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Review

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Review

Overview of the Genetics of Alcohol Use Disorder

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Abstract

Aims: Alcohol Use Disorder (AUD) is a chronic psychiatric illness characterized by harmful drinking patterns leading to negative emotional, physical, and social ramifications. While the underlying pathophysiology of AUD is poorly understood, there is substantial evidence for a genetic component; however, identification of universal genetic risk variants for AUD has been difficult. Recent efforts in the search for AUD susceptibility genes will be reviewed in this article.
Methods: In this review, we provide an overview of genetic studies on AUD, including twin studies, linkage studies, candidate gene studies, and genome-wide association studies (GWAS).
Results: Several potential genetic susceptibility factors for AUD have been identified, but the genes of alcohol metabolism, alcohol dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*), have been found to be protective against the development of AUD. GWAS have also identified a heterogeneous list of SNPs associated with AUD and alcohol-related phenotypes, emphasizing the complexity and heterogeneity of the disorder. In addition, many of these findings have small effect sizes when compared to alcohol metabolism genes, and biological relevance is often unknown.
Conclusions: Although studies spanning multiple approaches have suggested a genetic basis for AUD, identification of the genetic risk variants has been challenging. Some promising results are emerging from GWAS studies; however, larger sample sizes are needed to improve GWAS results and resolution. As the field of genetics is rapidly developing, whole genome sequencing could soon become the new standard of interrogation of the genes and neurobiological pathways which contribute to the complex phenotype of AUD.
Short summary: This review examines the genetic underpinnings of Alcohol Use Disorder (AUD), with an emphasis on GWAS approaches for identifying genetic risk variants. The most promising results associated with AUD and alcohol-related phenotypes have included SNPs of the alcohol metabolism genes *ADH* and *ALDH*.

INTRODUCTION

Alcohol Use Disorder (AUD) is a chronic psychiatric condition characterized by drinking patterns that lead to detrimental emotional, physical, and social outcomes. The Centers for Disease Control and Prevention (CDC) has reported that alcohol use contributes to approximately 88,000 deaths annually in the United States (Stahre *et al.*, 2014), reflecting high morbidity and mortality. To diagnose individuals with AUD, the Diagnostic and Statistical Manual of Mental Disorders,

Fifth Edition (Mizokawa *et al.*, 2013) utilizes 11 criteria pertaining to excessive alcohol use, alcohol abuse, and alcohol dependence. The range of symptoms encompassed in the criteria for AUD diagnosis, including drinking more or for longer than intended or continuing to drink despite psychological or health problems, for instance, demonstrates the disorder's heterogeneous clinical presentation.

Recent investigations of the intersection of AUD with epidemiological factors and comorbid psychiatric disorders indicate the high and rising prevalence of AUD in the United States. Findings from the National Epidemiologic Survey on Alcohol and Related Conditions (NESARC) Waves 1 and 2 have revealed that lifetime DSM-IV AUD is as high as 36.7% in persons aged 30–44, 42.0% in men compared to women (19.5%), and 34.1% in non-Hispanic whites (Grant *et al.*, 2016). Furthermore, AUD frequently co-occurs with other psychiatric disorders, including mood and anxiety disorders (Regier *et al.*, 1990), post-traumatic stress disorder (Sampson *et al.*, 2015), and other substance use disorders (Kessler *et al.*, 1997). According to the Epidemiologic Catchment Area Study, roughly half of individuals with AUD have a 'dual diagnosis', or comorbid psychiatric disorder (Regier *et al.*, 1990), and the National Comorbidity Study presented the finding that ¾ of men and women with AUD met lifetime criteria for the diagnosis of a psychiatric illness (Kessler *et al.*, 1997). These data highlight the heterogeneity of AUD and overlap with other psychiatric disorder that often also have strong genetic heritability estimates.

