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Genome-wide association study for maximum number of alcohol drinks in European Americans and African Americans

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Abstract

Background—We conducted a genome-wide association study (GWAS) for *maximum number of alcoholic drinks consumed in a 24-hour period* (“MaxDrinks”), in two independent samples comprised of over 9,500 subjects, following up on our GWAS for alcohol dependence (AD) in European Americans (EAs) and African Americans (AAs).

Methods—The samples included our GWAS samples (Yale-UPenn) recruited for studies of the genetics of drug or alcohol dependence, and a public available sample: the Study of Addiction: Genetics and Environment (SAGE). Genome-wide association analysis was performed for ~890,000 single nucleotide polymorphisms (SNPs) using linear association random effects models. European Americans and African Americans were separately analyzed.

Results—The results confirmed significant associations of the well-known functional loci at *ADH1B* with MaxDrinks in EAs (rs1229984 *Arg48His* $p=5.96 \times 10^{-15}$) and AAs (rs2066207 *Arg370Cys*, $p=2.50 \times 10^{-10}$). The region of significant association on chromosome 4 was extended to *LOC100507053* in AAs but not EAs. We also identified potentially novel significant common SNPs for MaxDrinks in EAs in the Yale-UPenn sample: rs1799876 at *SERPINC1* on chromosome 1 (4.00×10^{-8}) and rs2309169 close to *ANKRD36* on chromosome 2 ($p=5.58 \times 10^{-9}$). After adjusting for the peak SNP rs1229984 on *ADH1B*, rs1799876 was nearly significant ($p=1.99 \times 10^{-7}$) and rs2309169 remained highly significant (2.12×10^{-9}).

Conclusions—The results provide further support that *ADH1B* modulates alcohol consumption. Future replications of potential novel loci are warranted. This is the largest MaxDrinks GWAS to date, the first in AAs.

Keywords

Alcohol maximum drinks; genome-wide association; African American; European American

Introduction

Alcohol consumption is a moderately heritable phenotype, with heritability estimated at approximately 0.5 (Agrawal et al., 2009). Quantitative measures of the heaviness of alcohol consumption have greater power for gene discovery than dichotomous measures of alcohol phenotypes, such as alcohol dependence (AD), as they better represent the underlying continuum of alcohol-related problems. The maximum number of alcoholic drinks consumed in a 24-hour period (MaxDrinks) is a quantitative measure that can be considered an intermediate phenotype or a proxy for AD for gene detection; Diagnostic and Statistical Manual of Mental Disorders (DSM) criterion count is another such measure (Agrawal et al., 2012, Wang et al., 2013, Gelernter et al., 2014, Kendler et al., 2010, Quillen et al., 2014). Although MaxDrinks is highly correlated with alcohol dependence, MaxDrinks is directly associated with alcohol metabolism and physiopathology, which AD is more heterogeneous diagnosis including social functions and legal problems. Genes identified for MaxDrinks may more likely be biologically relevant to alcohol consumption.

Previous linkage studies have located genetic variants associated with MaxDrinks on chromosomes 2, 4, and 7 in European Americans (EAs)(Saccone et al., 2000, Saccone et al., 2005), and chromosomes 12 and 18 in a study of Irish affected sibpairs (Kuo et al., 2006). Consistent with gene-based association studies for AD, the strongest and most consistent signals for MaxDrinks in European-ancestry populations are located on *ADH1B*, a gene that encodes an alcohol dehydrogenase, a key enzyme in alcohol metabolism (Macgregor et al., 2009, Bierut et al., 2010). A functional single nucleotide polymorphism (SNP) at *ADH1B*, rs1229984 (A>G *Arg48His*), has been associated with MaxDrinks in Asian, Israeli, Australian, and European American populations (Bierut et al., 2012, Macgregor et al., 2009, Meyers et al., 2013), with the *His48* allele conferring a protective effect against heavy consumption (i.e., lower MaxDrinks). This variant is uncommon in individuals of African descent and has not been found to be associated with MaxDrinks in AA samples; however, another functional variant rs2066207 with similar functional effects associated with AD diagnostic traits in AA samples (Edenberg et al., 2006). Gene-based studies have reported an association between rs671 at *ALDH2* and reduced MaxDrinks in Asian populations, but not in EAs or AAs, where this variant is uninformative. Our recent GWAS in a relatively isolated Chinese population confirmed this locus on *ALDH2* for MaxDrinks that explained 22.9% of the phenotypic variation in our sample (Quillen et al., 2014).

