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Authors

Edwards, Alexis C
Deak, Joseph D
Gizer, Ian R
et al.

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Meta-Analysis of Genetic Influences on Initial Alcohol Sensitivity

Alexis C. Edwards, Joseph D. Deak, Ian R. Gizer, Dongbing Lai, Chris Chatzinakos, Kirk P. Wilhelmsen, Jonathan Lindsay, Jon Heron, Matthew Hickman, Bradley T. Webb, Silviu-Alin Bacanu, Tatiana M. Foroud, Kenneth S. Kendler, Danielle M. Dick*, and Marc A. Schuckit*

Background: Previous studies indicate that low initial sensitivity to alcohol may be a risk factor for later alcohol misuse. Evidence suggests that initial sensitivity is influenced by genetic factors, but few molecular genetic studies have been reported.

Methods: We conducted a meta-analysis of 2 population-based genome-wide association studies of the Self-Rating of the Effects of Alcohol scale. Our final sample consisted of 7,339 individuals (82.3% of European descent; 59.2% female) who reported having used alcohol at least 5 times. In addition, we estimated single nucleotide polymorphism (SNP)-based heritability and conducted a series of secondary aggregate genetic analyses.

Results: No individual locus reached genome-wide significance. Gene and set based analyses, both overall and using tissue-specific expression data, yielded largely null results, and genes previously implicated in alcohol problems and consumption were overall not associated with initial sensitivity. Only 1 gene set, related to hormone signaling and including core clock genes, survived correction for multiple testing. A meta-analysis of SNP-based heritability resulted in a modest estimate of $h^2_{SNP} = 0.19$ (SE = 0.10), though this was driven by 1 sample ($N = 3{,}683$, $h^2_{SNP} = 0.36$, SE = 0.14, $p = 0.04$). No significant genetic correlations with other relevant outcomes were observed.

Conclusions: Findings yielded only modest support for a genetic component underlying initial alcohol sensitivity. Results suggest that its biological underpinnings may diverge somewhat from that of other alcohol outcomes and may be related to core clock genes or other aspects of hormone signaling. Larger samples, ideally of prospectively assessed samples, are likely necessary to improve gene identification efforts and confirm the current findings.

Key Words: Avon Longitudinal Study of Parents and Children, Genetics, Genome-Wide Association Studies, Heritability, Initial Alcohol Sensitivity, Self-Rating of the Effects of Alcohol.

ALCOHOL MISUSE IS a common and costly human behavior, accounting for 3.3 million deaths worldwide in 2012 (World Health Organization, 2014) and over $220 billion in annual economic tolls in the United States alone (Bouchery et al., 2011). Alcohol-related outcomes are influenced by genetic factors: The heritability of alcohol use disorder (AUD) was estimated at 0.50 in a meta-analysis of twin studies (Verhulst et al., 2015), and recent genome-wide association studies (GWAS) of alcohol consumption and symptoms have reported single nucleotide polymorphism (SNP)-based heritabilities of 0.13 (Clarke et al., 2017) and 0.12 (Sanchez-Roige et al., 2017), respectively. Variation in genes involved in alcohol metabolism (e.g., alcohol and aldehyde dehydrogenases) is known to impact liability to problems (Edenberg, 2007) and consumption (Jorgenson et al., 2017); there is support for a role of genes outside of this metabolic pathway as well (Schumann et al., 2011, 2016). However, much remains unclear about the mechanisms underlying genetic influences on alcohol outcomes, necessitating further study and consideration of precursors in addition to the outcomes themselves.

Sensitivity to alcohol's effects, particularly during initiation of voluntary alcohol consumption, has been associated

From the Department of Psychiatry (ACE, CC, BTW, S-AB, KSK), Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, Richmond, Virginia; Department of Psychological Sciences (JDD, IRG), University of Missouri, Columbia, Missouri; Department of Medical and Molecular Genetics (DL, TMF), Indiana University, Indianapolis, Indiana; Departments of Neurology and Genetics (KPW), University of North Carolina, Chapel Hill, North Carolina; Department of Pharmacology and Toxicology (JL), Virginia Commonwealth University, Richmond, Virginia; Population Health Sciences (JH, MH), University of Bristol, Bristol, UK; Department of Psychology (DMD), Virginia Commonwealth University, Richmond, Virginia; Department of Human and Molecular Genetics (DMD), Virginia Commonwealth University, Richmond, Virginia; College Behavioral and Emotional Health Institute (DMD), Virginia Commonwealth University, Richmond, Virginia; and Department of Psychiatry (MAS), University of California, San Diego, La Jolla, California.

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Reprint requests: Alexis C. Edwards, PhD, Department of Psychiatry, Virginia Institute for Psychiatric and Behavioral Genetics, Virginia Commonwealth University, VCU, Box 980126, Richmond, VA 23298-0126; Tel.: 804-828-8591; Fax: 804-828-1471; E-mail: alexis.edwards@vcuhealth.org

**Joint senior authors.*

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with later alcohol use and misuse (Schuckit, 1994; Schuckit et al., 2008a,b). Under a model in which an individual consumes alcohol in part to experience its pleasant physiological effects (e.g., a "buzz"), it follows that those who are less sensitive to these effects will consume more than their peers (Trela et al., 2016). Higher consumption, in turn, is positively associated with alcohol problems (Barnett et al., 2014; Dick et al., 2011; Heath et al., 1999; Schuckit et al., 2007) in some but not all (Heath et al., 1999) studies, raising the possibility that those whose subjective response to alcohol is low have higher liability to later misuse.

