brain	Cerebellum, frontal cortex, pons, and temporal cortex (n = 382)	phs000249.v2.p1	-
Gene network	Multiple tissues spanning humans, mice (BXD, AXB, LXS, etc.), rats (HXB), and drosophila.	http://genenetwork.org/webqtl/main.py	

Author Manuscript Author Manuscript Author Manuscript Author Manuscript

Table 3

Overview of pathway or network-based GWAS of alcohol-related behaviors

Author, Date	Sample	Phenotype	Analysis tool/method	Gene sets	Summary of key enrichment domains
Kendler et al. (2011)	Molecular Genetics of Schizophrenia control sample	Factor score derived from alcohol craving, DSM-IV alcohol abuse and dependence	ALIGATOR	KEGG, GO	Response to hormone stimulus, membrane raft organization, anatomical structure homeostasis, phosphorylation, and regulation of transporter activity; lipid catabolic processes, regulation of protein transport, regulation of cell adhesion, and cholesterol metabolic process
Kos et al. (2013)	SAGE	DSM-IV alcohol dependence	Permuted Fisher's exact tests	ResNet Mammalian v. 7.0 database curated by Ariadne Genomics	Maf transcription factors, Hox AbdB genes, chloride transport, glycine and serine metabolism
Biernacka et al. (2013)	SAGE	DSM-IV alcohol dependence	Permutation-based self-contained SNP or gene set analysis	KEGG	Synthesis and degradation of ketone bodies; neuroactive ligand-receptor interaction
Han et al. (2013)	Discovery: COGA+SAGE; Replication: Yale-Penn, OZ-ALC	DSM-IV alcohol dependence	R-package dmGWAS	Protein-protein interaction network, GO	cation transport, synaptic transmission, and transmission of nerve impulses
Juraeva et al. (2015)	Discovery: German sample; replication: COGA, SAGE	DSM-IV alcohol dependence	R-package globaltest version 5.12.0	KEGG, GO, BioCarta, microRNA targets, transcription factor targets, positional gene-sets	Nineteen gene-sets identified in discovery sample, five of which were nominally significant in replication sample, including DNA integration, focal adhesion, leucocyte transendothelial migration, chr7q32, and MIR-377
Adkins et al. (2015)	GSMS, CHDS, VTSABD	Longitudinal alcohol consumption	Hypergeometric test	ConsensusPathDB	Neuronal system, Transmission across chemical Synapses, pharmacodynamics, Adherens junctions interactions, G protein signaling pathways, Axon guidance