Genomewide Association Study for Maximum Number of Alcoholic Drinks in European Americans and African Americans

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Background: We conducted a genomewide association study (GWAS) for *maximum number of alcoholic drinks consumed in a 24-hour period* ("MaxDrinks"), in 2 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 AD, and a publicly available sample: the Study of Addiction: Genetics and Environment (SAGE). Genomewide association analysis was performed for ~890,000 single nucleotide polymorphisms (SNPs) using linear association random effects models. EAs and AAs 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 (rs2066702 *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.

Key Words: Alcohol Maximum Drinks, Genomewide Association, African American, European American.

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; Gelernter et al., 2014; Kendler et al., 2010; Quillen et al., 2014; Wang et al., 2013). Although MaxDrinks is highly correlated with AD, MaxDrinks is directly associated with alcohol metabolism and physiopathology, which AD is a 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, 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

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key enzyme in alcohol metabolism (Bierut et al., 2010; Macgregor et al., 2009). A functional single nucleotide polymorphism (SNP) at *ADH1B*, rs1229984 (A>G *Arg48His*), has been associated with MaxDrinks in Asian, Israeli, Australian, and EA populations (Bierut et al., 2012; Macgregor et al., 2009; Meyers et al., 2015), 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 African American (AA) samples; however, rs2066702 is another functional variant 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 genomewide association study (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 (Gelernter et al., 2014a; Park et al., 2013; Schumann et al., 2011; Treutlein et al., 2009), GWAS for the "MaxDrinks" trait have not yielded robust associations in EAs or AAs (Heath et al., 2011; Kapoor et al., 2013; Pan et al., 2013). 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 genomewide significant markers for this phenotype (Kapoor et al., 2013; Pan et al., 2013). However, multiple loci approached significance, including SNPs at *LMO1, PLCL1, 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 publicly available (via dbGAP application) SAGE data set. 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 AD. The SAGE sample consists of the COGA (Edenberg, 2002; Edenberg and Foroud, 2006), the Family Study of Cocaine Dependence (Grucza et al., 2008), and the Collaborative Genetic Study of Nicotine Dependence (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., 2014a,b). 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 (Gelernter et al., 2005; Pierucci-Lagha 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 ± 0.84 in the EA sample and 2.57 ± 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 Fig. S1.

The SAGE Sample. SAGE is funded as part of the Gene Environment Association Studies initiative supported by the National Human Genome Research Institute (http://www.ncbi.nim.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 >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 were transformed to LogM for genetic analysis. A detailed description of this sample was reported previously (Gelernter et al., 2014a; Wang et al., 2013).

Table 1. Sample Characteristics

	EA			AA			
	M	F	Total	M	F	Total	Total
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

EA, European American; AA, African American; SAGE, Study of Addiction: Genetics and Environment.

Genotyping and Quality Control

The Yale-UPenn samples were genotyped using the Illumina HumanOmnil-Quad v1.0 microarray (Illumina, San Diego, CA) containing 988,306 autosomal SNPs, at the Center for Inherited Disease Research and the Yale Center for Genome Analysis. Genotypes were called using Illumina GenomeStudio software V2011.1 and genotyping module V 1.8.4. 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: (i) call rate >98%; (ii) minor allele frequency (MAF) >1%; and *p*-value for Hardy-Weinberg Equilibrium $>5 \times 10^{-6}$. 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 MAF <1%. After data cleaning and quality control, 5,543 individuals and 889,659 SNPs remained for the imputation. After applying the same quality control 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 (http://pngu.mgh.harvard.edu/~purcell/plink/). Individuals who shared more than 25% of their alleles IBD were assigned to the same family.

Genotyping Imputation. We used the 1000 Genomes data set (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 (https://mathgen.stats.ox.ac.uk/impute/impute_v2.html#home). EA and AA populations were imputed separately within each data set.

Statistical Analysis

Population Stratification. We conducted principal component (PC) analysis to address population structure in the Yale-UPenn samples and SAGE samples using the program Eigensoft (Patterson et al., 2006; Price et al., 2006). SNPs were pruned for linkage disequilibrium 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 3 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 genomewide for the LogM. In this analysis, MaxDrinks was the dependent variable and each SNP was a predictor. Each model was adjusted for age, sex, and the first 3 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 genomewide 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—3 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 rs2066702 in the AA samples—the 2-peak significant SNPs in those populations.

