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Genome-wide association study identifies loci associated with liability to alcohol and drug dependence that is associated with variability in reward-related ventral striatum activity in African- and European-Americans.

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Author Contributions: LW, DL, ECJ, RB and AAgrawal conducted analyses, drafted and revised the manuscript. TF, RB and AAgrawal supervised all aspects of the study. TF and AMG provided oversight with genomic analyses and interpretation. ARH collected the DNS sample. KKB, DMD, VH, CK, JK, SK, JLM, JIN, MS, DMS, RET, JT, BP and HJE provided feedback on development of phenotypes and in statistical analyses.

Conflict of Interest:

Alison Goate is listed as an inventor on Issued U.S. Patent 8080,371, “Markers for Addiction” covering the use of certain variants in determining the diagnosis, prognosis, and treatment of addiction.

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Abstract

Genetic influences on alcohol and drug dependence partially overlap, however specific loci underlying this overlap remain unclear. We conducted a genome-wide association study (GWAS) of a phenotype representing alcohol or illicit drug dependence (*ANYDEP*) among 7,291 European-Americans (EA; 2,927 cases) and 3,132 African-Americans (AA: 1,315 cases) participating in the family-based Collaborative Study on the Genetics of Alcoholism. *ANYDEP* was heritable (h^2 in EA=0.60, AA=0.37). The AA GWAS identified 3 regions with genome-wide significant (GWS; p<5E-08) single nucleotide polymorphisms (SNPs) on chromosomes 3 (rs34066662, rs58801820) and 13 (rs75168521, rs78886294), and an insertion-deletion on chromosome 5 (chr5:141988181). No polymorphisms reached GWS in the EA. One GWS region (chromosome 1: rs1890881) emerged from a trans-ancestral meta-analysis (EA+AA) of *ANYDEP*, and was attributable to alcohol dependence in both samples. Four genes (AA: *CRKL, DZIP3, SBK3*; EA: *P2RX6*) and 4 sets of genes were significantly enriched within biological pathways for hemostasis and signal transduction. GWS signals did not replicate in two independent samples but there was weak evidence for association between rs1890881 and alcohol intake in the UK Biobank. Among 118 AA and 481 EA individuals from the Duke Neurogenetics Study, rs75168521 and rs1890881 genotypes were associated with variability in reward-related ventral striatum activation. This study identified novel loci for substance dependence and provides preliminary evidence that these variants are also associated with individual differences in neural reward reactivity. Gene discovery efforts in non-European samples with distinct patterns of substance use may lead to the identification of novel ancestry-specific genetic markers of risk.

Keywords

African American; alcohol dependence; drug dependence; European American; genetics; GWAS; heritability; neural reward; ventral striatum; fMRI

INTRODUCTION

Reducing the widespread prevalence[1–3] and devastating worldwide impact[4,5] of alcohol and illicit drug dependence is hindered by limited etiologic insight that impedes prevention and

treatment advances. In the United States (US), 12.5% of the population meets criteria for a lifetime history of alcohol dependence[3] while 2.6% meet criteria for DSM-IV drug dependence during their lifetime[2]. Notably, individuals are often comorbid for multiple substance use disorders[2], and common latent genetic factors[6,7] explain a large proportion of the moderate to high heritability of dependence on individual substances (h^2=50–70%[8–10]). The common genetic architecture of dependence liability is also underscored by evidence from genome-wide association studies (GWAS) documenting genetic correlations between alcohol-related measures and cannabis and cigarette use[11,12]. Leveraging the common genetic architecture underlying general substance dependence liability to identify markers of dependence risk through GWAS would complement existing efforts targeting individual substances (e.g., [12–16]) to elucidate underlying etiologic risk factors for general and specific substance dependence liability.

It is estimated that an overwhelming proportion of participants in existing GWAS are of European ancestry.[17,18] Data generated from GWAS of individuals of European ancestry are less applicable to other ancestral groups and when applied to non-European cohorts may result in inaccurate estimations of risk that may further perpetuate racial health and healthcare disparities. Studies suggest that even when discovery samples of non-European individuals are small, including them in individual discovery analyses and trans-ancestral analyses can result in novel insights into the genetic architecture of the disorder and in polygenic prediction[12,19,20]. Differences in prevalence and patterns of substance dependence across ancestrally diverse groups in the United States[21] underscore the importance of conducting GWAS on these phenotypes in these groups. In particular, the study of African-Americans, one the largest minorities represented in GWAS data in the US, provides an opportunity to address this notable disparity in genomic research.