The etiology of AUD, which encompasses a variety of behavioral, environmental, psychological, and physiological factors, may be genetically predisposed. Lifetime drinking history is an important behavioral risk factor, which includes the age of first alcohol use, average number of drinks per day, and number of years of heavy drinking (Grant and Dawson, 1998). Environmental factors, such early life stressors (including physical or sexual abuse) increase the risk for AUD later in life (Enoch, 2011). High trait anxiety and other psychological factors are associated with an increased risk for developing AUD (Poikolainen, 2000). Physiological factors, such as alcohol withdrawal, also influence the risk of developing AUD (Becker and Mulholland, 2014). In addition to family history, all of the above risk factors for AUD have been previously predicted to have an underlying genetic cause. Approximately 50% of the risk for developing AUD is due to genetics, while the remaining percent may be due to either environmental factors, or gene-environment interactions. Therefore, a genetic predisposition to the addictive effects of alcohol, combined with other environmental risk factors, may cause harmful lifetime drinking patterns and AUD development. The current review provides an overview of the diverse types of genetic studies conducted on AUD, with an emphasis on recent genome-wide association studies (GWAS). Because AUD is a common psychiatric disorder characterized by a complex array of traits, GWAS may provide an effective experimental approach to identifying associated genetic variants. Further exploration of SNPs and other genetic variants associated with AUD could be used to advise at-risk individuals and develop more effective pharmacological interventions.

METHODS

A PubMed search was conducted for original GWAS published from 1/1/2005 to 2/1/2016 using a combination of the terms 'genetics', 'GWAS', 'alcohol dependence', and/or 'alcohol use disorder'. These initial papers and recent reviews on the genetics of alcohol dependence were used to identify additional literature on twin studies, linkage studies, and candidate gene association studies of AUD. The GWAS section of the current paper focuses on findings with a P-value $< 10^{-8}$.

RESULTS

Twin studies

Research using family, adoption, and twin studies was the first to demonstrate the role of genetics in AUD. The Australian twin-family study of alcohol use disorder (OZALC) found a greater concordance of alcohol dependence in monozygotic (56% for males) compared to dizygotic twins (33% for males) and a heritability estimate of 64% (Heath *et al.*, 1997). More recent twin studies have established that AUD heritability ranges from 40% to 70% (Enoch and Goldman, 2001; Agrawal and Lynskey, 2008; Kendler *et al.*, 2012) , with similar heritability estimates in both males and females (Heath *et al.*, 1997; Prescott *et al.*, 1999). One sample using male twins from the Vietnam Era Twin Registry reported different heritability estimates for 23 symptoms of alcohol dependence, further highlighting the heterogeneity of AUD (Slutske *et al.*, 1999). Overall, the heritability estimates of these studies strongly suggest that genetic components contribute to AUD; however, given that the concordance rates are below 50%, other factors such as rare somatic de novo mutations, environmental influences, and gene-environment interactions might contribute to the remaining heritability.

Linkage studies

Because of the epidemiological evidence for genetic factors in AUD, the field has hoped for a straightforward identification of AUD risk alleles. The first comprehensive investigation into the genetics of AUD used linkage studies. Linkage studies are useful for identifying broad regions of the genome associated with large increases in risk for a disorder. The term *linkage* refers to the observation that two genetic markers on the same chromosome are often inherited together. This approach uses families with multiple affected members to determine chromosomal regions with genetic risk variants. Previous linkage studies have been most successful in rare autosomal dominant diseases with high penetrance, such as cystic fibrosis.

The first linkage studies on alcohol dependence from the Collaborative Study on the Genetics of Alcoholism (COGA) (Reich *et al.*, 1998) and a sib-pair study from a Southwest American Indian tribe (Long *et al.*, 1998) reported a broad risk locus on chromosome 4q. This region contains the genes that encode the isoforms of alcohol dehydrogenase. Additional studies have implicated chromosomal regions containing GABA-A (Wang *et al.*, 2004) and CHRM2 (Wang *et al.*, 2004) among other variants (Edenberg and Foroud, 2014). However, these linkage studies were unable to identify specific genetic risk variants associated with AUD and instead, found chromosomal regions with multiple linkage peaks. These results highlight the complex, polygenetic biology of AUD.