Although several GWAS, including our recent study in EAs and AAs, identified risk loci for AD (Park et al., 2013, Gelernter et al., 2014, Treutlein et al., 2009, Schumann et al., 2011), GWAS for the “MaxDrinks” trait have not yielded robust associations in EAs or AAs

(Kapoor et al., 2013, Pan et al., 2013, Heath et al., 2011). A nominally significant association (rs9512637 on chromosome 13, $p=1.2 \times 10^{-7}$) for a heaviness of alcohol drinking factor score was found in a large Australian twin population (Heath et al., 2011), in which the SNP density was relatively low. Several recent meta-analyses from large consortium samples (the Collaborative Study on the Genetics of Alcoholism (COGA) and the Study of Addiction: Genes and Environment (SAGE) revealed no genome-wide significant markers for this phenotype (Kapoor et al., 2013, Pan et al., 2013). However, multiple loci approached significance, including SNPs at *LMO1*, *PLCLI*, *KCNB2*, and *DDC* (Kapoor et al., 2013, Pan et al., 2013). One study identified a locus on *C12orf51* associated with alcohol consumption in Korean men at an extremely high level of significance ($p=5.8 \times 10^{-46}$) (Baik et al., 2011). Interestingly, the majority of these GWAS have not replicated the association of the *ADH1B* gene with MaxDrinks that was previously reported from several studies (Bierut et al., 2012, Macgregor et al., 2009) except for one (2.04×10^{-8}) in the combination of COGA and SAGE samples (Kapoor et al., 2013). Thus, in order to identify genetic influences on MaxDrinks and to establish the role of *ADH1B* on Maxdrinks, additional GWAS in large sample populations with high-density genotyping arrays in EAs and AAs are warranted.

Here we report a GWAS with MaxDrinks in our EA and AA samples and the publically available (via dbGAP application) SAGE dataset. We combined sample sets through meta-analysis, yielding a total sample of more than 9,500 subjects. This is the largest GWAS for MaxDrinks to date and is particularly noteworthy for its large AA representation.

Materials and Methods

Subject Characteristics

The study samples included our GWAS samples (Yale-UPenn) (N=5,543) and the public samples (SAGE: phs000092.v1.p1, N=4,012). Sample characteristics are shown in Table 1. The Yale-UPenn sample was recruited for studies of the genetics of drug (opioid or cocaine) or alcohol dependence. The SAGE sample consists of the Collaborative Study on the Genetics of Alcoholism (COGA) (Edenberg and Foroud, 2006, Edenberg, 2002), the Family Study of Cocaine Dependence (FSCD) (Grucza et al., 2008), and the Collaborative Genetic Study of Nicotine Dependence (COGEND) (Bierut, 2007) samples. We obtained the SAGE data via dbGAP.

The Yale-UPenn sample—Detailed descriptions of recruitment and assessment procedures from this multi-site study can be found in previous publications (Gelernter et al., 2014, Gelernter et al., 2013). The sample consisted of small nuclear families originally collected for linkage studies and unrelated individuals. Briefly, subjects were interviewed using the Semi-Structured Assessment for Drug Dependence and Alcoholism (SSADDA) to assess symptoms of drug use and other major psychiatric disorders (Pierucci-Lagha et al., 2005, Gelernter et al., 2005). MaxDrinks was assessed with the question “In your lifetime, what is the largest number of drinks you have ever had in a 24-hour period, including all types of alcohol?” The number of drinks was coded as standard drinks. We used log-transformed MaxDrinks (LogM) values to represent MaxDrinks in the analysis models. A total of 2,328 EA and 3,215 AA subjects had MaxDrinks data. The mean of LogM was

2.95 $\pm$ 0.84 in the EA sample, and 2.57 $\pm$ 1.01 in the AA samples. The LogM in AAs was significantly lower than in EAs ($p < 0.0001$). The distributions of LogM for EA and AA samples are shown in supplemental Figure 1.