RESULTS

Genomewide Association

We identified multiple significant loci on chromosome 4 for MaxDrinks in both EA and AA populations (Figs 1–3). Within a 236-kb region on chromosome 4, 1 SNP was significantly associated with MaxDrinks in EAs, and 8 SNPs were significantly associated in AAs (Table 2). Three of the 9 SNPs were uniquely associated with MaxDrinks; the other 6 were also strongly associated with AD in our previous study (Gelernter et al., 2014a). In this 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 (MAF = 0.06, $p = 5.96 \times 10^{-15}$, $\beta = -0.26$) (Table 2, Fig. 1*A,B*). 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 (Fig. 4*A*). 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) (Fig. 2*A,B*). Individuals carrying Cys allele reported less number of maximum drinks (Fig. 4*B*). Two other SNPs in *ADH1B*, 1 synonymous SNP, rs1789882 (*Ile251Ile*) (MAF = 0.23, $p = 2.10 \times 10^{-9}$, $\beta = -0.14$), and 1 intronic SNP, rs1693457 (MAF = 0.23; $p = 4.24 \times 10^{-9}$, $\beta = -0.14$), were significant in AAs but were monomorphic in EAs. All 4 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 *ADH* cluster are showed in Fig. 3*A* for EAs and in Fig. 3*B* 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 (Fig. 3*B*). This uncharacterized RNA gene was previously associated with AD symptom count (Gelernter et al., 2014a). Three of these 5 significant SNPs were associated in this 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., 2014a). None of those SNPs were associated with MaxDrinks, although there was 1 marginally significant SNP in the AA population in the combined Yale-UPenn and SAGE samples (rs34009511, $p = 8.11 \times 10^{-8}$).

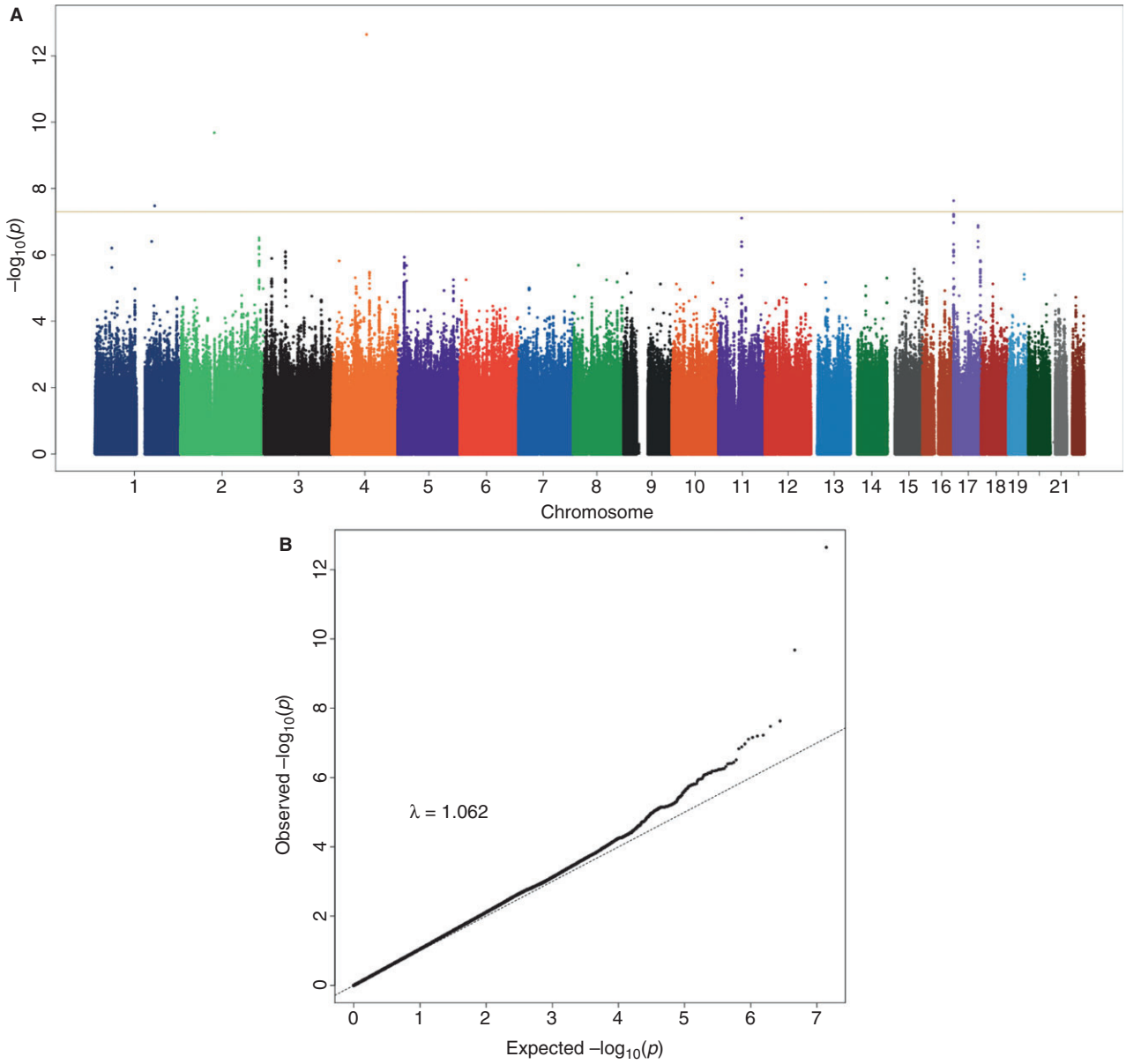

Fig. 1. Manhattan plot **(A)** and QQ plot **(B)** in European Americans (EAs) in the Yale-UPenn sample. The peak single nucleotide polymorphism (SNP) was rs1229984 (*ADH1B*) on chromosome 4 ($p = 5.96 \times 10^{-15}$). Two novel loci were rs1799876 on chromosome 1 ($p = 4.00 \times 10^{-8}$) and rs2309169 on chromosome 2 (5.58×10^{-9}). After adjusting for rs122984 on *ADH1B*, rs2309169 remained significant ($p = 2.12 \times 10^{-9}$), but rs1799876 was nearly significant ($p = 1.99 \times 10^{-7}$).