The Self-Rating of the Effects of Alcohol (SRE) scale was developed by Schuckit and colleagues (1997) to operationalize an individual's alcohol sensitivity by quantifying the number of standard alcoholic drinks necessary to experience physiological effects of alcohol, such as dizziness and slurring; it does not capture all dimensions of intoxication. Higher scores reflect the need to consume a higher volume of alcohol to experience those effects—that is, lower sensitivity to alcohol per drink. Importantly, there is evidence that this sensitivity is heritable: Individuals with a family history of alcohol problems exhibit less pronounced alcohol sensitivity (Schuckit, 1980, 1984; Schuckit et al., 1996, 2000, 2003) across a variety of assessments, including the SRE (Schuckit et al., 2003). We are aware of only 1 twin study of alcohol response (Heath et al., 1999) in adulthood, which reported a heritability of 0.6. Furthermore, linkage studies have identified loci associated with SRE (Ehlers et al., 2010; Schuckit et al., 2001), and variants in *GABRA2* were nominally associated with both subjective and objective measures of alcohol sensitivity in an Australian sample (Lind et al., 2008). A variety of gene sets are hypothesized to influence alcohol sensitivity (Schuckit, 2018), but in the absence of molecular genetic analyses, these remain speculative.

The current study aimed to expand information available on genetic influences underlying initial sensitivity to alcohol. We conducted a meta-analysis of GWAS on SRE scores from 2 independent, population-based cohorts. While both samples are predominantly of European (EUR) descent, 1 sample also included individuals of African (AFR) and American (AMR) descent. By elucidating the biological underpinnings of initial sensitivity to alcohol, we can improve existing models of mechanisms underlying risk of alcohol misuse and potentially inform personalized intervention and prevention programming that might be used even before the first drink.

MATERIALS AND METHODS

Samples

Avon Longitudinal Study of Parents and Children. We used 2 population-based cohort samples: the Avon Longitudinal Study of Parents and Children (ALSPAC) and Spit for Science (S4S). ALSPAC recruited 14,541 pregnant women residing in Avon, UK, with expected dates of delivery April 1, 1991, to December 31, 1992; 14,541 is the initial number of pregnancies for which the mothers enrolled in the ALSPAC study and had either returned at least 1 questionnaire or attended a "Children in Focus" clinic by July 19, 1999. Of these initial pregnancies, there were a total of 14,062 live births and 13,988 children who were alive at 1 year of age. Subsequent phases of enrollment increased the sample size over time. The phases of enrollment are described in more detail elsewhere (Boyd et al., 2013; Fraser et al., 2013). For the current analyses, full or partial phenotypic data were available for 5,626 participants, in part reflecting the need for a subject to have had experience with alcohol in order to fill out the SRE. The study website contains details of all the data that is available through a fully searchable data dictionary (http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary/). Ethical approval for the study was obtained from the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees.

Spit for Science. S4S is an ongoing longitudinal study of college students enrolled in a large, urban university in the mid-Atlantic (Dick et al., 2014). Briefly, incoming students age 18 or older were eligible to complete phenotypic assessments, which covered a wide range of topics but focused on alcohol use. Study data were collected and managed using REDCap electronic data capture tools (Harris et al., 2009) hosted at Virginia Commonwealth University. Follow-up assessments were completed in subsequent spring semesters. Individuals who did not participate in the first wave of data collection (including those who turned 18 after the end of the first wave of data collection) had the opportunity to join the study the following spring; those who participated during their first year were eligible to complete follow-up assessments each spring. Participants who completed the phenotypic assessments were eligible to provide a DNA sample. The current study includes 3 cohorts, which matriculated in Fall 2011 (N = 2,714), 2012 (N = 2,486), and 2013 (N = 2,403), for a total N = 7,603. Ethical approval was obtained from the local Institutional Review Board.

Phenotypes

Outcome. The SRE consists of 4 items; for the current study, each item referred to the *first 5 or so times* a participant used alcohol. Participants were asked to report the number of standard drinks they consumed before they experienced signs of alcohol's effect (from feeling any effect of the alcohol on to slurring words, feeling unsteady on their feet, to unwanted falling asleep). Consistent with prior papers, responses were winsorized to limit extreme values and reduce the effect of possibly spurious outliers. SRE scores were calculated by summing drinks needed for effects across items and dividing by the number of the effects experienced (i.e., the 4 items that the participant could have experienced), as recommended by Schuckit and colleagues (1997). The final score was used as a continuous outcome in subsequent GWAS.

Both ALSPAC and S4S participants were administered the SRE items across multiple assessments. For ALSPAC, we examined data from questionnaires/clinic visits at average ages 15.5, 16.5, and 17.5. S4S participants were administered the SRE items at average ages 18.5, 19.0, 19.9, and 21.0. For both samples, only participants who reported having initiated alcohol use were administered the SRE items; others were coded as "NA." Where scores were available for a participant across multiple waves, the first score was used for GWAS, reasoning that this wave represented the assessment most temporally proximal to the initiation of alcohol use and was therefore least susceptible to recall bias. The size of a standard drink, to which respondents are asked to refer when completing the SRE items, differs in the United States and the United Kingdom (14 g vs. 8 g of ethanol [EtOH], respectively). Therefore, raw SRE scores were standardized for GWAS analyses, ensuring that effect sizes observed across the ALSPAC and S4S samples were on the same scale.