The SAGE sample—The Study of Addiction: Genetics and Environment (SAGE) is funded as part of the Gene Environment Association Studies (GENEVA) initiative supported by the National Human Genome Research Institute (http://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs000092.v1.p1). A total of 2,736 EA and 1,276 AA unrelated individuals were included for this study. Some cocaine users were recruited for SAGE and the sample informative for alcohol MaxDrinks was also partially comorbid with cocaine use. SAGE also included the individuals with Fagerstrom Test for Nicotine Dependence greater than 3. Alcohol and nicotine dependence are common in population. We did not exclude subjects with nicotine dependence. The number of MaxDrinks was assessed by inquiring as to the number of standard drinks consumed within a 24-hour period. Consistent with the MaxDrinks variable from Yale-UPenn, the MaxDrinks reported in the SAGE data and transformed to LogM for genetic analysis. A detailed description of this sample was reported previously (Gelernter et al., 2014, Wang et al., 2013).

Genotyping and Quality control

The Yale-UPenn samples were genotyped using the Illumina HumanOmni-Quad v1.0 microarray containing 988,306 autosomal SNPs, at the Center for Inherited Disease Research (CIDR) and the Yale Center for Genome Analysis. Genotypes were called using Illumina GenomeStudio software V2011.1 and genotyping module V 1.8.4. (Illumina, San Diego, CA, USA). The SAGE samples were genotyped on the Illumina Human 1M array containing 1,069,796 total SNPs. We used the following criteria for genotyping quality control filtering: 1) call rate $>98\%$; 2) MAF $> 1\%$; and p value for HWE $> 5 \times 10^{-6}$. After data cleaning, 44,644 SNPs on the microarray and 135 individuals with call rates $< 98\%$ were excluded; 62,076 additional SNPs were removed due to minor allele frequencies (MAF) $< 1\%$. After data cleaning and quality control, 5,543 individuals and 889,659 SNPs remained for imputation. After applying the same QC procedures to the SAGE sample, 39 subjects with call rates $< 98\%$ were excluded. Thus, in the SAGE sample, 4,012 individuals and 726,191 SNPs remained for analysis.

In addition, we examined the genetic relationships in the family-based samples for the Yale-UPenn samples by estimating pairwise identity by descent (IBD) proportion using PLINK v1.90a. Individuals who shared more than 25% of their alleles IBD were assigned to the same family.

Genotyping imputation—We used the 1000 Genomes dataset (<http://www.1000genomes.org/>) as a reference to impute genotyping for our Yale-UPenn and SAGE samples. The program IMPUTE2 was applied for genotyping imputation. EA and AA populations were imputed separately within each dataset.

Statistical analysis

Population stratification—We conducted principal components analysis to address population structure in the Yale-UPenn samples and SAGE samples using the program Eigensoft (Price et al., 2006, Patterson et al., 2006). SNPs were pruned for linkage disequilibrium (LD) estimated by $r^2 > 0.8$. The first PC score distinguished AAs and EAs. Thus, we analyzed AAs and EAs separately to minimize population admixture. Within each population, the top three PCs were then used in all analyses to correct for residual population stratification.

Association analysis—A linear association model was applied for association analyses between SNPs genome-wide for the log-transformed MaxDrinks. In this analysis, MaxDrinks was the dependent variable and each SNP was a predictor. Each model was adjusted for age, sex and the first three ancestry PCs. Consider body weight may influence alcohol distribution and consumption, we conducted a separate analysis with body mass index (BMI) as an additional covariate. The p-value for genome-wide significance was set at 5.0×10^{-8} . To further limit the confounding factors, we tested models for moderately associated SNPs ($p < 1 \times 10^{-4}$) for dependence on cocaine, opioids, and alcohol, three major comorbid substance use in our samples

Meta-analysis of the Yale-UPenn and SAGE samples—We used the program METAL to meta-analyze the combined results of the Yale-UPenn and SAGE samples (Willer et al., 2010). Inverse variance was applied to the analysis.