In addition to the genomic region on chromosome 4q, we identified 2 significant loci for EAs. In the EA samples, rs1799876 on chromosome 1 and rs2309169 on chromosome 2 showed significant association (rs1799876: MAF = 0.38, $p = 4.00 \times 10^{-8}$, $\beta = -0.16$; rs2309169, $p = 5.58 \times 10^{-9}$, $\beta = -0.28$) (Fig. S2*A*,*B*). Rs1799876 maps to *SERPINC1*, which encodes a plasma protease inhibitor. Rs2309169 is located 10 kb downstream to Ankyrin Repeat Domain 36 (*ANKRD36*). In AAs, a SNP rs1140833 *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 neither 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

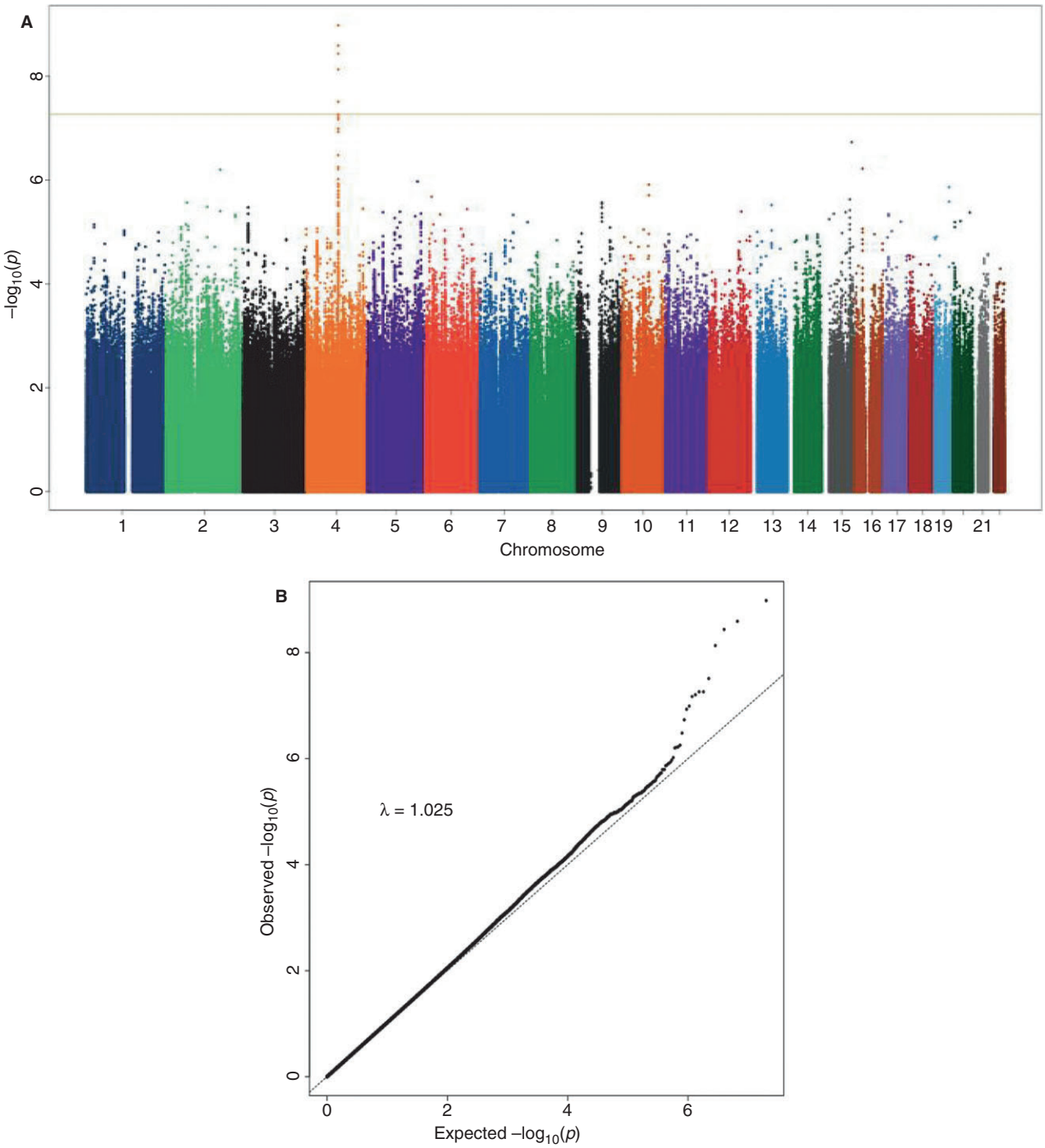

Fig. 2. Manhattan plot (**A**) and QQ plot (**B**) in African Americans (AAs) in the combination of the Yale-UPenn samples and the Study of Addiction: Genetics and Environment (SAGE) samples. Eight single