Covariates. Sex was included as a covariate (individuals whose self-reported gender was inconsistent with genetic sex were excluded from these analyses) in initial GWAS within both samples. For

ALSPAC, wave at which the SRE items were first completed was included as a covariate; assessments are age-standardized but precise date of completion was unavailable. For S4S, age at which the SRE items were first completed was included as a covariate (mean [SD] age across all samples = 18.80 [0.79]). To account for population structure, 10 ancestry-informative principal components (PCs) were included in the ALSPAC GWAS, consistent with prior analyses using this sample (Edwards et al., 2017). S4S participants are of diverse ancestry and were first assigned to empirically based ancestry groups using PCs derived from 1000 Genomes (phase 3) reference populations, as described by Peterson and colleagues (2017). Subsequently, within-ancestry PCs were calculated to capture fine-grained stratification; PCs were retained as covariates in the GWAS using a stepwise regression approach.

Imputation and Quality Control Filters

Imputed genotypes for both samples were derived using the 1000 Genomes reference panels as previously reported (Edwards et al., 2017; Webb et al., 2017). Quality control procedures for genetic analyses of both the ALSPAC and S4S samples have been previously described (Fatemifar et al., 2013; Webb et al., 2017), and those within-sample approaches were applied for the current analyses (see Appendix S1). Briefly, individual DNA samples and markers were excluded based on excess missingness (>5% for both samples), deviations from Hardy–Weinberg equilibrium ($p < 5e\text{-}7$ for ALSPAC, $p < 5e\text{-}6$ for S4S), and minor allele frequency (MAF; <0.01 for ALSPAC, <0.005 for S4S). Cryptic relatedness was calculated using pi-hat, and related individuals were excluded.

Genetic Analyses

GWAS and Meta-Analysis. For ALSPAC, phenotypic (including outcome and covariate) and genetic data were available for 3,683 individuals, all of EUR ancestry. GWAS was run in Plink 1.9 (Chang et al., 2015). For S4S, we included only groups with $N \geq 400$ to reduce the likelihood of spurious results, yielding the following sample sizes: AFR = 892; AMR = 408; and EUR = 2,356 (total S4S N = 3,656). The S4S GWAS were run in SNPTest version 2.5.2 (Marchini et al., 2007), separately by ancestry group, as described previously. Results across samples (total N = 7,339) were meta-analyzed using METAL (Willer et al., 2010), using inverse variance weighting by sample size. Markers with MAF < 0.01 within sample/ancestry group and/or INFO < 0.5 were excluded from further analysis.

Gene and Gene Set Analyses. We applied 2 approaches to gene and set based analyses, FUMA (Watanabe et al., 2017) and JEPEGMIX (Lee et al., 2016). The former conducts gene-based tests across all markers followed by gene set analysis. We submitted meta-analysis summary statistics to the FUMA pipeline, which requires selection of a reference panel in order to account for linkage disequilibrium (LD) among markers. We selected the EUR subsample of the 1000 Genomes reference panel as this group constituted >82% of the sample, and correcting for EUR LD is a more conservative approach than correcting for AFR LD (AFR being the next-largest component of the full sample). JEPEGMIX differs from FUMA in that its gene and set based analyses are tissue specific: Using GWAS summary statistics, it tests the joint effect of functional SNPs known to affect the expression of a gene, effectively predicting whether tissue-specific gene expression is associated with an outcome of interest (here, SRE score). The method can be extended to estimate the joint effects across gene sets.

Heritability and Genetic Correlations. GCTA (Yang et al., 2011) was used to assess SNP-based heritability (h^2_{SNP}). Analyses were conducted within group (ALSPAC, AFR, AMR, and EUR), using unrelated individuals and markers with MAF ≥ 0.01. To assess genetic correlations between SRE and other relevant traits assessed in independent samples, summary statistics were uploaded to LD Hub (Zheng et al., 2017). We tested whether SRE was genetically correlated with traits in selected categories: anthropometric, brain volume, cognitive, education, hormone, metabolites, personality, psychiatric, reproductive, and smoking behaviors.

Polygenic Risk Scores. Polygenic risk scores (PRS) were derived to test whether aggregate risk for SRE in the discovery set was associated with phenotype in the test set. We used weights from 2 discovery sets: (i) the full meta-analysis; and (ii) the ALSPAC-specific results, given evidence of heritability in ALSPAC but not S4S. The first test set involved participants (N = 1,080; 61.1% male) with both genotype and phenotype data from the UCSF Family Alcoholism Study (Vieten et al., 2004). The sample was composed of small family pedigrees, which ranged in size from 3 to 20 individuals. The subsample used in the present study had an average age of 48.9 (SD = 12.1) years. PLINK 1.9 (Chang et al., 2015) was used to derive PRS. We employed a linear mixed model with a kinship matrix fitted as a random effect to control for the relatedness among participants. Age, sex, and 4 ancestry PCs were included as covariates. The second data set was from the Collaborative Study on the Genetics of Alcoholism (COGA). COGA is a multicenter study of families with alcohol dependence. African American (AA) and European American (EA) subsamples of COGA were included in analysis. For each COGA subsample, all individuals (N = 1,527 in AA; N = 4,717 in EA) and COGA prospective samples (N = 326 in AA; N = 822 in EA), which were offspring of COGA families that were born after 1982 (Bucholz et al., 2017) to match the ALSPAC/S4S samples, were tested separately. PRSice-2 (Euesden et al., 2015) was used to calculate PRS. Sex, the first 4 ancestry PCs, and genotyping array indicators were included as covariates. For analyses of all individuals, birth cohorts were also included as covariates. Linear mixed models were fit to adjust family clustering using SAS9.4 (SAS Institute Inc., Cary, NC). For both the UCSF and COGA samples, we tested for associations at 8 p-value thresholds: 0.001, 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, and 0.5.