Conditional association analysis—To identify additional association signals after accounting for the known loci, we ran conditional analyses, in which we included the significant allele counts at the conditioning loci as covariates to calculate conditional p values for EAs and AAs. We adjusted for the effect of rs1229984 in the EA samples and for rs2066207 in the AA samples, the two peak significant SNPs in those populations.

Results

Genome-wide association

We identified multiple significant loci on chromosome 4 for MaxDrinks in both EA and AA populations. Within a 236 kb region on chromosome 4, one SNP was significantly associated with MaxDrinks in EAs, and eight SNPs were significantly associated in AAs (Table 2). Three of the nine SNPs were uniquely associated with MaxDrinks; the other six were also strongly associated with AD in our previous study (Gelernter et al., 2014). In the present study, the significant signals were mostly driven by the Yale-UPenn sample.

The peak GWAS hits were from the *ADH1B* locus. As expected, the functional SNP rs1229984 (A>G *Arg48His*) showed the strongest association with MaxDrinks in the EAs from combined Yale-UPenn and SAGE samples (minor allele frequency, MAF=0.06, $p=5.96 \times 10^{-15}$, $\beta=-0.26$) (Table 2, Figures 1A, 1B). This SNP remained highly significant after adjusting for BMI ($p=4.27 \times 10^{-12}$). Individuals with *His48* allele reported less number of maximum drinks within 24 hours than homozygote *Arg48* individuals (Figure 4A). This

SNP is minimally informative in AA samples. However, another functional SNP in *ADH1B*, rs2066702, was strongly associated with MaxDrinks in the AAs from the combination of Yale-UPenn and SAGE samples (MAF=0.19, $p=2.5 \times 10^{-10}$, $\beta=-0.16$, $p=1.89 \times 10^{-8}$ adjusting for BMI) (Figures 2A, 2B). Individuals carrying Cys allele reported less number of maximum drinks (Figure 4B). Two other SNPs in *ADH1B*, one synonymous SNP, rs1789882 (*Ile12520Ile*) (MAF=0.23, $p=2.1 \times 10^{-9}$, $\beta=-0.14$), and one intronic SNP, rs1693457 (MAF=0.23; $p=4.2 \times 10^{-9}$, $\beta=-0.14$), were significant in AAs but were monomorphic in EAs. All four SNPs in *ADH1B* were previously shown to be significantly associated with AD. These findings confirm the contribution of *ADH1B* to the development of alcohol-related pathophysiological phenotypes. Our prior GWAS used AD criteria and diagnosis as traits, however, MaxDrinks is not among the AD diagnostic criteria. Regional Manhattan plots on chromosome 4 close to AHD cluster are showed in Fig 3A for EAs and in Fig 3B for AAs.

Consistent with our previous findings for AD on chromosome 4, the region that was associated with MaxDrinks also extended to the locus *LOC100507053*. Five SNPs at *LOC100507053*, which is in the same genomic region as the *ADH* cluster, were significant only in the AA sample (Figure 3B). This uncharacterized RNA gene was previously associated with AD symptom count (Gelernter et al., 2014). Three of these five significant SNPs were associated in the present study but were not observed in our previous AD GWAS. We also previously found that 11 SNPs in *ADH1C* were associated with AD symptom count (Gelernter et al., 2014). None of those SNPs were associated with MaxDrinks, though there was one marginally significant SNP in the AA population in the combined Yale-UPenn and SAGE samples (rs34009511, $p=8.1 \times 10^{-8}$).

In addition to the genomic region on chromosome 4q, we identified two significant loci for EAs. In the EA samples, rs1799865 on chromosome 1 and rs2309169 on chromosome 2 showed significant association (rs1799865: MAF=0.38, $p=4.00 \times 10^{-8}$, $\beta=-0.16$; rs2309169, $p=5.58 \times 10^{-9}$, $\beta=-0.28$) (Supplemental figure 2A and 2B). Rs1799865 maps to *SERPINC1*, which encodes a plasma protease inhibitor. Rs2309169 is located 10kb downstream to *ANKRD36*. In AAs, a SNP rs1040883 *SMPD1* on chromosome 11 showed significant association with MaxDrinks, which was mostly driven by the SAGE sample ($p=1.10 \times 10^{-8}$). However, this locus was not significantly associated in either our AA samples nor in the meta-analysis in the combination samples, and the effects of the SNP were opposite direction in the SAGE sample and the combined sample, suggesting a potential spurious significant SNP.