Candidate gene association studies

Case/control association studies can be performed to test candidate genes within chromosomal regions identified through linkage studies or based on neurological plausibility. These studies can detect small increases in genetic risk variants associated with a disorder. However, many candidate gene studies on AUD have found results with small effect sizes and are often difficult to replicate.

Based on previous linkage studies, the strongest associations have been identified in the alcohol metabolism genes, alcohol

dehydrogenase (*ADH*) and aldehyde dehydrogenase (*ALDH*). Alcohol metabolism is a two-step process where ethanol is first oxidized to acetaldehyde by ADH and then further oxidized to acetate by ALDH. Accumulation of the toxic intermediate acetaldehyde can cause adverse physiological symptoms, including flushing syndrome, tachycardia, and nausea. The rate at which acetaldehyde is produced and converted to the waste product acetate is influenced by genetic variations encoding the isoenzymes of ADH and ALDH. Individuals with isoforms of ADH that oxidize ethanol at a faster rate and/or isoforms of ALDH that oxidize acetaldehyde at a slower rate are protected against AUD due to the unpleasant effects that result from acetaldehyde accumulation.

There are several isoforms of ADH that metabolize alcohol in the liver (ADH1A, ADH1B, ADH1C, and ADH4-7). A majority of alcohol oxidation is performed by the alcohol dehydrogenase isoform ADH1B. Individuals with the ADH1B*2 single-nucleotide polymorphism (SNP) rs1229984 (Arg48His) metabolize alcohol at a much faster rate than those with the ADH1B*1 variant. ADH1B*2 is more common in East Asians than other populations and has been found to have a protective effect on the risk for developing AUD (Luczak *et al.*, 2006; Edenberg, 2007; Li *et al.*, 2012). Although rare outside of East Asian populations, the ADH1B*2 variant was also found to have a protective effect in a sample of European-Americans (Bierut *et al.*, 2012). In addition, the ADH1B*3 variant was associated with a decreased risk of AUD in African-American and Native American samples (Wall *et al.*, 2003; Edenberg *et al.*, 2006). While ADH1B is the primary enzyme, the isoforms ADH1A and ADH1C contribute to alcohol metabolism, but at lower concentrations of ethanol. Several studies have found a protective effect of the ADH1C*2 SNP rs698 (Ile350Val) (Biernacka *et al.*, 2013b).

Variations in the isoforms of ALDH are also associated with the risk for AUD. A majority of the oxidation of acetaldehyde to acetate is performed by the acetaldehyde dehydrogenase isoform ALDH2. Individuals with the ALDH2*2 SNP rs671 (Glu504Lys) metabolize acetaldehyde at a much slower rate. Slow metabolism of acetaldehyde creates an unpleasant alcohol flushing syndrome, which is only found in East Asian populations. Therefore, the ALDH2*2 variant has been associated with a decreased risk of AUD (Luczak *et al.*, 2006; Hurley and Edenberg, 2012; Li *et al.*, 2012). When combined, both the ADH and ALDH2 variants are highly protective against the risk of developing AUD.

Although alcohol metabolizing enzyme polymorphisms are the most consistent candidate genes, genetic variants in several neurotransmitter systems have been associated with AUD. These include the glutamate receptor (*GIRK1*), gamma-aminobutyric acid receptor (*GABA-A*), D2 dopamine receptor (*DRD2*), dopamine transporter (*SLC6A3*), serotonin transporter (*SLC6A4*), tryptophan hydroxylase 1 (*TPH1*), catechol-O-methyltransferase (*COMT*), cholinergic muscarinic receptor (*CHRM2*), and u-opioid receptor (*OPRM1*, among others) (Edenberg *et al.*, 2004; Ray and Hutchison, 2004; Wang *et al.*, 2004; Munafo *et al.*, 2007; Kranzler *et al.*, 2009; McHugh *et al.*, 2010; Tammimaki and Mannisto, 2010; Du *et al.*, 2011; Xu and Lin, 2011; Chen *et al.*, 2012). However, many of these genetic risk variants are dependent on gene-environment interactions and replication of findings has been difficult due to the generally small expected effect size, lack of power, and clinical and genetic heterogeneity.