RESULTS

Descriptive Statistics

Descriptive statistics of the sample, including mean SRE scores by group prior to transformation, are provided in Table 1. Scores were significantly higher in the S4S sample relative to ALSPAC (t = 7.49, $p < 0.0001$). Differences were also observed across S4S ancestry groups, $F(2, 3{,}653) = 10.12$, $p < 0.0001$. Men's scores were higher than women's (t = 20.51, $p < 0.0001$). SRE scores were moderately correlated with later alcohol consumption (r = 0.20 to 0.25 in S4S; r = 0.18 to 0.28 in ALSPAC; all $p < 0.0001$), which was operationalized in S4S as grams of EtOH per month (derived from responses to alcohol use frequency and quantity; Salvatore et al., 2016) and in ALSPAC as Alcohol Use Disorders Identification Test-Consumption (AUDIT-C) scores (Bush et al., 1998).

Meta-Analysis of GWAS Results

Results from the meta-analysis of SRE scores are displayed in Figs 1 and 2. In each group-level analysis and in

Table 1. Descriptive Statistics by Sample and Sex

	N	Mean (SD) Age	Mean (SD) Self-Rating of the Effects of Alcohol score
Sample			
Combined	7,339	17.4 (1.58)[a]	5.30 (2.54)
ALSPAC	3,683	16.03 (0.74)[a]	5.08 (2.75)
S4S	3,656	18.78 (0.79)	5.53 (2.29)
African	892	18.82 (0.80)	5.24 (2.41)
American	408	18.76 (0.89)	5.77 (2.43)
European	2,356	18.78 (0.77)	5.59 (2.21)
Sex			
Women	4,347	17.45 (1.54)	4.81 (2.31)
Men	2,992	17.33 (1.62)	6.02 (2.70)

[a]Because precise ages were not available for Avon Longitudinal Study of Parents and Children (ALSPAC) participants, expected average age for the wave of data collection was used for these values.

the meta-analysis, there was no evidence of inflation due to population stratification (λ_{1000} = 0.99 to 1.00). A total of 15,642,250 markers were analyzed in the meta-analysis; 10,752,408 were assessed in at least 1,000 individuals. No individual locus met genome-wide significance criteria ($p < 5 \times 10^{-8}$). The top marker was rs146298733 ($p = 3.16 \times 10^{-7}$), which maps to an intron in *DLGAP1* on chromosome 18; this result may be spurious as surrounding markers do not have similar *p*-values. The minor allele was only of sufficient frequency to test in the ALSPAC and EUR groups (N = 6,039; MAF = 0.02 in both samples).

Meta-analysis summary statistics were uploaded to FUMA, which identified 35 lead SNPs based on *p*-value and LD; these are presented in Table 2 alongside functional information derived from Combined Annotation Dependent Depletion (CADD; Kircher et al., 2014) scores and RegulomeDB (Boyle et al., 2012). Overall, these markers are not predicted to be especially deleterious—only 1 marker has a CADD score >10—nor are they predicted to have meaningful regulatory roles—no marker has a RegulomeDB score of 1a to 1f.

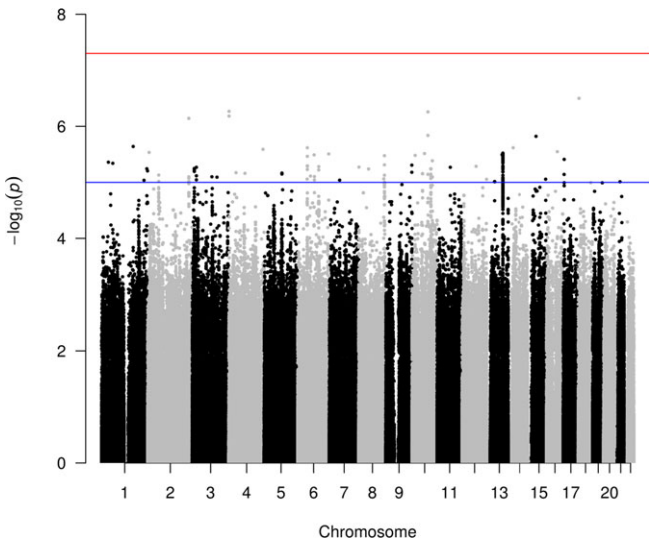

Fig. 1. Manhattan plot of Self-Rating of the Effects of Alcohol scores for markers assessed in at least 1,000 individuals. Red line indicates genome-wide significance threshold ($p < 5 \times 10^{-8}$); blue line indicates suggestive significance ($p < 1 \times 10^{-5}$).