Conditional analysis

To evaluate whether identified SNPs contribute independently to MaxDrinks, we repeated the analyses, conditioning on the most significant *ADH1B* SNPs in each population: rs1229984 in EAs and rs2066702 in AAs. In EAs, after adjusting for rs1229984, rs2309169 on chromosome 2 remained highly significant ($p=2.12 \times 10^{-9}$) and rs1799876 on chromosome 1 was nearly significant ($p=1.99 \times 10^{-7}$). In AAs, after adjusting for rs2066702, among the eight SNPs identified as significant in the combined Yale-UPenn and the SAGE samples, only one SNP remained significant. Rs200475889 on chromosome 4 showed

significant association in the Yale-UPenn AAs after adjusting for rs2066702 ($p=4.80 \times 10^{-8}$), suggesting that both of these SNPs contributed to genetic risk for MaxDrinks in this population. Thus, independent loci other than *ADH1B* contributed to Maxdrinks in both AAs and EAs.

Discussion

We identified multiple significant loci for peak daily alcohol consumption (MaxDrinks) in a large sample of EAs and AAs, thus expanding the existing literature in several ways. First, the association of the functional *ADH1B* SNP rs1229984 with MaxDrinks in EAs is consistent with previous observations. This finding provides additional support for a role of that locus in determining alcohol consumption, as well as increasing confidence in our findings implicating other loci relevant to drinking behavior. Second, this is the first report of a significant association of rs2066702 at *ADH1B* with MaxDrinks in an AA population – this finding is not unexpected, as it is similar to the finding in EAs. Third, additional protective loci, i.e., alleles mapped to genes not previously associated with either MaxDrinks or AD, were identified in both EA and AA populations. It is also noteworthy that the significant genes or alleles associated with MaxDrinks were population-specific (in all cases except *ADH1B*), suggesting that different genes and perhaps different biological pathways may be involved in this trait in different populations. This pattern of findings also reinforces the importance of stratifying populations for gene discovery when using a GWAS approach.

The *ADH1B* gene has been previously associated with MaxDrinks and related alcohol phenotypes in EA populations using linkage and candidate gene approaches. A genome scan of MaxDrinks identified significant loci in the 4q21.3 region (the maximal LOD=3.5), a region to which the *ADH* gene cluster maps. Drinkers with the *ADH1B* rs1229984**Arg48* allele were less likely to have a flushing response to alcohol (Bierut et al., 2012, Chen et al., 1999), report a higher Maxdrinks count (Bierut et al., 2012, Macgregor et al., 2009), and greater past year alcohol consumption (Macgregor et al., 2009, Edenberg, 2007) than those with the rs1229984**His48* allele. Consistent with earlier studies (Bierut et al., 2012), the genome-wide significance of rs1229984 in the current study was 10^{-15} in EAs, reflecting a relatively large effect size ($\beta = -0.25$). The individuals with *His48* allele reported less Maxdrinks in EA population from our Yale-UPenn samples (Fig 4). Rs2066702, also at *ADH1B*, has not previously been associated with MaxDrinks, likely because of its low frequency in EAs and Asians, who are most heavily represented in studies of *ADH1B* variants and alcohol phenotypes. This SNP has a higher minor allele frequency in populations of African descent (0.19 in the present study). The p-value was 10^{-10} in the AA population in the combined Yale-UPenn and SAGE samples. Rs2066702 (*Arg370Cys*) is located in exon 9 and encodes the $\beta 3$ subunit of *ADH1B*. The substitution of the cysteine residue, which results in more rapid alcohol metabolism than the common arginine residue, was associated with lower MaxDrinks. This finding is consistent with the substitution causing higher levels of acetaldehyde, which are aversive. In addition to the two known functional SNPs in *ADH1B*, rs20047889 was also associated with Maxdrinks, independent of the effect of rs2066702 in the AA samples. These findings, along with our previous findings of significant association of *ALDH2* with MaxDrinks in a Chinese population

(Quillen et al., 2014), provide additional strong evidence of a convergent biological pathway that involves alcohol metabolism in different populations.