nucleotide polymorphisms (SNPs) on chromosome 4 reached genomewide significance. The peak SNP was rs2066702 (*ADH1B*) on chromosome 4 ($p = 2.50 \times 10^{-10}$).

on chromosome 1 was nearly significant ($p = 1.99 \times 10^{-7}$). In AAs, after adjusting for rs2066702, among the 8 SNPs identified as significant in the combined Yale-UPenn and the SAGE samples, only 1 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.

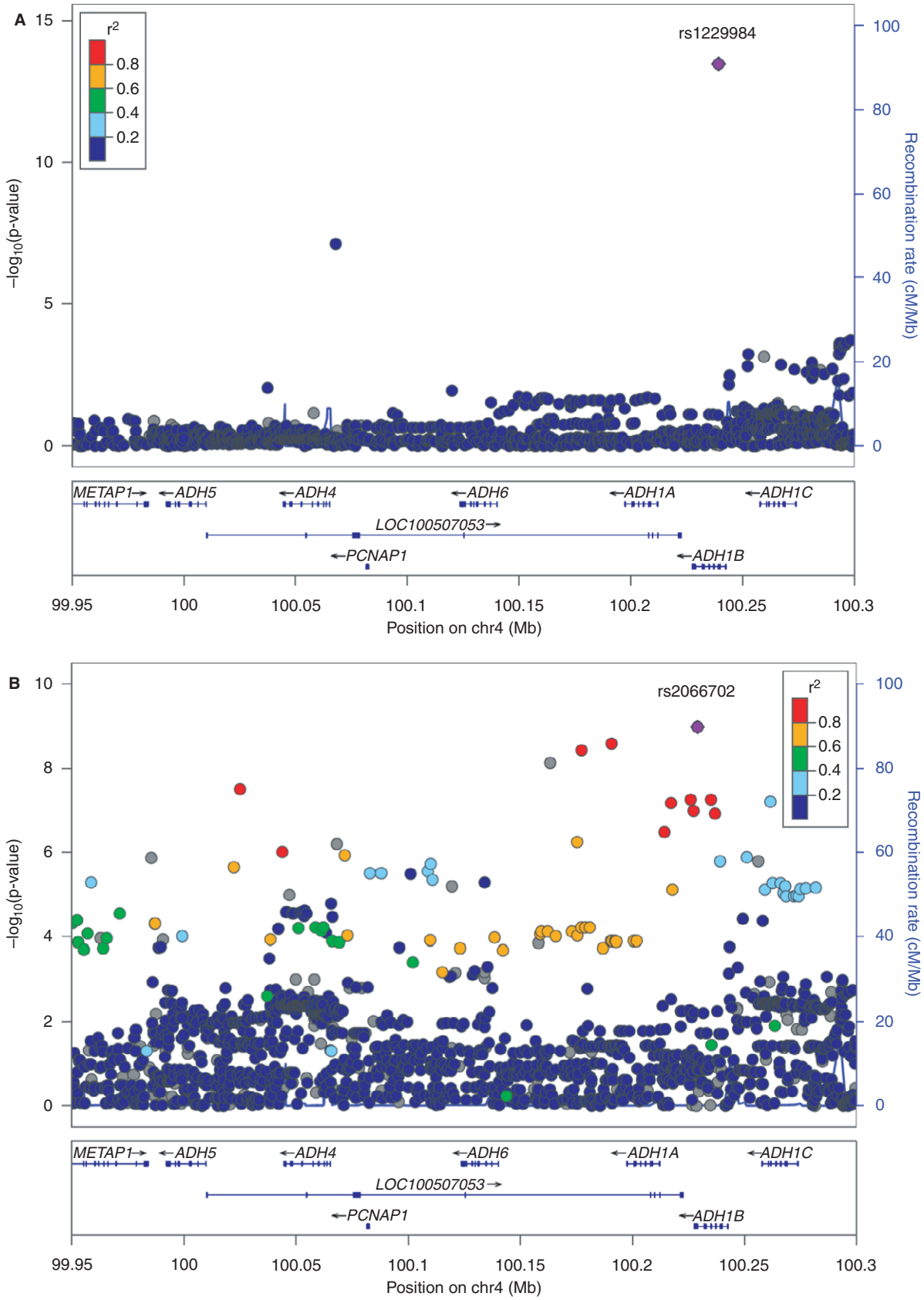

Fig. 3. Chromosome 4 regional Manhattan plots in the combination of the Yale-UPenn samples and the Study of Addiction: Genetics and Environment (SAGE) samples. (**A**) European Americans: The peak significant single nucleotide polymorphism (SNP) was rs1229984 on *ADH1B* ($p = 5.96 \times 10^{-15}$) (**B**) African Americans: The peak significant SNP was rs2066702 on *ADH1B* ($p = 2.50 \times 10^{-10}$)