Here, we conduct a GWAS of a phenotype representing alcohol or illicit drug (i.e., cannabis, cocaine, sedatives, stimulants and/or opioids) dependence (*ANYDEP*) among 7,291 European-Americans (EA; 2,927 cases) and 3,132 African-Americans (AA: 1,315 cases) participating in the family-based Collaborative Study on the Genetics of Alcoholism (COGA). COGA participants were recruited from extended families, most of which were ascertained for alcohol dependence. The *ANYDEP* phenotype is particularly well suited for this ascertained sample as drug dependence more commonly co-occurs with alcohol dependence than with dependence on any other substance[22,23]. We conducted ancestry-specific analyses in EAs and AAs followed by a trans-ancestral meta-analysis (EA+AA) to identify loci associated with *ANYDEP*, i.e., dependence on any one or a combination of alcohol, cannabis, cocaine, sedatives, stimulants and/or opioids. For genome-wide significant (GWS) associations, we performed secondary analyses evaluating associations with individual alcohol and drug dependence diagnoses, and to examine whether the exclusion of those cases who met criteria only for alcohol dependence altered the association. Replication was attempted in two small independent samples that contained EA and AA individuals and substance dependence phenotypes, the Study of Addiction: Genes and Environment (EA: 630 cases, 1,020 controls: AA 387 cases, 415 controls)[24] and the Yale-Penn AA study (AA: 1,525 cases, 485 controls)[25]. Further, any GWS associations with *ANYDEP* in the EA sample were tested for association with alcohol intake among 452,264 individuals from the UK Biobank[26] and cannabis use from a meta-analysis conducted on

184,765 individuals[27]. Finally, given the proposed role of reward-related neural response in the etiology of addiction[28,29], we examined whether GWS loci were correlated with reward-related ventral striatum reactivity as measured with blood-oxygen-level dependent (BOLD) functional magnetic resonance imaging (fMRI) in the independent Duke Neurogenetics Study (EA n=481, AA n=118)[30,31].

MATERIALS AND METHODS

Sample:

COGA is a large family-based study that recruited alcohol dependent probands from treatment facilities across seven sites in the United States[32,33]. Probands and their extended families were invited to participate. Additional individuals and their families were recruited from the same communities using a variety of resources (e.g., dental clinics). Institutional review boards at all sites approved the study, and all participants provided informed consent. All participants were administered a version of the Semi-Structured Assessment for the Genetics of Alcoholism interview (SSAGA; those aged <18 years were administered a child version, the C-SSAGA)[34,35]. Phenotypic data were available on 16,809 individuals. A substantial portion of the sample (n=12,146) has been genotyped. Because the number of individuals of other ethnicities was small, only EA (n=7,983) and AA (n=3,685) individuals were included in these analyses. As the study was ascertained for alcohol dependence, individuals who reported never drinking alcohol even once in their life were excluded from analyses (n=550). The majority of those individuals reported not ever using other drugs, with the exception of cannabis (n=63). The final analytic sample (n=7,291 EA and 3,132 AA) included those with both genotypic and phenotypic data.

Measures:

ANYDEP was defined as a binary variable where cases met lifetime criteria for DSM-IV dependence[36] on alcohol, cannabis, cocaine, sedatives, stimulants and/or opioids (for prescription drugs, non-prescription use was specified) or any combination thereof. We did not include nicotine dependence as it was not assessed in earlier versions of the SSAGA, and was therefore missing for those who were only interviewed using older SSAGAs. Controls did not meet DSM-IV dependence criteria for alcohol or any drug listed above but were required to have consumed at least 1 drink of alcohol. Of the controls, 32.9% met lifetime criteria for DSM-IV alcohol or drug abuse (analyses excluding these individuals are described in the Discussion). For GWS SNPs, alcohol dependence and each individual DSM-IV drug dependence diagnosis was also examined against this uniform set of controls. As COGA was primarily ascertained for alcohol dependence, we created a variable for secondary analysis, *drug_noalc*, where individuals with alcohol dependence were excluded from the *ANYDEP* cases (and remained excluded from controls). Numbers of individuals for each phenotype are in Table 1.

Phenotype analysis:

The prevalence of alcohol and drug dependence were compared across ancestral groups using chi-square tests. The number of DSM-IV criteria endorsed by individuals in AA and EA families were compared (total number and for each drug) using an ordinary least squares

regression that accounted for sex. Birth cohorts (1890–1929, 1930–1949, 1950–1969, ≥1970) were included in all COGA analyses as covariates to account for secular trends (see[37]) across this wide range of birth years. Over and above birth cohort, age was not a significant predictor of *ANYDEP (p > 0.3)*. A confirmatory factor analysis of substance dependence diagnoses was fitted to the data separately for EAs and AAs, to determine phenotypic patterns of comorbidity using MPLUSv8[38]. The comparative fit index (CFI), standardized root mean square residual (SRMR), and the root mean square error of approximation (RMSEA) were used to assess model fit. Heritability for *ANYDEP* and *drug_noalc* was estimated using SOLAR[39] in the EA and AA families separately, using familial relatedness (but not GWAS data) alone.