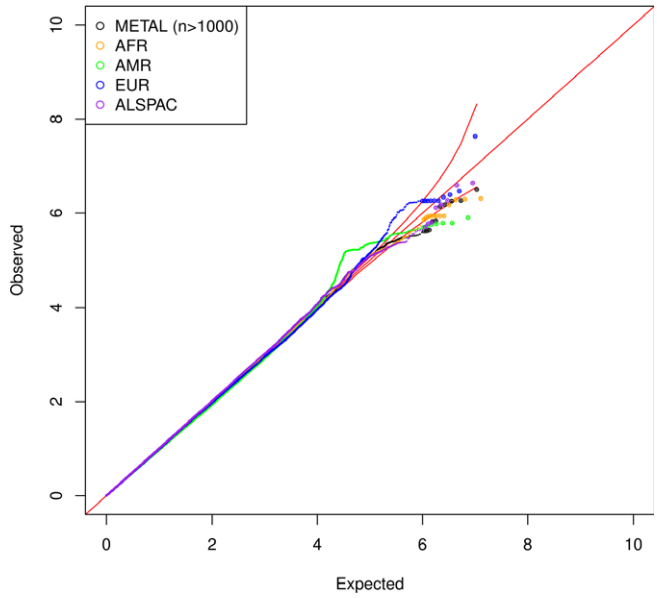

Fig. 2. Quantile–quantile plot for each sample, as well as for meta-analysis results for markers assessed in at least 1,000 individuals.

Gene and Gene Set Analyses

Using FUMA, markers were mapped to 18,363 protein coding genes; none met genome-wide significance criteria ($p < 2.72 \times 10^{-6}$). Complete results are available in Table S1, while the top 10 genes are listed in Table 3. The FUMA pipeline also uses the complete distribution of SNP *p*-values to conduct a gene set analysis (N_{set} = 10,891) using MSigDB (Subramanian et al., 2005). Only 1 gene set had a corrected *p*-value of <0.05: *regulation of intracellular steroid hormone receptor signaling pathway* (Table 4; corrected p = 0.03). Complete results are available in Table S2.

Using JEPEGMIX, we assessed whether tissue-specific expression for individual genes was predicted to be associated with SRE scores. No gene met the corrected significance threshold (Table S3). We next tested whether expression levels of genes in canonical gene sets were jointly predicted to be associated with SRE scores. No gene set met the corrected significance threshold (Table S4).

Heritability, Genetic Correlations, and PRS

Heritability estimates for each S4S ancestry group were not significantly different from 0 (h^2_{SNP}<0.001; SE = 0.16 to 0.59; p = 0.13 to 0.50). However, for ALSPAC, heritability was moderate (h^2_{SNP} = 0.36, SE = 0.14, p = 0.04). Although the meta-analytic h^2_{SNP} was different from 0 (h^2_{SNP} = 0.19,

Table 2. Lead SNPs from Functional Mapping and Annotation of Genome-Wide Association Studies (FUMA) and Corresponding Functional Annotation

rsID	Chr	Position	*p*-Value	Nearest gene	Distance from gene	Function	CADD score	RDB
rs145005509	1	244472953	5.73E-06	C1orf100	42,984	intergenic	11.47	7
rs10788734	1	248075398	6.26E-06	OR2T8	8,922	intergenic	1.717	7
rs72806266	2	59501865	7.42E-06	ENSG00000233891	0	ncRNA_intronic	0.442	6
rs112834343	2	224599695	7.22E-07	AP1S3	16,708	intergenic	4.525	6
rs17033567	3	10913738	5.65E-06	SLC6A11	0	intronic	2.597	7
rs2336522	3	22023520	5.40E-06	ZNF385D-AS2	2,200	intergenic	0.718	7
rs112368179	3	133217908	8.09E-06	ENSG00000214301	7,559	intergenic	5.85	5
rs75536499	4	536426	6.58E-07	PIGG	3,108	intergenic	0.127	5
rs115496994	4	86353705	6.92E-06	ARHGAP24	42,562	intergenic	7.664	7
rs10020261	4	184171187	2.57E-06	WWC2	0	intronic	8.42	4
rs4869281	5	95651353	6.84E-06	CTD-2337A12.1	0	ncRNA_intronic	3.662	3a
rs75886551	6	51028172	8.77E-06	FTH1P5	147,203	intergenic	0.435	4
rs11465543	6	52108584	2.41E-06	IL17F	0	intronic	2.813	NA
rs76563242	6	88277132	3.24E-06	RARS2	0	intronic	0.719	7
rs62421504	6	113654797	5.24E-06	ENSG00000223811	23,408	intergenic	0.663	7
rs206972	6	167689552	3.11E-06	UNC93A	0	intronic	0.043	6
rs73133463	7	55119501	9.18E-06	EGFR	0	intronic	3.336	5
rs2100160	8	427140	5.35E-06	ENSG00000272005	0	ncRNA_exonic	0.355	NA
rs16905012	8	134905738	8.65E-06	RP11-157E21.1	0	ncRNA_intronic	4.592	7
rs11777857	8	138546064	3.35E-06	ENSG00000254076	162,903	intergenic	3.359	5
rs28373932	9	139998042	4.94E-06	MAN1B1	0	intronic	4.023	5
rs76238752	10	16614401	3.30E-06	RSU1	18,209	intergenic	6.354	7
rs10825405	10	56592865	7.00E-06	PCDH15	0	intronic	1.107	5
rs75752490	10	67293784	3.05E-06	ENSG00000228065	36,312	intergenic	5.932	6
rs61866256	10	85682627	5.53E-07	ENSG00000233258	9,949	intergenic	4.181	7
rs7076325	10	101868347	5.73E-06	TPM4P1	5,827	intergenic	0.899	6
rs184338590	10	109779481	4.11E-06	RP11-215N21.1	0	ncRNA_intronic	3.103	7
rs75794081	11	71069255	5.39E-06	AP002387.1	24,392	intergenic	1.829	5
rs41287003	13	41910631	9.71E-06	NAA16	0	intronic	5.577	7
rs9547398	13	86417640	3.01E-06	SLITRK6	44,017	intergenic	2.744	7
rs1016246	14	26679400	2.42E-06	CYB5AP5	1,770	intergenic	2.935	NA
rs116879015	15	45492952	1.51E-06	SHF	0	exonic	7.285	4
rs146087183	16	57934080	2.83E-06	CNGB1	0	intronic	1.436	4
rs7214066	17	4678855	3.89E-06	TM4SF5	0	intronic	2.927	7
rs146298733	18	4114529	3.16E-07	DLGAP1	0	intronic	2.39	7