Genome-wide association studies

Unlike candidate gene studies, which require prior knowledge about the neurobiology underlying potential genetic risk variants, GWAS provide high resolution analysis of the entire genome without an *a priori* hypothesis. GWAS are used to analyze several hundred thousand or millions of SNPs across the genome to identify differences in genotype frequencies between case-controlled individuals, without selecting for only a few specific genes. This is a useful approach for identifying genetic risk variants in complex disorders where small effect sizes are expected. GWAS on other complex phenotypes, such as rheumatoid arthritis, have been successful in identifying SNPs associated with disease processes (Suzuki *et al.*, 2011). Promising results are also emerging from GWAS on mental illnesses, such as schizophrenia and bipolar disorder (Moskvina *et al.*, 2009; Williams *et al.*, 2011; Schizophrenia Working Group of the Psychiatric Genomics, 2014). Thus, this strategy may also be successful in identifying the genetic underpinnings of AUD.

In 2006, Johnson et al. analyzed 104,268 SNPs from 120 patients and 160 controls in a European-American population from the COGA sample (Johnson *et al.*, 2006). This study identified 51 chromosomal regions in the genome that harbor at least three genetic risk markers, including genes of the cell adhesion molecules cadherin 11 (*CDH11*) and cadherin 13 (*CDH13*). However, these results were only nominally significant ($P = 3.4 \times 10^{-4}$). In 2009, the first positive GWAS analysis of alcohol dependence, published by Treutlein *et al.* (2009), examined 487 alcohol dependent patients and 1358 controls from a German population (Treutlein *et al.*, 2009). This study found genome-wide significance for two intergenic loci on chromosome 2q35 (rs7590720 and rs1344694) close to the gene for peroxisomal trans-2-enoyl-CoA reductase (*PECR*). A follow-up study with 1024 patients and 966 controls replicated 15 significant SNPs, including rs11640875 in *CDH13*, rs1614972 in *ADH1C*, and rs13273672 in the GATA binding protein 4 (*GATA4*) (Table 1).

In an effort to increase sample size, several GWAS publications have analyzed genome-wide SNPs from the COGA, OZALC, and the Study of Addiction: Genetics and Environment (SAGE) samples. In 2010, Edenberg et al. performed the first complete GWAS of the COGA. Although this study found no single SNPs with genome-wide significance, it provided converging evidence for a chromosome 11 gene cluster. In 2011, Wang et al. conducted a low density GWAS with 11,120 SNPs from the COGA and OZALC samples and found genome-wide significance at $P < 10^{-8}$ for DSCMAL1 (Table 1). However, other groups have found only nominally significant SNPs ($P > 10^{-8}$) or no genome–wide significant markers by analyzing the COGA, OZALC, and SAGE samples (Bierut *et al.*, 2010; Lind *et al.*, 2010; Wang *et al.*, 2011; Zuo *et al.*, 2011, 2012b, 2013; Biernacka *et al.*, 2013a) (Table 1).

The most consistent GWAS findings for AUD have confirmed previously reported associations for genetic risk variants in *ADH* and *ALDH* genes. In 2012, Frank et al. identified genome-wide significance for rs1789891 in the *ADH1* gene cluster in a German population. This SNP was found to be in linkage disequilibrium with the functional variant ADH1C (Arg272Gln). Park *et al.* (2013) also found multiple nominally significant SNPs in the ADH gene cluster on chromosome 4q22-q23, as well as genome-wide significance for rs1442492 and rs10516441 in *ADH7* and rs671 in *ALDH2* in an East Asian sample. Likewise, other GWAS have shown that the *ALDH2*2* variant rs671 (Glu504Lys) is associated with a decreased risk of AUD in East Asian populations (Takeuchi *et al.*, 2011; Frank *et al.*, 2012; Quillen *et al.*, 2014). Additionally, GWAS have found that the ADH1B*2 SNP rs1229984 has a protective effect for AUD development (Takeuchi *et al.*, 2011; Park *et al.*, 2013). A recent study by Gelernter *et al.* (2014) found that the SNP rs1229984 decreased the risk of AUD in a European-