Genotyping, Quality Review, Ancestry and Imputation

Multiple genome-wide arrays were used to genotype the COGA sample[23,40–42] (see Supplemental Text). A subset of 47,000 common (minor allele frequency (MAF) > 0.1 in the combined sample), independent (defined as $R^2 < 0.5$) and high quality (missing rate < 2% and Hardy-Weinberg Equilibrium (HWE) p-values > 0.001) SNPs that were genotyped across all arrays were used to assess duplicate samples, confirm the reported pedigree structure and compute ancestral principal components (see Supplemental Text for details). After assignment of individuals in a family to a specific population, family-wise ancestry was designated according to the majority of individual family members (see Lai et al, accompanying paper). Only AA and EA families were included in subsequent analyses, due to low numbers of other groups. Only variants with non A/T or C/G alleles, missing rates < 5%, MAF > 3%, and HWE p values > 0.0001 were used for imputation. Genotypes were imputed to 1000 Genomes using the cosmopolitan reference panel (Phase 3, version 5, NCBI GRCh37; Supplemental Text) using SHAPEIT2[43] and Minimac3[44]. Imputed SNPs with $R^2 < 0.30$ were excluded, and genotype probabilities were converted to best-guess genotypes if ≥ 0.90. Because some individuals within a family were genotyped on different arrays, families were again evaluated for Mendelian inconsistencies using Pedcheck[45], and imputed SNPs were cleaned as described above. All genotyped and imputed SNPs with missing rates <25%, MAF ≥1% and HWE $p > 1 \times 10^{-6}$ were included in analyses.

Genome wide association studies and meta-analysis:

Association analysis was performed separately in AA and EA families using a generalized estimating equation (GEE) framework to account for family relatedness by considering each family as a cluster. The GEE employs a logistic regression model (i.e., binomial distribution) to account for relatedness in the R package GWAF[46]. Gender, birth cohort, GWAS array indicator, and the first four principal components (as in[23]) were included as covariates in the model. A trans-ancestral (EA+AA) GWAS was performed by meta-analyzing summary statistics from the EA and AA GWAS using inverse-variance weighting in METAL[47], with genomic control.

Annotation of results and gene-based analyses:

Overall plotting (e.g., regional association) and annotation of individual loci was conducted in FUMA[48]. For gene and gene-set based analysis, MAGMA, as implemented in FUMA, was used. FUMA was utilized to conduct gene-set analyses that examined whether genes

were enriched in curated classification systems, by molecular function, biological process or other criteria. Gene sets were defined for 4,728 curated gene sets (including canonical pathways) and 6,166 GO terms. Differential expression of prioritized genes was conducted using the GENE2FUNC option in FUMA, which examines whether genes of interest from the GWAS are overrepresented in differentially expressed gene sets in 53 specific tissue types from The Genotype-Tissue Expression (GTEx) database[49]. Although this database is comprised of primarily EA individuals, it is one of few publicly available databases available, and therefore was utilized for the AA results as well. To further prioritize possible causal genes, we used S-PrediXcan[50] to impute genetically-regulated gene expression in twelve brain tissues and whole blood. The prediction models were trained on reference transcription data from GTEx (brain) and the Depression Genes and Network (DGN) (whole blood) (all available from the PredictDB Data Repository, http://predictdb.org, downloaded on 6/6/2018). Analyses were restricted to the EA data, as the prediction models used by the tools were built using only individuals of European ancestry. We used GTEx v7 to extract gene expression values. Finally, individual genome-wide significant (GWS) SNPs and genes were examined against SNP and gene-based summary statistics for 3,798 GWAS of 2,824 traits, available through http://atlas.ctglab.nl/ (accessed on 10/1/2018).

Replication:

Data from two dbGaP samples with individuals of EA and AA ancestry that were ascertained for alcohol and substance dependence were utilized for replication of GWS SNPs. These included the Study of Addiction: Genes and Environment (phs000092.v1.p1, SAGE: non-overlapping individuals numbered EA: 630 cases and 1,020 controls; AA: 387 cases and 415 controls)[24] and the Yale-Penn AA sample (phs000425.v1.p1) with 1,525 cases and 485 controls[25]. Any overlapping participants as well as the first and second degree of relatives ($\pi \geq 0.2$) of COGA members in SAGE or Yale-Penn were excluded from the replication samples. Cases and controls were defined as described above. Covariates included sex and the first 3 principal components. For SAGE, birth cohorts as defined in COGA were included as covariates while for Yale-Penn AA, age was used (as recommended in prior publications of this sample[51,52]). Effect sizes across COGA and replication samples were meta-analyzed in METAL [47]. For SNPs associated with *ANYDEP* in the EA families, we also examined summary statistics for association with alcohol intake frequency in 452,264 individuals from the UK Biobank (http://geneatlas.roslin.ed.ac.uk/, accessed 11/26/2018)[26] and with cannabis use from the current largest GWAS of the phenotype [n=184,765[27]].

Neuroimaging analysis of GWS loci:

We examined whether GWS SNPs identified in our COGA GWAS of *ANYDEP* (i.e., rs34066662, rs75168521, rs1890881; the *indel* was not available) were associated with reward-related brain function in the Duke Neurogenetics Study (DNS), an independent neuroimaging sample containing non-Hispanic AA (n=118) and EA (n=481) undergraduate students aged 18–22 years[31] (see Supplemental Text). A number guessing paradigm was used to elicit ventral striatum (VS) reactivity associated with positive and negative feedback linked to monetary gains and losses while bold-oxygen-level dependence (BOLD) functional magnetic resonance imaging (fMRI) data were acquired[53]. Statistical Parametric Mapping