Beyond the *ADH* cluster, we identified two potential novel loci for MaxDrinks in the EA population. Rs1799876 at *SERPINC1* on chromosome 1 was significantly associated with Maxdrinks in our Yale-Upenn EA sample ($p=4.0 \times 10^{-8}$). It became nearly significant after adjusting for rs1229984 ($p=1.99 \times 10^{-7}$). *SERPINC1*, serpin peptidase inhibitor, clade C gene has been associated with thrombosis-related phenotypes (Fischer et al., 2013). Rs2309169 on chromosome 2 was significantly associated with Maxdrinks in the Yale-Upenn EA samples ($p=5.6 \times 10^{-9}$). It remained highly significant after adjusting rs1229984 ($p=2.1 \times 10^{-9}$). This SNP is close to Ankyrin Repeat Domain 36 (*ANKRD36*). The link of *ANKRD36* with Maxdrinks is still unknown.

We previously found a significant association of *ALDH2* with MaxDrinks in a Chinese population (Quillen et al., 2014). No such relationship was observed in either population in the present study, which is not surprising inasmuch as the allele that is protective in some Asian populations is not observed in either AAs or EAs.

Two other GWAS of alcohol consumption phenotypes have been reported in European-ancestry populations. Heath et al (2011) conducted a GWAS analysis with a well characterized quantitative phenotype for heaviness of alcohol consumption that included MaxDrinks in the Australian Twin Registry sample (a total of 8,300 subjects), and failed to identify a genome wide significant association (Heath et al., 2011). Our success in identifying potential novel loci for MaxDrinks is likely due to a higher density genotyping array and a greater proportion of affected subjects in the present study. Approximately 890,000 SNPs were genotyped in our samples compared to only 30K SNPs in Heath et al.'s study. More recently, Pan et al (2013) reported several SNPs at *DDC* that were associated with MaxDrinks in the SAGE sample ($p=2.87 \times 10^{-5}$) (Pan et al., 2013). They also reported an association of rs1128951 on chromosome 3 with MaxDrinks in the combined SAGE and COGA samples ($p=4.3 \times 10^{-8}$). It is noteworthy that the p value in that study did not reach the conventional value of 5×10^{-8} . We did not replicate either finding. The SAGE sample is composed of both EA and AA populations and their study did not address population stratification, which makes it difficult to interpret these findings. As discussed above, risk loci for MaxDrinks may differ across populations. Thus, population admixture may contribute to inconsistent findings.

The present loci identified via GWAS for MaxDrinks overlap partially with loci identified in our previous GWAS for AD, but independent loci were also observed in each study. These findings suggest that shared genetic influences on MaxDrinks and AD phenotypes are attributable in large part to alcohol metabolism genes. These results are consistent with prior genetic epidemiologic findings from a twin study of alcohol consumption phenotypes, where a high degree of genetic overlap between the heaviness of consumption and AD symptoms was observed (Grant et al., 2009). In our Yale-UPenn sample, significant correlation of Maxdrinks and AD symptoms were also observed in both EAs and AAs (Sartor et al., 2014). Numerous genes that were reported genome wide significant in GWAS for AD (Gelernter et al., 2014), including *PDLIM5* in EAs, *METAP* in AAs, *MTIF2* and *CCDC88A* in both EAs

and AAs, were not associated with MaxDrinks in the current study. Similarly, several of the genes associated with MaxDrinks in this study have not been reported previously to be associated with AD, suggesting specific genetic loci contribution to Maxdrinks.

Despite the strengths of the study, certain limitations should be noted. Some of our Yale-UPenn sample was recruited for studies of drug dependence rather than alcohol dependence, while the SAGE sample was primarily recruited for alcohol dependence. Although we adjusted for these comorbidities, greater substance dependence comorbidity in the Yale-UPenn sample may have contributed to the differences in findings with respect to the SAGE sample. We note that many prior studies have not taken note of substance use comorbidity at all, and the assumption that there is less comorbidity in those cases where it is not reported, is not necessarily warranted. We also acknowledged the proportion of sex and races are slightly different between two datasets. In the meta-analysis, we analyzed associations separately in EAs and AAs, and adjusted sex and other demographics.