Table 1. Positive findings from GWAS on alcohol use disorder and alcohol-related phenotypes

Author	Phenotype	Gene/SNP	*P* Value	Sample	Ethnicity
Baik *et al.*, 2011	Alcohol Consumption	*C12ORF24* (rs2074356)	$P = 9.49e{-59}$	1721	Korean Men
Biernacka *et al.*, 2013a,b	Alcohol Dependence	KEGG pathway ID 72- Synthesis and degredation of keton bodies	$P = 0.003$	SAGE (2544)	European- and African-American
Bierut *et al.*, 2010	Alcohol Dependence	*GABRA2*	$P < 0.05$	SAGE (3829)	European- and African-American
		PBX/knotted 1 homeobox 2, *PKNOX2*	$P = 1.93e{-07}$		
Chen *et al.*, 2012	Alcohol Drinking	Ankyrin repeat domain 7, *ANKRD 7*, and Cytokine-like 1, *CYTL1*, (rs6466686-rs4295599-rs12531086) (halotype)	$P = 6.51e{-8}$	904	Caucasian
Frank *et al.*, 2012	Alcohol Dependence	*ALDH2* (rs671)	$P = 1.27e{-08}$	3501	German descent
		ADH1 between *ADH1B* and *ADH1C* (rs1789891)	$P = 1.27e{-08}$		
Gelernter *et al.*, 2014	Alcohol Dependence	*ADH1B* (rs1229984)	$P = 1.17e{-31}$	379 European Americans,	European- and African-American
		ADH1B (rs1789882)	$P = 6.33e{-17}$	3318 African Americans (total = 16,087)	
		ADH1C (Thr151Thr)	$P = 4.94e{-10}$		
		Between *MTIF2* and *CCDC88A* on chromosome 2 (rs1437396)	$P = 1.17e{-10}$		
Johnson *et al.*, 2006	Alcohol Dependence	51 gene loci, including *CDH11*, *CDH13*	$P = 0.00034$	COGA (280)	European-American
Lind *et al.*, 2010	Alcohol and Nicotine co-dependence	Near MAP/microtubule affinity-regulating kinase 1, *MARK1*	$P = 1.90e{-09}$	1087	Australian
		Near DEAD (Asp-Glu-Ala-Asp) box helicase 6, *DDX6*	$P = 2.6e{-09}$		
		KIAA1409	$P = 4.86e{-08}$		
Lind *et al.*, 2010	Alcohol and nicotine co-dependence	Near semaphorin 3E, *SEMA3E*	$P = 6.23e{-06}$	OZALC (2386)	Australian and Dutch
Park *et al.*, 2013	Alcohol Dependence	*ALDH2* (rs671)	$P = 8.42e{-08}$	396	Korean
		ADH1B (rs1229984)	$P = 2.63e{-21}$		
		ADH7 (rs1442492)	$P = 6.28e{-8}$		
Quillen *et al.*, 2014	Alcohol Dependence	*ALDH2* (rs671)	$P = 4.55e{-08}$	595	Chinese
Schumann *et al.*, 2011	Alcohol Consumption	*AUTS2* (rs6943555)	$P = 4e{-08}$	12 population-based samples (26,316)	European
Takeuchi *et al.*, 2011	Alcohol Consumption	*ALDH2* (rs 671)	$P = 3.6e{-211}$	2974 drinkers,	Japanese
		ADH1B (rs1229984)	$P = 3.6e{-4}$	1521 occasional drinkers, 1351 non-drinkers	
Treutlein *et al.*, 2009	Alcohol Dependence	Near peroxisomal trans-2-enoyl-CoA reductase, *PECR* (rs7590720 and rs1344694)	$P = 9.72e{-09}$	1845	German
		CDH13 (rs11640875)	$P = 1.84e{-5}$	2020	German
		ADH1C (rs1614972)	$P = 1.41e{-4}$		
		GATA4 (rs13273672)	$P = 4.75e{-4}$		
Wang *et al.*, 2011	Alcohol Dependence	*DSCMAL1*	$P < 10e{-08}$	COGA, OZALC 272 nuclear families	European-American and Australian
Wang *et al.*, 2011	Alcohol Dependence	near endothelin receptor type B, *EDNRB*	$P = 8.51e{-06}$	COGA, OZALC 272 nuclear families	European-American, African-American, and Australian
		TPARP, CYFIP2, THEMIS, PSG11	$P = 2.31e{-5}$		
Wang *et al.*, 2011	Alcohol Dependence	*KIAA0040, THSD7B, NRD1*	$P = 1.86e{-07}$	COGA (1594), SAGE (1669), OZALC (3334)	European-American, African-American, and Australian
Wang *et al.*, 2013	Alcohol Dependence Symptom Count	3 SNPs in *C15ORF53* gene	$P = 4.5e{-8}$	COGA (2322)	European-American