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	0.10
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**	***ADH1B***	**a**	**a**	**a**	**a**	**a**	**a**	**a**
4	100235053	A	G	rs1789882	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**	***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**	***ADH1B***	4.93E-07	**−0.154**	9.82E-04	**−0.19**	**2.50E-10**	**−0.16**	0.19
4	100235053	A	G	rs1789882	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**
4	**100239319**	**A**	**G**	**rs1229984**	**NSM**	***ADH1B***	**a**	**a**	**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	−0.20	0.09
4	177782899	A	AAAAC	rs200475889	a	a	**5.32E-08**	−0.235	a	a	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**	***ADH1B***	**2.50E-10**	**−0.16**
4	100235053	A	G	rs1789882	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**	***ADH1B***	**5.96E-15**	**−0.26**
4	100261524	C	T	rs34009511	INT	*ADH1C*	8.11E-08	−0.20
4	177782899	A	AAAAC	rs200475889	a	a	a	a

Chr, chromosome; BP, base pair; SNP, single nucleotide polymorphism; NSM, non-synonymous; SYN, synonymous; INT, intronic; Beta, beta coefficient for MaxDrinks; MAF, minor allele frequency; SAGE, Study of Addiction: Genetics and Environment.

a: Not tested because of low MAF or poor imputation quality.

Highlighted as red SNPs were identified associations for alcohol dependence in our previous study (Gelernter et al., 2014a).

Bold numbers are genomewide significance.

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, that is, 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 logarithm of odds = 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 (Edenberg, 2007; Macgregor et al., 2009) 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 MAF 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 β3 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 2 known functional SNPs in *ADH1B*, rs200475889 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.

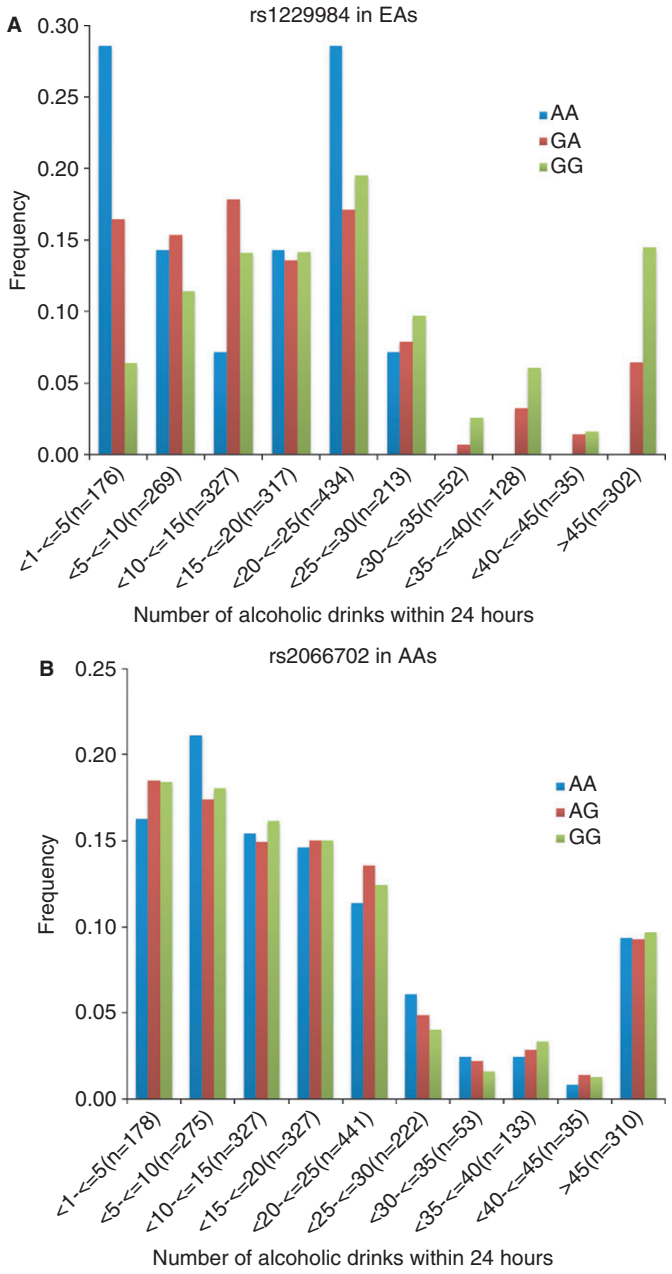

Fig. 4. The distribution of number of maximum drinks within 24 hours by 2-peak significant single nucleotide polymorphisms. (**A**) Rs1229984 in European Americans; (**B**) Rs2066702 in African Americans. The number of drinks was binned by 5 drinks. The number of drinks >45 was combined into 1 group.