Nevertheless, this is among the first study to apply GWAS in a large independent sample for MaxDrinks that included both EA and AA populations. The replication of findings related to the *ADH1B* validates the analytic strategies. Efforts to replicate the newly identified loci in independent samples are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Manhattan plot and QQ plot in European Americans (EA) in the Yale-UPenn sample. The peak SNP was rs1229984 (ADH1B) on chromosome 4 ($p=5.96 \times 10^{-15}$). Two novel loci were rs1799876 on chromosome 1 ($p=4.00 \times 10^{-8}$) and rs2309169 on chromosome 2 (5.58×10^{-9}). After adjusting for rs122984 on ADH1B, rs2309169 remained significant ($p=2.1 \times 10^{-9}$), but rs1799876 was nearly significant ($p=1.99 \times 10^{-7}$).

Figure 2.

Manhattan plot and QQ plot in African Americans (AAs) in the combination of the Yale-UPenn samples and the SAGE samples. Eight SNPs on chromosome 4 reached genome-wide significance. The peak SNP was rs2066702 (ADH1B) on chromosome 4 ($p=2.50 \times 10^{-10}$).

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Figure 3.

Chromosome 4 regional Manhattan plots in the combination of the Yale-UPenn samples and the SAGE samples. Left: European Americans: The peak significant SNP was rs1229984 on ADH1B ($p=5.96 \times 10^{-15}$) Right: African Americans: The peak significant SNP was rs2066702 on ADH1B ($p=2.50 \times 10^{-10}$)

Figure 4. The distribution of number of maximal drinks within 24 hours by two-peak significant SNPs. Left: rs1229984 in European Americans; Right rs2066702 in African Americans. The number of drinks was binned by five drinks. The number of drinks greater than 45 was combined into one group.

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Table 1

Sample characteristics

	EA		AA		Total	
	M	F	M	F	M	F
Yale-UPenn	1374	954	2328	1683	1532	3215
					5543	
SAGE	1213	1523	2736	625	651	1276
					4012	
Total	2587	2477	5064	2308	2183	4491
					9555	

Note:

EA: European American

AA: African American

SAGE: the Study of Addiction: Genetics and Environment

Table 2
Summary of significant results associated with Maxdrinks in European Americans and African Americans

European Americans													
Chr	BP	a1	a2	SNP	SNP Type	Gene	Yale-UPenn p	Yale-Upenn Beta	SAGE p	SAGE Beta	Meta p	Meta beta	MAF
1	173878471	G	A	rs1799876	INT	SERPINC1	4.00E-08	-0.161	2.62E-02	-0.0522	1.32E-07	-0.08	0.38
2	98239537	A	C	rs2309169	a	a	5.58E-09	-0.276	a	a	a	a	0.1
4	100025015	T	C	rs28470942	INT	LOC100507053	a	a	a	a	a	a	a
4	100163386	CT	C	rs33948359	INT	LOC100507053	a	a	a	a	a	a	a
4	100177417	C	A	rs28600890	INT	LOC100507053	a	a	a	a	a	a	a
4	100190805	T	C	rs28864441	INT	LOC100507053	a	a	a	a	a	a	a
4	100214164	A	G	rs904092	INT	LOC100507053	9.29E-01	-0.00306	2.26E-02	0.0674	2.67E-01	0.02	0.17
4	100229017	A	G	rs2066702	NSM,REF	ADH1B	.	a	a	a	a	a	a
4	100235053	A	G	rs1789882	REF,SYN	ADH1B	9.97E-01	-0.00014	2.68E-02	0.0652	2.42E-01	0.02	0.17
4	100236762	C	T	rs1693457	INT	ADH1B	9.37E-01	-0.00268	2.19E-02	0.067	2.55E-01	0.02	0.18
4	100239319	A	G	rs1229984	NSM,REF	ADH1B	1.09E-09	-0.335	1.82E-04	-0.222	5.96E-15	-0.26	0.06
4	100261524	C	T	rs34009511	INT	ADH1C	a	a	a	a	a	a	a
4	177782899	A	AAAAC	rs200475889	a	a	a	a	a	a	a	a	a