Continued

Table 1. Continued

Author	Phenotype	Gene/SNP	*P* Value	Sample	Ethnicity
Zuo *et al.*, 2011	Alcohol Dependence	PHD finger protein 3, *PHF3*, - Protein tyrosine phosphatase type IVA 1, *PTP4A1*, locus	*P* < 10e−4	COGA, SAGE (4116)	European- and African-American
Zuo *et al.*, 2012a,b	Alcohol Dependence	*KIAA0040*	*P* =2 .8e−07	COGA, SAGE (4116)	European- and African-American
Zuo *et al.*, 2012a,b	Alcohol and Nicotine co-dependence	SH3 domain binding protein 5, *SH3BP5*	*P* = 6.9e−6	SAGE (3143)	European- and African-American
		Nuclear receptor subfamily 2, group C, member 2, *NR2C2*	*P* = 5.3e−4		
		Plasminogen-like B2, *PLGLB2*	*P* = 3.1e−08		
Zuo *et al.*, 2013	Alcohol Dependence	*NKAIN1-SERINC2*	*P* = 1.7e−07	COGA, SAGE (2927)	European- and African-American
Zuo *et al.*, 2013	Alcohol and Nicotine co-dependence	IPO11-HTR1A region on chromosome 5q	*P* = 6.2e−9	COGA, SAGE (2214)	European- and African-American

American population as well. This study reported that the ADH1C variant (Thr151Thr) and the ADH1B SNP rs1789882 (Arg369Cyc) decreased the risk of AUD in an African-American population (Gelernter *et al.*, 2014) (Table 1).

Several other GWAS have found genome-wide significant SNPs for other alcohol-related phenotypes, such as alcohol and nicotine codependence, and alcohol consumption. When analyzed for alcohol and nicotine comorbidity, Lind *et al.* (2010) found genome-wide significance for rs7530302 near MAP/microtubule affinity-regulating kinase 1 (*MARK1*), rs1784300 near DEAD box helicase 6 (*DDX6*), and rs12882384 in *KIAA1409* (Lind *et al.*, 2010). Likewise, Zuo *et al.* (2012a,b) identified SNPs in SH3 domain binding protein 5 (*SH3BP5*), nuclear receptor subfamily 2 group C member 2 (*NR2C2*), Plasminogen-like B2 (*PLGLB2*), and rs7445832 in IPO11-HRT1A region on chromosome 5q associated with alcohol and nicotine dependence codependence (Zuo *et al.*, 2012a; Zuo *et al.*, 2013). Schumann *et al.* (2011) identified genome-wide significance of alcohol consumption for *AUT2* rs6943555 in a sample of 26,316 individuals from 12 population-based samples (Schumann *et al.*, 2011). Additionally, Baik *et al.* (2011) measured genome-wide significance of alcohol consumption in a sample of 1,721 males and replicated SNPs on chromosome 12q24, including *C12ORF51* rs2074356, which is LD with *ALDH2*, *CCDC63*, and *MYL2* (Baik *et al.*, 2011). In 2012, Chen et al. found genome-wide significance for SNP clusters in Ankyrin repeat domain 7 (*ANKRD7*) Cytokine-like 1 (*CYTL1*) associated with alcohol drinking. A 2013 study by Wang et al. also found significance for three SNPs in the *C15ORF53* gene when analyzed for alcohol dependence symptom count (Wang *et al.*, 2013) (Table 1).