version 8 (SPM8) software was used to extract parameter estimates for the contrast of Positive Feedback > Negative Feedback from maximal voxels within left and right VS regions of interest (ROIs). Imaging acquisition protocol, task, ROIs, and preprocessing details are described in the Supplemental Text. Extracted parameter estimates from VS activity in each hemisphere were regressed on genotype (coded as 0 vs 1 or more copies of the minor allele) of GWS loci while co-varying for sex, and 3 (AA) or 2 (EA) ancestral principal components using Full Information Maximum Likelihood in MPlus v7.3[38]. Confidence intervals on estimates were derived via bootstrapping (n=10,000). To adjust for multiple comparisons, we used a Bonferroni-corrected p-value threshold ($p<0.00625$), to account for our hypothesized 8 tests [i.e., rs34066662 and rs75168521 in both brain hemispheres among AAs (4 tests); rs1890881 in both brain hemispheres among AAs and EAs (4 tests)]. As rates of drug dependence, but not alcohol problems (see[31]), are low in the DNS sample, structural equation models linking genotype to substance dependence with reward-related response as a mediator were not fitted to these data.

RESULTS

Phenotypic analyses:

Alcohol dependence was the largest contributor to *ANYDEP,* followed by cannabis and cocaine dependence (Table 1). Alcohol and drug dependence were correlated with each other in both EAs and AAs (Supplemental Table S1). However, correlations between dependence on alcohol and individual drugs were higher ($r = 0.55 – 0.82$) in EA relative to AA ($r = 0.33 – 0.77$), especially for cannabis dependence (see Supplemental Table S1). A single factor solution fit the lifetime dependence diagnoses data adequately in both EAs and AAs (EA: Comparative Fit Index=0.989, Standardized Root Mean-square Residual=0.046, Root Mean Square Error of Approximation=0.042; AA: Comparative Fit Index=0.978, Standardized Root Mean-square Residual=0.09, Root Mean Square Error of Approximation: 0.047) and factor loadings were greater than 0.75, with the exception of cannabis dependence in AA (loading=0.35).

Other phenotypic differences across ancestral groups in this sample were apparent. For instance, while alcohol dependence was the most common contributing diagnosis in EAs (81%, vs. 67% in AAs), cannabis (EA: 42%; AA: 52%) and cocaine dependence (EA: 27%; AA: 44%) were more common in AAs. Across ancestral groups, *ANYDEP* cases endorsed a similar number of criteria across all substances (i.e., 7 criteria x 6 substances; mean = 10.8, SD = 7.3; beta=0.11, SE=0.24, p=0.66). Among *ANYDEP* cases, EAs endorsed significantly more alcohol dependence criteria than AAs (EA: mean 4.44, SD 2.09; AA; mean 3.89 SD 2.21; beta=0.45, SE=0.06, $p < 0.0001$). Conversely, AAs endorsed a greater number of cannabis (AA: mean 2.76, SD 2.29; EA: mean 2.24, SD 2.27; beta=0.41, SE=0.07, $p < 0.0001$) and cocaine dependence (AA mean 2.82 SD 3.10; EA: mean 1.65, SD: 2.59, beta=1.22, SE=0.09, $p < 0.0001$) criteria than EAs, and these differences were significant even after accounting for sex and birth cohorts. The heritability of *ANYDEP* and *drug_noalc* were 0.60 (standard error (SE) = 0.043) and 0.59 (SE=0.085) respectively, in the EA families. Although the heritability of *ANYDEP* was lower in the AA families (0.37; SE=0.065), the heritability of *drug_noalc* was slightly higher (0.63; SE=0.106).

GWAS findings:

No GWS loci emerged in the EA GWAS (Figure 1a; Supplemental Figure S1A). The lowest p-value (p = 8.6E-08; Table 2) was obtained for rs74611272, an intergenic SNP on chromosome 7. In contrast, three GWS regions were identified in the AA GWAS: on chromosome 3 (rs34066662: p = 1.77E-08 & rs58801820: p = 1.89E-08; Figure 1b; Supplemental Figure S1B), chromosome 13 (rs75168521: p = 3.31E-08 & rs78886294: p = 4.38E-08) and an insertion–deletion (*indel*) on chromosome 5 (5:141988181, mapped to rs527904740, p = 4.48E-08). As shown in Table 2, the effects of these variants were ancestry-specific. In addition, one locus on chromosome 1 was GWS in the trans-ancestral (EA+AA) meta-analysis (Figure 1c, Supplemental Figure S1C), with the most significant SNP, rs1890881 (p = 3.77E-08; EA p = 8.95E-05; AA p = 1.94E-05) in an intron of *RABGAP1L* (RAB GTPase Activating Protein 1 Like).

Specificity of GWAS SNPs to alcohol or drug dependence:

In the AA GWAS, all drugs contributed to the chromosome 3 GWS signal, albeit at nominal levels of significance, while all drugs except opioids and sedatives contributed to the chromosome 5 and 13 signals (Table 3). Alcohol dependence was also associated with these loci (Table 3); however, when a smaller subset of individuals who met criteria for drug but not alcohol dependence was studied (i.e., *drug_noalc*), the loci on chromosome 3, 5 and 13 remained nominally associated (all p<5.04×10–4; Table 4), suggesting that these signals were only partially attributable to shared genetic liability between alcohol and illicit drug dependence. In contrast, the trans-ancestral signal on chromosome 1 was due primarily to association with cocaine and alcohol dependence in both the EA and AA subsamples (Table 3). When individuals with alcohol dependence only were excluded from the study (*drug_noalc*), there was no association (p=0.42) in the EAs and the association in AAs decreased in significance to p=0.04.