Beyond the *ADH* cluster, we identified 2 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 ($p = 5.58 \times 10^{-9}$). It remained highly significant after adjusting rs1229984 ($p = 2.12 \times 10^{-9}$). This SNP is close to *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 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 and colleagues (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 genomewide 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 and colleagues' (2011) study. More recently, Pan and colleagues (2013) reported several SNPs at *DDC* that were associated with MaxDrinks in the SAGE sample ($p = 2.87 \times 10^{-5}$). They also reported an association of rs1128951 on chromosome 3 with MaxDrinks in the combined SAGE and COGA samples ($p = 4.3 \times 10^{-8}$). We did not replicate either finding. The SAGE sample is composed of both EA and AA populations and Pan and colleagues (2013) selected only EA samples in the study. Within each population, we adjusted 3 ancestry PCs to address residual population admixture and Pan and colleagues' study (2013) did not. As discussed above, risk loci for MaxDrinks may differ across populations. Thus, potential 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 genomewide significant in GWAS for AD (Gelernter et al., 2014a), including *PDL1M5* 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 AD, while the SAGE sample was primarily recruited for AD. 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 2 data sets. 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.

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REFERENCES

Agrawal A, Freedman ND, Cheng YC, Lin P, Shaffer JR, Sun Q, Taylor K, Yaspan B, Cole JW, Cornelis MC, DeSensi RS, Fitzpatrick A, Heiss G, Kang JH, O'Connell J, Bennett S, Bookman E, Bucholz KK, Caporaso N, Crout R, Dick DM, Edenberg HJ, Goate A, Hesselbrock V, Kittner S, Kramer J, Nurnberger JI Jr, Qi L, Rice JP, Schuckit M, van Dam RM, Boerwinkle E, Hu F, Levy S, Marazita M, Mitchell BD, Pasquale LR, Bierut LJ, Consortium G (2012) Measuring alcohol consumption for genomic meta-analyses of alcohol intake: opportunities and challenges. Am J Clin Nutr 95:539–547.

Agrawal A, Grant JD, Littlefield A, Waldron M, Pergadia ML, Lynskey MT, Madden PA, Todorov A, Trull T, Bucholz KK, Todd RD, Sher K, Heath AC (2009) Developing a quantitative measure of alcohol consumption for genomic studies on prospective cohorts. J Stud Alcohol Drugs 70:157–168.

Baik I, Cho NH, Kim SH, Han BG, Shin C (2011) Genome-wide association studies identify genetic loci related to alcohol consumption in Korean men. Am J Clin Nutr 93:809–816.

Bierut LJ (2007) Genetic variation that contributes to nicotine dependence. Pharmacogenomics 8:881–883.

Bierut LJ, Agrawal A, Bucholz KK, Doheny KF, Laurie C, Pugh E, Fisher S, Fox L, Howells W, Bertelsen S, Hinrichs AL, Almasy L, Breslau N, Culverhouse RC, Dick DM, Edenberg HJ, Foroud T, Grucza RA, Hatsukami D, Hesselbrock V, Johnson EO, Kramer J, Krueger RF, Kuperman S, Lynskey M, Mann K, Neuman RJ, Nothen MM, Nurnberger JI Jr, Porjesz B, Ridinger M, Saccone NL, Saccone SF, Schuckit MA, Tischfield JA, Wang JC, Rietschel M, Goate AM, Rice JP, Gene EASC (2010) A genome-wide association study of alcohol dependence. Proc Natl Acad Sci USA 107:5082–5087.

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.

Chen CC, Lu RB, Chen YC, Wang MF, Chang YC, Li TK, Yin SJ (1999) Interaction between the functional polymorphisms of the alcohol-metabolism genes in protection against alcoholism. Am J Hum Genet 65:795–807.

Edenberg HJ (2002) The collaborative study on the genetics of alcoholism: an update. Alcohol Res Health 26:214–218.

Edenberg HJ (2007) The genetics of alcohol metabolism: role of alcohol dehydrogenase and aldehyde dehydrogenase variants. Alcohol Res Health 30:5–13.

Edenberg HJ, Foroud T (2006) The genetics of alcoholism: identifying specific genes through family studies. Addict Biol 11:386–396.

Edenberg HJ, Xuei X, Chen HJ, Tian H, Wetherill LF, Dick DM, Almasy L, Bierut L, Bucholz KK, Goate A, Hesselbrock V, Kuperman S, Nurnberger J, Porjesz B, Rice J, Schuckit M, Tischfield J, Begleiter H, Foroud T (2006) Association of alcohol dehydrogenase genes with alcohol dependence: a comprehensive analysis. Hum Mol Genet 15:1539–1549.

Fischer R, Sachs UJ, Heidinger KS, Eisenburger D, Kemkes-Matthes B (2013) Prevalence of hereditary antithrombin mutations is higher than estimated in patients with thrombotic events. Blood Coagul Fibrinolysis 24:444–448.