While the recent use of GWAS to identify the underlying genetic components of AUD has been promising, there are several limitations of GWAS that must be considered. GWAS use a 'hypothesis-free' design by genotyping hundreds of thousands to 2 million markers simultaneously in cases and controls. This approach generates large amounts of data and creates issues with regard to multiple testing. The current stringent statistical correction for GWAS is a *P* value of 10^{-8}. As a result, early GWAS in psychiatric phenotypes yielded negative findings (Sklar *et al.*, 2008; Craddock and Sklar, 2013). In retrospect, those studies (despite sample sizes in the range of 1000–2000) were largely underpowered to detect risk variants of small effect. Current power and sample size estimates for GWAS with effect sizes of 1.05–1.2 range from 30,000 – 120,000 (Owen *et al.*, 2010; Schizophrenia Working Group of the Psychiatric Genomics, 2014). While the use of a stringent *P*-value for GWAS avoids the detection of false positive findings, it might also miss 'true' variants. Recent attempts to address this issue have used pathway analysis and polygenic risk score approaches (Gelernter *et al.*, 2014) but have not been widely applied to AUD genetic analyses.

As the field moves forward, it is important to identify expected findings. AUD is a complex disorder, and likely hundreds if not thousands of genes contribute to its broad and varied phenotype. Therefore, it is unlikely that GWAS will detect genes of large effect . In addition, given the current chip-based methodology of GWAS, this technology by design misses rare de novo mutations or insertion/deletion variants (Stefansson *et al.*, 2008; Walsh *et al.*, 2008; Clarke and Cooper, 2010). Furthermore, several findings have been for intronic SNPs with no clear understanding of their underlying biological relevance. It is expected that GWAS will continue to be the standard of investigation of current genetic efforts to understand AUD. As it has been done for other psychiatric phenotypes, GWAS in AUD will need a collaborative approach in the form of large meta-analyses (Cichon *et al.*, 2009; Sklar *et al.*, 2011). While efforts are ongoing (Dick and Agrawal, 2008), no AUD GWAS meta-analysis currently exists.

A changing definition of the heterogeneous phenotype of AUD may also pose a challenge to identifying genetic variants through GWAS. The above studies used the DSM-IV-TR criteria for alcohol dependence in order to define the phenotype. As the field of psychiatry transitions to the DSM-5 criteria for AUD, there may also be changes in the functional variants identified by GWAS. Future GWAS should focus on the endophenotypes of AUD in order to better understand the genetic connections to specific behavioral symptoms. Likewise, it will be important to separate the role of genetic variants due other substance use disorders and to comorbid psychiatric disorders. Defining specific phenotypes and separating comorbid disorders will be useful in order to parse genetic variants involved in multiple disorders and addictions from those only involved in AUD. Future studies may also focus on pathway analysis in order to better understand the heterogeneous group of variants currently identified by GWAS.

GCTA/GREML methods

To address the 'missing heritability' problem, or the fact that variations in single genes have not accounted for much of the heritability in diseases, phenotypes, or behavioral pathologies, researchers have adopted Genome-Wide Complex Trait Analysis (GCTA)/Genomic

Restricted Maximum Likelihood (GREML) (Yang *et al.*, 2011). GCTA/GREML, or GCTA, is a statistical method which estimates variance in genetics by quantifying the chance genetic similarity of individuals and comparing their similarity in trait measurements. By quantifying the additive contributions of a subset of genetic variants (SNPs) to a trait's heritability, GCTA can corroborate the findings of GWAS studies. If the GCTA estimate of SNP heritability is consistent with the total genetic heritability, it is implicated that those genetics variants have a causal effect on the observed phenotype (Yang *et al.*, 2011).