Biological annotation:

Regional association plots for the chromosome 3 and chromosome 13 GWS loci from the AA GWAS are presented in Figure 2. The two SNPs on chromosome 3, rs34066662 and rs58801820, are in complete linkage disequilibrium (LD; HapMap AFR sample: r2>0.996; D'=1). There was evidence that one or both of these SNPs were eQTLs for Nephrocystin 3 *(NPHP3)* in the sigmoid colon (GTEx v6: p = 4E-06) and the adrenal gland (GTEx v7: p = 7E-06; reference expression data primarily drawn from Europeans, see chromatin interactions in Appendix Supplemental Figure S3A).

The regional association plot for the GWS *indel* on chromosome 5 (chr5:141988181, mapped to rs527904740) is presented in Supplemental Figure S2A. The next most significantly associated variant, rs74911483 (chr5:141990602, p=6.13E-8), is shown in Supplemental Figure S2B, and was in high LD with this variant (AFR: r2=0.87, D'=0.94[54]). Both variants were in the intron of the Fibroblast Growth Factor 1 (*FGF1*) gene. While FUMA could not be utilized for *indel* annotation, individual searches for the proxy SNP, rs74911483 in RegulomeDB[55] (score of 5), Combined Annotation Dependent Depletion[56] (maximum CADD score of 5), and GTEx[57] (no eQTLs) did not provide persuasive support

for regulatory effects of this variant. However, chromatin interactions were noted with neighboring genes (Supplemental Figure S3).

The strongest signal on chromosome 13 was from rs75168521, a non-coding intergenic SNP downstream of *SLITRK5* (SLIT and NTRK like family member 5); there was no evidence that rs75168521 is an eQTL for *SLITRK5* or any other gene. rs75168521 is a perfect LD proxy for rs78886294 which was also genome-wide significant. Both SNPs were also in high D', but low r2 (D'=1; r2=0.33) with numerous SNPs in the 3' region of *MIR4500HG* with the closest SNP being 108 bp from rs75168521. The SNP rs75168521 made chromatin contact with *MIR4500HG* in bladder, liver and the left ventricle (although the gene is only appreciably expressed in the liver). Several additional distal points of contact were also identified (Supplemental Figure S4). Conditional analyses of the lead variants on chromosomes 3, 5 and 13 indicated that the remaining genome-wide significant SNPs did not represent additional independent loci on each chromosome (Supplemental Figure S5A – S5C). However, additional SNPs in the region did show p-values indicative of potential independent signals that might be clarified with increase in sample size.

The trans-ancestral (EA+AA) analyses identified rs1890881 as genome-wide significant (Figure 3). The SNP is in an intron of *RABGAP1L*; however, it is also an eQTL for several neighboring genes. According to the GTEx (v7) database, rs1890881 is an eQTL for 7 genes (48 signals), that included several signals in brain tissue (Supplemental Table S2). rs1890881 also made chromatin contact with several of these genes, including *SERPINC1* (Supplemental Figure S6; genes identified using eQTL and chromatin interaction mapping are in red) as well as other distal contacts across tissues. There was no support for independent loci in the region in either ancestral group (Supplemental Figure S5D and S5E).

Gene-based and gene-set analyses:

Gene-based analyses in the AA data identified 3 genes that surpassed genome-wide correction ($P_{significance}$ = 2.76E-06, corrected for 18,125 protein coding genes; Supplemental Figure S7; Supplemental Table S3). The genes were SH3 Domain Binding Kinase Family Member 3 (*SBK3*; chromosome 19), DAZ Interacting Zinc Finger Protein 3 (*DZIP3*; chromosome 3) and CRK Like Proto-Oncogene, Adaptor Protein (*CRKL*; chromosome 22). *DZIP3* and *CRKL* are ubiquitously expressed with appreciable expression in brain regions, while *SBK3* is expressed in cardiac tissue (Supplemental Figure S8). Gene-set analyses did not identify any GO terms that surpassed multiple testing correction. We also performed gene function analyses with 26 genes that mapped to the region of 2 of the GWS loci (including, but not limited to *SLITRK5, NPHP3* and *NPHP3-AS1, LINC00433*) on chromosomes 3 and 13. Two positional gene sets (MSigDB_c1) on chromosome 3 and one on chromosome 13 were significantly enriched for prioritized genes (chr3q22, $P_{adjusted}$ = 8.3E-10: *NPHP3, NPHP3-AS1, BFSP2-AS1, SRPRB, C3orf36*; chr3q21, $P_{adjusted}$ = 1.6E-4: *TMEM108, BFSP2, TF*; and chr13q31, $P_{adjusted}$ = 1.4E-09: *SLITRK5, PEX12P1, KRT18P27*). Of these, Transferrin (*TF*) in particular, showed higher average differential expression in brain tissue (Supplemental Figure S9).