Chr, chromosome; CADD, Combined Annotation Dependent Depletion; RDB, RegulomeDB; SNP, single nucleotide polymorphism.

Table 3. Top 10 Gene-Based Results From FUMA

Symbol	Chr	Start BP	End BP	*N* SNPs	*Z* statistic	*p*-Value
ZBTB44	11	130086572	130194581	397	4.2267	1.19e-05
BHLHE40	3	5010801	5037008	88	4.2012	1.33e-05
ISL1	5	50668921	50700564	77	3.8686	5.47e-05
NDNF	4	121946768	122004176	193	3.6830	1.15e-04
ACTN4	19	39128289	39232223	370	3.5510	1.92e-04
C1orf122	1	38262651	38285126	42	3.5426	1.98e-04
CYSLTR2	13	49270951	49293498	83	3.5399	2.00e-04
ATG4D	19	10644571	10674094	113	3.5359	2.03e-04
TMIGD1	17	28633351	28671077	90	3.4772	2.53e-04
FAM159A	1	53089016	53145355	181	3.4280	3.04e-04

Chr, chromosome; BP, base pair; *N* SNPs, number of single nucleotide polymorphisms.

SE = 0.10), this was clearly driven by the ALSPAC group. Because h^2_{SNP} exceeded 0 only in the ALSPAC sample, only ALSPAC-specific summary statistics (i.e., not the meta-analytic results) were uploaded to LD Hub. There were no significant genetic correlations between SRE and any of the traits assessed in LD Hub, though we note that the mean χ^2 (1.005) was flagged by the program as potentially too low. Complete results are available in Table S5. We also conducted bivariate GCTA between SRE and AUDIT-C and total scores at ages 16, 18, and 21; these analyses were limited to ALSPAC given null h^2_{SNP} estimates in S4S. Genetic correlations were not significant, but were largely positive (rG_{SNP} = 0.55 to 1.00) with 1 exception (SRE and age 21 AUDIT-C, $rG_{SNP} = -0.07$, n.s.). Finally, we tested whether markers implicated at 8 *p*-value thresholds in the meta-analysis were associated with SRE scores in 2 independent samples. We derived the PRS using meta-analysis SNP weights and using ALSPAC-specific weights, due to the detection of significant h^2_{SNP} in ALSPAC but not S4S groups. We observed several nominally significant associations ($0.01 < p < 0.05$) but no systematic effects.

DISCUSSION

Initial sensitivity to the effects of alcohol has been associated, in the ALSPAC sample (Schuckit et al., 2008a,b) among others, with later alcohol misuse and problems, such that individuals less sensitive to alcohol when they begin drinking are at higher risk of later misuse. Evidence from twin and family studies, alongside preliminary findings from gene identification efforts, has suggested that the association

Table 4. Top 10 Gene Set Based Results from FUMA

Full set name	*N* genes	Beta	Beta SD	SE	*p*-Value
GO bp:go regulation of intracellular steroid hormone receptor signaling pathway	57	0.4570	0.0254	0.1000	2.51e-06
Curated gene sets:dasu il6 signaling up	58	0.4140	0.0232	0.1030	2.89e-05
GO bp:go regulation of metal ion transport	315	0.1770	0.0229	0.0444	3.41e-05
Curated gene sets:kegg circadian rhythm mammal	13	0.7670	0.0204	0.1930	3.47e-05
GO bp:go regulation of cell proliferation involved in heart morphogenesis	15	0.9090	0.0260	0.2300	4.00e-05
GO bp:go cell-cell recognition	59	0.3640	0.0206	0.0943	5.73e-05
GO bp:go protein alpha 1 2 demannosylation	13	0.7530	0.0200	0.2040	1.13e-04
GO bp:go protein demannosylation	13	0.7530	0.0200	0.2040	1.13e-04
GO bp:go positive regulation of hydrolase activity	872	0.0995	0.0212	0.0271	1.22e-04
GO bp:go photoperiodism	23	0.5170	0.0183	0.1420	1.40e-04