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 (2014a) Genome-wide association study of alcohol dependence: significant findings in African- and European-Americans including novel risk loci. Mol Psychiatry 19:41–49.

Gelernter J, Panhuysen C, Weiss R, Brady K, Hesselbrock V, Rounsaville B, Poling J, Wilcox M, Farrer L, Kranzler HR (2005) Genomewide linkage scan for cocaine dependence and related traits: significant linkages for a cocaine-related trait and cocaine-induced paranoia. Am J Med Genet B Neuropsychiatr Genet 136B:45–52.

Gelernter J, Sherva R, Koesterer R, Almasy L, Zhao H, Kranzler HR, Farrer L (2014b) Genome-wide association study of cocaine dependence and related traits: FAM53B identified as a risk gene. Mol Psychiatry 9:717–723.

Grant JD, Agrawal A, Bucholz KK, Madden PA, Pergadia ML, Nelson EC, Lynskey MT, Todd RD, Todorov AA, Hansell NK, Whitfield JB, Martin NG, Heath AC (2009) Alcohol consumption indices of genetic risk for alcohol dependence. Biol Psychiatry 66:795–800.

Grucza RA, Wang JC, Stitzel JA, Hinrichs AL, Saccone SF, Saccone NL, Bucholz KK, Cloninger CR, Neuman RJ, Budde JP, Fox L, Bertelsen S, Kramer J, Hesselbrock V, Tischfield J, Nurnberger JI Jr, Almasy L, Porjesz B, Kuperman S, Schuckit MA, Edenberg HJ, Rice JP, Goate AM, Bierut LJ (2008) A risk allele for nicotine dependence in CHRNA5 is a protective allele for cocaine dependence. Biol Psychiatry 64:922–929.

Heath AC, Whitfield JB, Martin NG, Pergadia ML, Goate AM, Lind PA, McEvoy BP, Schrage AJ, Grant JD, Chou YL, Zhu R, Henders AK, Medland SE, Gordon SD, Nelson EC, Agrawal A, Nyholt DR, Bucholz KK, Madden PA, Montgomery GW (2011) A quantitative-trait genome-wide association study of alcoholism risk in the community: findings and implications. Biol Psychiatry 70:513–518.

Kapoor M, Wang JC, Wetherill L, Le N, Bertelsen S, Hinrichs AL, Budde J, Agrawal A, Bucholz K, Dick D, Harari O, Hesselbrock V, Kramer J, Nurnberger JI Jr, Rice J, Saccone N, Schuckit M, Tischfield J, Porjesz B, Edenberg HJ, Bierut L, Foroud T, Goate A (2013) A meta-analysis of two genome-wide association studies to identify novel loci for maximum number of alcoholic drinks. Hum Genet 132:1141–1151.

Kendler KS, Myers J, Dick D, Prescott CA (2010) The relationship between genetic influences on alcohol dependence and on patterns of alcohol consumption. Alcohol Clin Exp Res 34:1058–1065.

Kuo PH, Neale MC, Riley BP, Webb BT, Sullivan PF, Vittum J, Patterson DG, Thiselton DL, van den Oord EJ, Walsh D, Kendler KS, Prescott CA (2006) Identification of susceptibility loci for alcohol-related traits in the Irish Affected Sib Pair Study of Alcohol Dependence. Alcohol Clin Exp Res 30:1807–1816.

Macgregor S, Lind PA, Bucholz KK, Hansell NK, Madden PA, Richter MM, Montgomery GW, Martin NG, Heath AC, Whitfield JB (2009) Associations of ADH and ALDH2 gene variation with self report alcohol reactions, consumption and dependence: an integrated analysis. Hum Mol Genet 18:580–593.

Meyers JL, Shmulewitz D, Wall MM, Keyes KM, Aharonovich E, Spivak B, Weizman A, Frisch A, Edenberg HJ, Gelernter J, Grant BF, Hasin D (2015) Childhood adversity moderates the effect of ADH1B on risk for alcohol-related phenotypes in Jewish Israeli drinkers. Addict Biol 20:205–214.

Pan Y, Luo X, Liu X, Wu LY, Zhang Q, Wang L, Wang W, Zuo L, Wang KS (2013) Genome-wide association studies of maximum number of drinks. J Psychiatr Res 47:1717–1724.

Park BL, Kim JW, Cheong HS, Kim LH, Lee BC, Seo CH, Kang TC, Nam YW, Kim GB, Shin HD, Choi IG (2013) Extended genetic effects of ADH cluster genes on the risk of alcohol dependence: from GWAS to replication. Hum Genet 132:657–668.

Patterson N, Price AL, Reich D (2006) Population structure and eigenanalysis. PLoS Genet 2:e190.

Pierucci-Lagha A, Gelernter J, Feinn R, Cubells JF, Pearson D, Pollastri A, Farrer L, Kranzler HR (2005) Diagnostic reliability of the Semi-structured Assessment for Drug Dependence and Alcoholism (SSADDA). Drug Alcohol Depend 80:303–312.