Despite no individual SNP being genome-wide significant in the EA GWAS, one gene was genome-wide significant (*P2RX6*, p=7.11E-07; threshold $P_{significance}$ = 2.82E-06 for 17,757

coding genes; Supplemental Table S4). The Purinergic receptor P2X 6 (*P2RX6*) is expressed in brain tissue, although less robustly than in musculo-skeletal tissue (Supplemental Figure S10). In addition, 4 curated gene-set terms were statistically significant (Supplemental Table S5 for individual genes in the set). These gene sets were derived from Reactome and reflected gene sets involved in signal transduction (sets 1 and 2) and hemostasis (sets 3 and 4). When the correlation between *ANYDEP* and imputed, genetically-regulated gene expression was tested in the EA sample using S-PrediXcan, no genes met the multiple testing corrections; however, *P2RX6* was the most significant gene, with $p = 3.91e\text{-}05$ in the putamen basal ganglia tissue model.

Replication:

Despite these promising findings, the individual loci did not replicate in any of the replication samples (Supplemental Table S6; lowest p =0.06 for chr5:141988181 in Yale-Penn) and meta-analysis across COGA and the replication samples did not retain their genome-wide significance, although findings for the chromosome 3 locus were in the same direction in the AA samples, and for chromosome 1 in the EA samples. In the UK Biobank, there was weak evidence of association between alcohol intake frequency and rs1890881 (beta=−0.010, p=0.026). In addition, rs1890881 was marginally associated with cannabis use (beta=0.029, p=0.048) in the current largest GWAS of the trait[27]. There was evidence of association between alcohol intake and rs74611272 (beta=0.016, p=0.007;[26]), the strongest signal in the EA GWAS, in the UK Biobank.

Extension of Neuroimaging to Significant Loci:

As shown in Table 5, carriers of the minor (C) allele of rs75168521 (chr 13), which was associated with increased likelihood of *ANYDEP* in COGA, had blunted right (beta=−0.111, p=0.004), but not left (beta=−0.065, p=0.143), VS activation in the AA subsample. However, rs34066662 genotype (chr 3) was not associated with VS activity in either hemisphere among AAs, despite a similar directional pattern (betas>−0.075, ps >0.103). As expected, among EA there was no association between rs75168521 or rs34066662 genotype and VS activation in either hemisphere (|betas|<0.025, ps >0.247). Carriers of the minor (C) allele of rs1890881 (chr 1), which was associated with decreased likelihood of *ANYDEP* in the trans-ancestral meta-analysis (effect driven by alcohol dependence), were characterized by blunted reactivity of the left VS among AA (beta=−0.134, p=0.001). Nominally significant associations that were not robust to Bonferroni correction were observed between rs1890881 genotype and right VS activation in AA (beta = −0.098, p = 0.013) and left (beta=−0.036, p=0.029), but not right (beta=−0.004, p=0.801), VS activation among EA.

DISCUSSION

Alcohol and drug dependence tend to co-aggregate in families[58,59]. Based on a prior GWAS in a smaller subset of 118 COGA families[23], we developed an *ANYDEP* phenotype that represented a diagnosis of dependence on alcohol or any illicit drug. GWAS in the COGA AA families identified novel loci on chromosomes 3, 5, and 13 while the trans-ancestral EA +AA analysis identified a locus on chromosome 1. However, these signals failed to replicate in independent samples. In addition, across the AA and EA GWAS, a total of 4 genes (AA

and EA) and 4 gene sets (EA only) survived correction for multiple testing. These findings underscore the feasibility of using an aggregate substance dependence phenotype to identify underlying shared heritable influences for locus discovery.

Broadly, two categories of loci were identified. First, in the COGA AA families, loci on chromosome 3, 5, and 13 were GWS and appeared to be attributable to contributions from each individual illicit drug, as well as alcohol dependence. Exclusion of alcohol dependence diagnosis (*drug_noalc*) resulted in nominal significance in all three regions, despite the substantially reduced sample size and power. Thus, these loci may represent genetic liability that is common to alcohol and illicit drug dependence that cannot be entirely attributed to alcohol dependence. On the other hand, the locus on chromosome 1 which was GWS in the COGA EA+AA analysis and was supported by signals in both the EA and AA subsamples, showed nearly no evidence for association in the *drug_noalc* analyses (p=0.04; and only in the AA families), suggesting that this signal is primarily due to alcohol dependence. In EAs, this genome-wide significant SNP (rs1890881) is in perfect LD with rs61826952 which was genome-wide significant in the COGA GWAS of DSM-IV alcohol dependence (COGA EA +AA p=8.4E-11; see accompanying paper by Lai et al.). The r^2 in the AFR reference populations is 0.48, potentially indicating an independent signal in the AAs. However, conditional analyses did not support an effect of rs61826952, independent of rs1890881, on *ANYDEP* (Supplemental Figures 5D and 5E). Similarly, rs1890881 was not associated with alcohol dependence (Lai et al., accompanying paper), independent of rs61826952 (AA p=0.118; EA p=0.559).