GO, gene ontology; bp, biological process; SD, standard deviation; SE, standard error

may be due in part to genetic influences on sensitivity. In the current study, we meta-analyzed GWAS of initial sensitivity to alcohol, using the first 5 times drinking SRE scale in 2 large, population-based samples. Analyses yielded few genome-wide significant findings, and PRS were not consistently associated with SRE in 2 independent samples. Our limited positive results came from aggregate tests, which indicated moderate heritability (overall $h^2_{SNP} = 0.19$, SE = 0.10) and support for a role of genes involved in hormone signaling. Initial alcohol sensitivity may be more prominently environmentally influenced than previously thought. However, studies of other behavioral outcomes with heritable components have yielded null results until much larger sample sizes were amassed (e.g., Wray et al., 2018). Follow-up is warranted, preferably using samples assessed during adolescence, contemporaneous with initial alcohol use. Furthermore, assessment of multiple ancestry groups is critical for clarifying the extent to which phenotypic differences are influenced by genetic factors.

Although no marker met genome-wide significance criteria, this is not entirely unexpected given evidence that substantially larger sample sizes may be necessary to reliably identify loci of small effect in complex traits (Bacanu and Kendler, 2018; Sullivan et al., 2017). Suggestive loci localize to several genes of interest. For example, *SLC6A11* is a GABA transporter preferentially expressed in brain (Fagerberg et al., 2014); variation in this gene has been associated with intellectual and behavioral aberrations (Dikow et al., 2014) and resistance to epilepsy pharmacotherapy (Kim et al., 2011). Given the role of the GABAergic system in alcohol response and sensitization (Camarini and Pautassi, 2016; Koob, 2013), the biological plausibility of *SLC6A11* is compelling. While other GABAergic genes involved in alcohol-relevant processes had suggestive *p*-values (e.g., *GABARAP*, $p = 0.003$; *GABRB3*, $p = 0.001$), these did not survive a multiple testing correction. Genes implicated in recent large GWAS of alcohol-related outcomes (Clarke et al., 2017; Jorgenson et al., 2017; Sanchez-Roige et al., 2017; Schumann et al., 2016) were also not supported. Indeed, no locus implicated by lead SNPs or gene-based analyses has been previously associated with alcohol use/misuse; these novel candidates require further investigation to determine the mechanisms by which they may influence alcohol sensitivity. However, the dearth of loci meeting strict correction thresholds prevents extensive interpretation of the current findings.

As indicated by CADD scores, lead SNPs are overall not predicted to be deleterious. Only rs145005509 has a CADD score >10; this SNP is intronic to a predicted locus and upstream of an open reading frame, thus, its functional significance is unclear. Importantly, we evaluated only common alleles, which are relatively infrequently deleterious. Perhaps more interestingly, lead SNPs are not predicted to have clear regulatory functions, in contrast with findings for schizophrenia (Roussos et al., 2014), major depression (Wray et al., 2018), and nicotine dependence (Zanger and Schwab, 2013). RegulomeDB annotations were only obtained for lead SNPs through the FUMA pipeline, leaving open the possibility that less strongly implicated markers have regulatory functions.

One gene set survived the multiple test correction threshold (*regulation of intracellular steroid hormone reception signaling pathway*), and scrutiny of the genes in that category (obtained from MSigDB) revealed a potentially interesting avenue for further inquiry: They include core clock genes involved in circadian rhythms and/or photoperiodism, which were among the top 10 most strongly implicated gene sets (Table S2). Genes included in all 3 sets are *CLOCK*, *CRY1*, *CRY2*, and *PER1*; other clock genes are common to 2 of the 3 sets. *CLOCK* and *PER1* have been associated with AUDs (Partonen, 2015), and there is evidence that clock genes may have a regulatory role in reward circuitry (Parekh et al., 2015). Furthermore, mice with various perturbations in clock genes exhibit aberrant alcohol-related phenotypes (Dong et al., 2011; Gamsby et al., 2013; Perreau-Lenz et al., 2009; Spanagel et al., 2005; Wang et al., 2012).

The heritability of SRE scores in ALSPAC was moderate ($h^2_{SNP} = 0.36$) and differed significantly from 0, suggesting that aggregate genetic factors contribute substantially to initial alcohol sensitivity. However, the heritability estimates were effectively 0 for each S4S ancestry group. This pronounced difference may be due in part to assessment. ALSPAC participants were periodically assessed in the time frame during which they were likely to begin experimenting

with alcohol: While 62% responded to SRE items in wave 1 (age ~15.5), the remainder had not used alcohol 5 or more times until a later assessment. In contrast, 79% of S4S participants' reports were from wave 1 (age ~18.5) and it is likely that many were reporting on alcohol exposure several years in the past. This raises the possibility that the scores are quite sensitive to recall bias. Thus, it is unclear whether the null heritability estimates of SRE across S4S ancestry groups are due to a true absence of genetic influences on SRE in S4S, potential error introduced by retrospective reports, or other factors. We are further unable to determine whether ancestry-based differences in heritability exist.