Price AL, Patterson NJ, Plenge RM, Weinblatt ME, Shadick NA, Reich D (2006) Principal components analysis corrects for stratification in genome-wide association studies. Nat Genet 38:904–909.

Quillen EE, Chen XD, Almasy L, Yang F, He H, Li X, Wang XY, Liu TQ, Hao W, Deng HW, Kranzler HR, Gelernter J (2014) ALDH2 is associated to alcohol dependence and is the major genetic determinant of "daily maximum drinks" in a GWAS study of an isolated rural chinese sample. Am J Med Genet B Neuropsychiatr Genet 165B:103–110.

Saccone NL, Kwon JM, Corbett J, Goate A, Rochberg N, Edenberg HJ, Foroud T, Li TK, Begleiter H, Reich T, Rice JP (2000) A genome screen of maximum number of drinks as an alcoholism phenotype. Am J Med Genet 96:632–637.

Saccone SF, Saccone NL, Neuman RJ, Rice JP (2005) Genetic analysis of the maximum drinks phenotype. BMC Genet 6(Suppl 1):S124.

Sartor CE, Wang Z, Xu K, Kranzler HR, Gelernter J (2014) The joint effects of ADH1B variants and childhood adversity on alcohol related phenotypes in African-American and European-American women and men. Alcohol Clin Exp Res 38:2907–2914.

Schumann G, Coin LJ, Lourdusamy A, Charoen P, Berger KH, Stacey D, Desrivieres S, Aliev FA, Khan AA, Amin N, Aulchenko YS, Bakalkin G, Bakker SJ, Balkau B, Beulens JW, Bilbao A, de Boer RA, Beury D, Bots ML, Breetvelt EJ, Cauchi S, Cavalcanti-Proenca C, Chambers JC, Clarke TK, Dahmen N, de Geus EJ, Dick D, Ducci F, Easton A, Edenberg HJ, Esko T, Fernandez-Medarde A, Foroud T, Freimer NB, Girault JA, Grobbee DE, Guarrera S, Gudbjartsson DF, Hartikainen AL, Heath AC, Hesselbrock V, Hofman A, Hottenga JJ, Isohanni MK, Kaprio J, Khaw KT, Kuehnel B, Laitinen J, Lobbens S, Luan J, Mangino M, Maroteaux M, Matullo G, McCarthy MI, Mueller C, Navis G, Numans ME, Nunez A, Nyholt DR, Onland-Moret CN, Oostra BA, O'Reilly PF, Palkovits M, Penninx BW, Polidoro S, Pouta A, Prokopenko I, Ricceri F, Santos E, Smit JH, Soranzo N, Song K, Sovio U, Stumvoll M, Surakk I, Thorgeirsson TE, Thorsteinsdottir U, Troakes C, Tyrfingsson T, Tonjes A, Uiterwaal CS, Uitterlinden AG, van der Harst P, van der Schouw YT, Staehlin O, Vogelzangs N, Vollenweider P, Waeber G, Wareham NJ, Waterworth DM, Whitfield JB, Wichmann EH, Willemsen G, Witteman JC, Yuan X, Zhai G, Zhao JH, Zhang W, Martin NG, Metspalu A, Doering A, Scott J, Spector TD, Loos RJ, Boomsma DI, Mooser V, Peltonen L, Stefansson K, van Duijn CM, Vineis P, Sommer WH, Kooner JS, Spanagel R, Heberlein UA, Jarvelin MR, Elliott P (2011) Genome-wide association and genetic functional studies identify autism susceptibility candidate 2 gene (AUTS2) in the regulation of alcohol consumption. Proc Natl Acad Sci USA 108:7119–7124.

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 Psychiatry 66:773–784.

Wang JC, Foroud T, Hinrichs AL, Le NX, Bertelsen S, Budde JP, Harari O, Koller DL, Wetherill L, Agrawal A, Almasy L, Brooks AI, Bucholz K, Dick D, Hesselbrock V, Johnson EO, Kang S, Kapoor M, Kramer J, Kuperman S, Madden PA, Manz N, Martin NG, McClintick JN, Montgomery GW, Nurnberger JI Jr, Rangaswamy M, Rice J, Schuckit M, Tischfield JA, Whitfield JB, Xuei X, Porjesz B, Heath AC, Edenberg HJ, Bierut LJ, Goate AM (2013) A genome-wide association study of alcohol-dependence symptom counts in extended pedigrees identifies C15orf53. Mol Psychiatry 18:1218–1224.

Willer CJ, Li Y, Abecasis GR (2010) METAL: fast and efficient meta-analysis of genomewide association scans. Bioinformatics 26:2190–2191.

SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Fig. S1. Distribution of log MaxDrinksM value (LogM) in the Yale-UPenn samples.

Fig. S2. Regional Manhattan plot on chromosome 1 (**A**) and chromosome 2 (**B**) in the EAs from the Yale-UPenn samples.