For *ANYDEP*, rs1229984 in *ADH1B*, the most well-replicated signal for alcohol dependence[12] was not GWS. Even relative to findings from the companion paper by Lai et al., where rs1229984 was GWS in the EA+AA analysis (beta=−0.86, p=1.72E-8), the association between rs1229984 and *ANYDEP* was considerably weaker both in magnitude and significance (Supplemental Table S7), despite a considerably larger analytic sample. Additional loci identified for alcohol dependence diagnosis, symptom count and individual criteria in Lai et al., also did not achieve GWS for *ANYDEP*, although all signals were nominally significant with effects in an identical direction. An important distinction between these two companion studies is noteworthy. While Lai et al., excluded individuals with ≥ 2 alcohol or drug abuse/dependence criteria from their unaffected population, the current study allowed these individuals to remain in the unaffected group. Thus, variations across findings in the two studies might be due, not only to differing definitions of affecteds, but also the definition of unaffecteds. Finally, the current study did not identify the same SNPs as were noted in our prior study of *ANYDEP* and its quantitative equivalent in a much smaller subset of these data (N=1,170 – 2,183; Supplemental Table S7), which is not uncommon with the substantially increased sample size used here.

In addition to sources of genomic variation (e.g., allele frequencies, LD), distinctions in findings across the ancestral groups are possibly attributable to the pattern of comorbidities in these groups, which may be genetic and environmental in nature. Notably, a fair proportion of AA qualified for a diagnosis of *ANYDEP* due to cannabis or cocaine dependence, whereas the preponderance of EAs primarily endorsed alcohol dependence. In addition, cannabis and cocaine dependence diagnoses in AA were relatively more severe

(i.e., more criteria were endorsed). Furthermore, *drug_noalc* (*h2*=0.63) was more heritable than *ANYDEP* (*h2*=0.37) and alcohol dependence (*h2*=0.27) itself in the AA but not the EA families. Thus, despite the smaller sample size, the AA subsample may have been better powered to identify loci more closely related to drug dependence. These patterns of individual and comorbid drug use disorders are also quite consistent with the broader epidemiological literature[3,21]. For instance, AA are more likely to initiate use of cannabis prior to alcohol and are more likely to escalate to problem use[60,61]. Similarly, AA are at nearly 3.5 increased odds of transitioning from cocaine use to dependence than their EA counterparts, even after adjustment for sociodemographic features and psychiatric comorbidity[62]. However, these population differences may reflect socio-cultural trends or represent barriers to access to prevention programs among minority populations, thus increasing rates of lifetime drug dependence[63]. While we might speculate that the three loci identified in the AA GWAS are more likely to relate to liability to both alcohol and drug dependence, this observation may merely be an artifact of cultural effects on the expression of genetic susceptibility.

Alternatively, the AA findings might be false positives. Due to sparser LD in AA relative to EA, the application of a uniform threshold of $p < 5E\text{-}08$ for attributing GWS in AAs may not be sufficiently stringent[64,65]. However, gene-based tests were also successful at identifying three significant genes. Thus, at least for the gene-based tests, even after correction for differences in LD, significant findings in the smaller AA sample were identified.

Despite several findings at the level of individual loci, genes and even gene-set terms (for EA), none of the biological units identified in this GWAS were related to genes previously linked to alcohol or drug related phenotypes. The gene sets, for instance, were broadly related to hemostasis and signal transduction. Prior gene set enrichment analyses have identified other gene sets related to signal transduction more broadly but not specifically via our gene-set terms[66,67]. However, as shown in Supplemental Figure S11, loci on chromosome 1, 3, 13 and 7, as well as the 4 genes that surpassed genome-wide correction (*CRKL, DZIP3, SBK3, P2RX6*) did show associations ($p < 0.05$) with other psychiatric, cognitive and behavioral traits. For instance, based on comparisons with other published and unpublished GWAS across multiple phenotypes, *CRKL* variants have been linked to age at smoking cessation (p=0.002) while *DZIP3* variants have been related to bipolar disorder (p=3.3E-05). Nonetheless, the nature of the effect of these variants on alcohol and illicit drug dependence remains unknown.

The relatively small sample size for the replication cohorts, especially when examining AA individuals, may have contributed to our limited evidence for replication. However, the neuroimaging extension provides evidence for an interesting, albeit preliminary, link between GWS loci and ventral striatum (VS) reactivity. For rs75168521, African American carriers of the C allele, which was associated with increased likelihood of *ANYDEP* in the COGA GWAS, had blunted response to positive versus negative feedback. Decreased reward sensitivity to rewarding, non-drug stimuli has been well documented within addiction, with evidence that this may arise following persistent drug exposure[68]. However, as the DNS sample is characterized by relatively low levels of substance use and related problems (other

than alcohol[31]), the association between genotype and blunted VS response to reward may plausibly be viewed as a predisposing factor. One might speculate that individuals, particularly adolescents, with a blunted response to rewarding stimuli, in general, may require larger drug amounts or more potent drugs for reinforcement, and thus, be more susceptible to the development of severe addiction[69]. Such an interpretation is consistent with evidence that unaffected offspring of alcohol dependent individuals and adolescents who later develop problematic drug use have reduced VS response to anticipatory cues of monetary reward[70,71] as well as evidence that individuals who are less sensitive to the intoxicating effects of alcohol are at greater risk for dependence[72].