Additional tests of aggregate genetic influences did not yield significant findings. The absence of association between PRS derived from meta-analysis and ALSPAC-specific SNP weights and SRE scores in independent samples could be due to assessment, that is, recall bias within the older individuals in the samples. However, we cannot rule out the possibility that the signals from our GWAS were of insufficient precision for outcome prediction, or that nongenetic factors are simply more influential than genetic factors on initial alcohol sensitivity.

The analyses presented here suggest that genetic factors have a modest impact on initial sensitivity to alcohol, but are largely inconclusive with respect to underlying biology. This underscores the need for prospective assessments of large, diverse samples to clarify the biological mechanisms underlying alcohol sensitivity and how they may differ across ancestries. Prior evidence that low initial sensitivity is associated with later alcohol misuse (Barnett et al., 2014; Heath et al., 1999; Schuckit, 1994) suggests that SRE scores could be a useful risk indicator. Further elucidation of the biological processes impacting initial sensitivity is necessary and could be accomplished in part by characterizing loci implicated in the current study in model systems to determine whether, and how, genetic manipulations effect EtOH sensitivity. Another avenue for potential research is the identification of specific environmental factors that account for the balance of phenotypic variance in SRE scores; examples may include diet, pubertal status, or psychological stressors with physiological consequences. While we understand a great deal about alcohol metabolism via alcohol dehydrogenases and other pathways (Lieber, 2005; Marshall and Chambers, 2005), the subjective experience of drinkers is likely influenced by a wider range of genetic factors, the identification of which is critical to developing a comprehensive model of risk.

Limitations

Despite this being the largest genetic study of initial alcohol sensitivity to date, it is possible that the retrospective SRE assessment in approximately half of the total sample compromised our statistical power to detect influential loci. Individuals in both samples for whom multiple waves of data were available generally reported increasing SRE scores in later assessments despite the reporting period being constant (i.e., the first 5 or so times they drank alcohol); this raises the possibility that current drinking experiences influence reporting of past sensitivity. This may have contributed to the discrepancy in h^2_{SNP} estimates across the ALSPAC and S4S samples. The moderate estimate in ALSPAC encourages us that genetic factors are, indeed, influential, though power analyses indicated <10% power to detect a h^2_{SNP} of 0.30 in the smallest S4S subgroup, AMR. The EUR samples were more adequately powered: In ALSPAC, we estimated 64% power to detect h^2_{SNP} of 0.20 and 99% power to detect h^2_{SNP} of 0.36 (the actual estimate), and 61% power to detect h^2_{SNP} of 0.30 the S4S EUR ancestry group. Despite the lack of genome-wide significant variants, the current report represents an important initial effort to improve our understanding of the biological underpinnings of alcohol sensitivity.

Genetic studies are frequently limited to samples of EUR ancestry, precluding opportunities to assess differential genetic effects across ancestry groups. Although this study included a diverse sample, the non-EUR groups were of modest sample size and we lacked sufficient power to directly test such effects. However, efforts to recruit diverse samples are increasing, and meta-analytic approaches will enable the current samples to be incorporated into larger analyses in the future. Results from such approaches raise issues regarding the incorporation of LD in various secondary analyses; here, we elected to use EUR LD for FUMA, as this is likely a conservative approach and is appropriate for the majority (>82%) of the sample, but other methods may be preferable. JEPEGMIX was designed to be robust to the inclusion of cosmopolitan samples.

Finally, genetic analyses have consistently benefitted from larger sample sizes. Here, we report initial progress toward the identification of genetic factors influencing alcohol sensitivity, but these efforts must be bolstered by combining data across samples to increase statistical power. Given its ease of use and evidence of validity, the SRE represents a potentially powerful tool to employ to that end. However, the SRE does not capture all dimensions of alcohol sensitivity, and the subjective nature of the measure introduces uncertainty into analyses that are sensitive to measurement error, as is the case for most complex behavioral traits. Studies of more objective measures, such as body sway or motor coordination, would complement studies employing the SRE.

In conclusion, we report evidence of modest genetic influences on initial sensitivity to alcohol. Suggestive loci have not been previously implicated in alcohol outcomes, suggesting that the biology of sensitivity is not entirely parallel to that of alcohol consumption or problems. However, gene set analysis supports a role for core clock genes in initial sensitivity. Assessment of sensitivity is likely superior when conducted temporally proximal to initial alcohol experimentation; ideally, future studies will involve diverse samples such as that included here. Further investigation of loci identified in the current study is warranted to determine their impact and optimally arrange them in a comprehensive model of risk for alcohol misuse.

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AUTHOR CONTRIBUTIONS

ACE, S-AB, KSK, JL, TMF, DMD, and MAS were involved in study concept and design; ACE, JDD, DL, IRG, CC, and JH analyzed the data; BTW was involved in data management and analytic support; KPW, JH, MH, TMF, KSK, DMD, and MAS were involved in funding and data acquisition; all authors critically reviewed the manuscript.

CONFLICT OF INTERESTS

The authors have no conflict of interests to report.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Table S1. Complete gene-based association results from FUMA.

Table S2. Gene set analysis results from FUMA.

Table S3. Predicted associations between tissue-specific gene expression and SRE scores, derived using JEPEGMIX.

Table S4. Predicted associations between tissue-specific expression of canonical gene sets and SRE scores, derived in JEPEGMIX.

Table S5. Complete results from LD Hub.

Appendix S1. Supplementary material.