In the context of AUD, GCTA could be applied to the subsets of previously discussed SNPs that reached genome-wide significance and were correlated with alcohol-dependent phenotypes. GCTA estimates could be used for diagnostic purposes and provide further insight as to whether variants in *ADH* and *ALDH*, among other genes, in fact contribute to the genetic predisposition for AUD.

In a study examining the heritability of behavioral disinhibition, a trait which has been previously linked to substance use disorder development, twin-estimated heritabilities, GCTA-estimated heritabilities, and genome-wide scores were calculated to determine genetics correlations among various indicators of substance use and behavioral inhibition (Vrieze *et al.*, 2013). Vrieze *et al.* (2013) found that, in biometric twin models, behavioral inhibition was highly genetically correlated with all substance use traits (nicotine use/dependence, alcohol consumption, alcohol dependence, and drug use). Regarding alcohol dependence, heritability was as high as 56%, and the aggregate additive SNP effects estimated by GCTA on the parent sample accounted for 16% of the variance (Vrieze *et al.*, 2013). Hence, Vrieze *et al.* (2013) found that substance use phenotypes, including those pertaining to alcohol use, and behavioral disinhibition share a genetic etiology, and that measured genetic variants contribute to their heritability.

Another study investigating the heritability of assorted substance dependencies, including alcohol, tobacco, cannabis, and illicit drugs, used GCTA estimates to conclude that common SNPs contribute to at least 20% of the variance in substance dependence vulnerability (Palmer *et al.*, 2015). Because the GWAS findings on substance dependence broadly have been limited, Palmer *et al.* (2015) demonstrated the efficacy of GCTA in identifying the heritability of substance use disorders via aggregate effects of genetic variants. Overall, GCTA methods may greatly facilitate investigators' abilities to make causal attributions of common SNPs to complex psychiatric conditions, including alcohol use phenotypes and dependence.

Whole genome sequencing

As the field of genomics is rapidly expanding, with advances in technology and decreases in costs, whole genome sequencing is expected to become feasible in the near future. Although GWAS are much more economical, the financial burden of whole genome sequencing could be outweighed by the value of genetic information obtained. Unlike GWAS, whole-genome sequencing is more likely to identify rare mutations, particularly recessive mutations, in exonic regions of the genome. These coding regions may have a strong impact on disease etiology and shed new light into possible pathophysiological mechanisms (Cirulli and Goldstein, 2010; Ng and Kirkness, 2010; Kato, 2015). Since exome analysis has been successful in identifying de novo and inherited point mutations in autism spectrum disorders, and it has been applied to mood and psychotic disorders, there is hope that exome/whole genome sequencing could be a highly beneficial tool in mapping the genetic architecture of substance use and addiction disorders (Kato, 2015).

CONCLUSIONS

Despite the evidence supporting the prominence of genetic factors in AUD's etiology, the identification of genetic risk variants has been difficult and labor intensive. With recent advances in technology, the most promising results stem from recent GWAS, which have helped to identify new variants in the genetics of AUD. Among the variants identified, the most significant SNPs remain in the alcohol metabolism enzyme genes, *ADH* and *ALDH*. Importantly, the prevalence of the various isoforms of ADH and ALDH differs among ethnicities and populations. Therefore, lower alcohol consumption in certain populations, as a result of the protective effect of alcohol metabolism SNPs, may be due to gene-environment interactions.

AUD prevention could be enhanced with a growing knowledge of the disorder's neurobiology and genetics. A growing body of literature on AUD genetics will improve both the understanding of at-risk individuals' biology and the development of new medications. Although information such as family history can currently be used to identify at-risk individuals, understanding the genetic architecture of AUD could enable us to pinpoint these individuals with greater certainty. Understanding of the genetic risk factors involved could be important to guide personalized treatments of patients who have already developed AUD and to inform the development of new pharmacological and other novel interventions.

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CONFLICT OF INTEREST STATEMENT

None declared.

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