African Americans													
Chr	BP	a1	a2	SNP	SNP Type	Gene	Yale-Upenn p	Yale-Upenn Beta	SAGE p	SAGE Beta	Meta p	Meta beta	MAF
1	173878471	G	A	rs1799876	INT	SERPINC1	6.02E-01	-0.0162	2.31E-01	0.06	6.27E-01	-0.01	
2	98239537	A	C	rs2309169	a	a	a	a	a	a	a	a	a
4	100025015	T	C	rs28470942	INT	LOC100507053	7.67E-06	-0.142	1.67E-03	-0.19	4.23E-08	-0.15	0.18
4	100163386	CT	C	rs33948359	INT	LOC100507053	2.55E-06	-0.145	1.25E-03	-0.19	1.04E-08	-0.15	0.18
4	100177417	C	A	rs28600890	INT	LOC100507053	1.71E-06	-0.147	9.03E-04	-0.19	5.22E-09	-0.16	0.19
4	100190805	T	C	rs28864441	INT	LOC100507053	1.24E-06	-0.149	9.03E-04	-0.19	3.68E-09	-0.16	0.18
4	100214164	A	G	rs904092	INT	LOC100507053	1.85E-05	-0.122	8.75E-03	-0.14	1.45E-08	-0.13	0.24
4	100229017	A	G	rs2066702	NSM,REF	ADH1B	4.93E-07	-0.154	9.82E-04	-0.19	2.50E-10	-0.16	0.19
4	100235053	A	G	rs1789882	REF,SYN	ADH1B	5.06E-06	-0.13	5.33E-03	-0.15	2.10E-09	-0.14	0.23
4	100236762	C	T	rs1693457	INT	ADH1B	7.05E-06	-0.128	8.41E-03	-0.15	4.24E-09	-0.14	0.23

African Americans											
Chr	BP	a1	a2	SNP	SNP Type	Gene	Yale-Upenn p	Yale-Upenn Beta	SAGE p	SAGE Beta	Meta p
4	100239319	A	G	rs1229984	NSM,REF	ADH1B	a	a	a	a	a
4	100261524	C	T	rs34009511	INT	ADH1C	1.38E-06	-0.21	2.43E-02	-0.18	8.11E-08
4	177782899	A	AAAAC	rs200475889	a	a	5.32E-08	-0.235	a	a	a

All											
Chr	BP	a1	a2	SNP	SNP Type	Gene	Meta p	Meta beta			
1	173878471	G	A	rs1799876	INT	SERPINC1	3.17E-06	-0.06			
2	98239537	A	C	rs2309169	a	a	a	a			
4	100025015	T	C	rs28470942	INT	LOC100507053	4.23E-08	-0.15			
4	100163386	CT	C	rs33948359	INT	LOC100507053	1.04E-08	-0.15			
4	100177417	C	A	rs28600890	INT	LOC100507053	5.22E-09	-0.16			
4	100190805	T	C	rs28864441	INT	LOC100507053	3.68E-09	-0.159			
4	100214164	A	G	rs904092	INT	LOC100507053	5.02E-03	-0.042			
4	100229017	A	G	rs2066702	NSM,REF	ADH1B	2.50E-10	-0.16			
4	100235053	A	G	rs1789882	REF,SYN	ADH1B	3.25E-03	-0.04			
4	100236762	C	T	rs1693457	INT	ADH1B	4.07E-03	-0.04			
4	100239319	A	G	rs1229984	NSM,REF	ADH1B	5.96E-15	-0.26			
4	100261524	C	T	rs34009511	INT	ADH1C	8.11E-08	-0.2			
4	177782899	A	AAAAC	rs200475889	a	a	a	a			

Abbreviations: Chr: chromosome; BP: base pair; SNP: single nucleotide polymorphism; NA: other; NSM: non-synonymous; SYN: synonymous; INT: intronic; Beta: beta coefficient for Maxdrinks; MAF: Minor allele frequency

^aNot tested because of low minor allele frequency or poor imputation quality\Not tested because

Note: highlighted as red SNPs were identified associations for alcohol dependence in our previous study