In direct contrast to the results for rs75168521, rs1890881 (chromosome 1) major T allele homozygotes, who were at increased risk for *ANYDEP* (driven by the association with alcohol dependence) in the COGA GWAS, had elevated reward-related VS response (identical to Lai et al.). While these findings may, on the surface, appear to be inconsistent with one another, literature suggests that both relatively reduced and heightened VS response to reward may be associated with substance involvement and dependence liability according to unique and shared mechanisms[31]. For example, evidence that reward-related VS activity is positively coupled with problematic drinking[73] as well as behavioral and self-reported impulsivity[74], converges with stage-based theories of addiction postulating that elevated impulsivity may lead to greater substance use exposure and experimentation that may lay the foundation for the development of problematic substance use. On the other hand, a parallel literature has also linked relative hypoactivity of the VS to drug-seeking behaviors, which has often been theorized to reflect compensation for blunted reactivity to reward[71,75]. Thus, it is plausible that blunted VS reward response associated with rs75168521 may confer susceptibility to extreme and generalized forms of drug dependence. On the other hand, the finding for rs1890881 might typify individuals at high neurobiological susceptibility for substance use engagement, particularly with alcohol which is easily accessible and socially accepted. Given the heterogeneity of substance use-related phenotypes, it is plausible that different genetic risk markers may impact disease risk through distinct mechanisms, and that these seemingly divergent theoretical models (e.g., impulsivity vs reward deficiency) may not be mutually exclusive.

Lastly, laterality differences were apparent following multiple testing correction (i.e., rs75168521 genotype was associated with blunted right, but not left, VS response while rs1890881 genotype was associated with blunted left, but not right VS response in AA). While prior reports have found evidence of lateralized associations with reward-related processing in the VS[76,77], the directionality of associations in our study were consistent across hemispheres and in some cases reached nominal levels of significance (Table 5). As such, it is possible that our lateralized findings resulted from limited power. Overall, our neuroimaging findings, while preliminary, showed ancestral specificity consistent with the GWAS, and suggest a putative role for the *ANYDEP*-associated variants in general reward responsiveness. However, despite correction for multiple testing, it is also plausible that these findings represent a false positive given our small sample, which also prevented us from testing potential quadratic effects which might be expected given that both relatively reduced and heightened VS response to reward were associated with genetic risk for substance use phenotypes.

Several limitations are worth noting. First, despite interesting findings and the partially high risk sample design, our sample size is underpowered to detect the modest effect sizes typically associated with substance use disorders[12]. For instance, in the AA subsample, 80% power to detect a common variant (MAF ≥ 35%) is only expected for genotype relative risks≥1.28[78], which is fairly high for psychiatric disorders. Second, due to the high degree of relatedness in our data, ascertainment, and the relatively small number of "cases", methods such as GCTA[79] or LD Score regression[80] that are typically used to assess SNP-heritability were not appropriate. Instead, we report heritability using familial relatedness. Third, we elected to derive diagnoses based on the DSM-IV nomenclature for dependence instead of DSM-5 definitions for substance use disorders. Even though the DSM-5 definition of substance use disorders is more contemporary, it relies on a lower symptom burden (e.g., ≥2 of 11 vs ≥3 of 7 criteria for DSM-IV dependence), which may dilute identification of genetic effects on more severe forms of the disorder. However, when we examined the association between GWS variants and a count of DSM-5 criteria across alcohol and illicit drugs, these SNPs were associated with that count but not at GWS levels (p>5E-5). Finally, 39% of the controls met criteria for substance abuse – we elected to include these individuals to maintain the sample size. Consistent with this, results for chromosome 3 (AA p=2E-8) remained GWS while those for chromosomes 1 (EA p=3E-4; AA p=6E-5), 5 (AA p=7E-8) and 13 (AA p=4E-5) were attenuated in statistical significance but not in magnitude upon exclusion of individuals with abuse from the controls. Finally, the lack of replication was discouraging, although it is noteworthy that sample sizes for the replication cohorts were modest, and they may not have had sufficient power to replicate findings with these effect sizes. Nonetheless, upon meta-analysis, these variants did not show consistent genome-wide support indicating considerable heterogeneity across-samples, low power, or raising the possibility that the current findings are false positives.

In conclusion, we leveraged the high degree of comorbid substance dependence in COGA to identify novel loci that may confer risk for both alcohol and drug dependence and parse them from those variants that relate more specifically to alcohol dependence liability (Lai et al., accompanying paper). Our results provide preliminary evidence for ancestrally-specific effects of loci that undergird addiction to alcohol and illicit drugs. Further, we find preliminary ancestry-specific evidence that GWS loci associated with dependence liability are also associated with reward-related VS response providing a compelling putative neural mechanism through which genetic risk might influence dependence liability. Notably, large scale GWAS of psychiatric disorders, with the exception of substance use disorders, have traditionally focused on populations of European ancestry. While the genetics of substance use disorders has been examined in AAs (e.g., [81,82]), sample sizes remain fairly modest, especially given the potential expectation of a higher burden of multiple testing. To delineate the role of genetic influences on substance use disorders in such minority populations, who also may further suffer due to restricted access to treatments, targeted data collection is needed.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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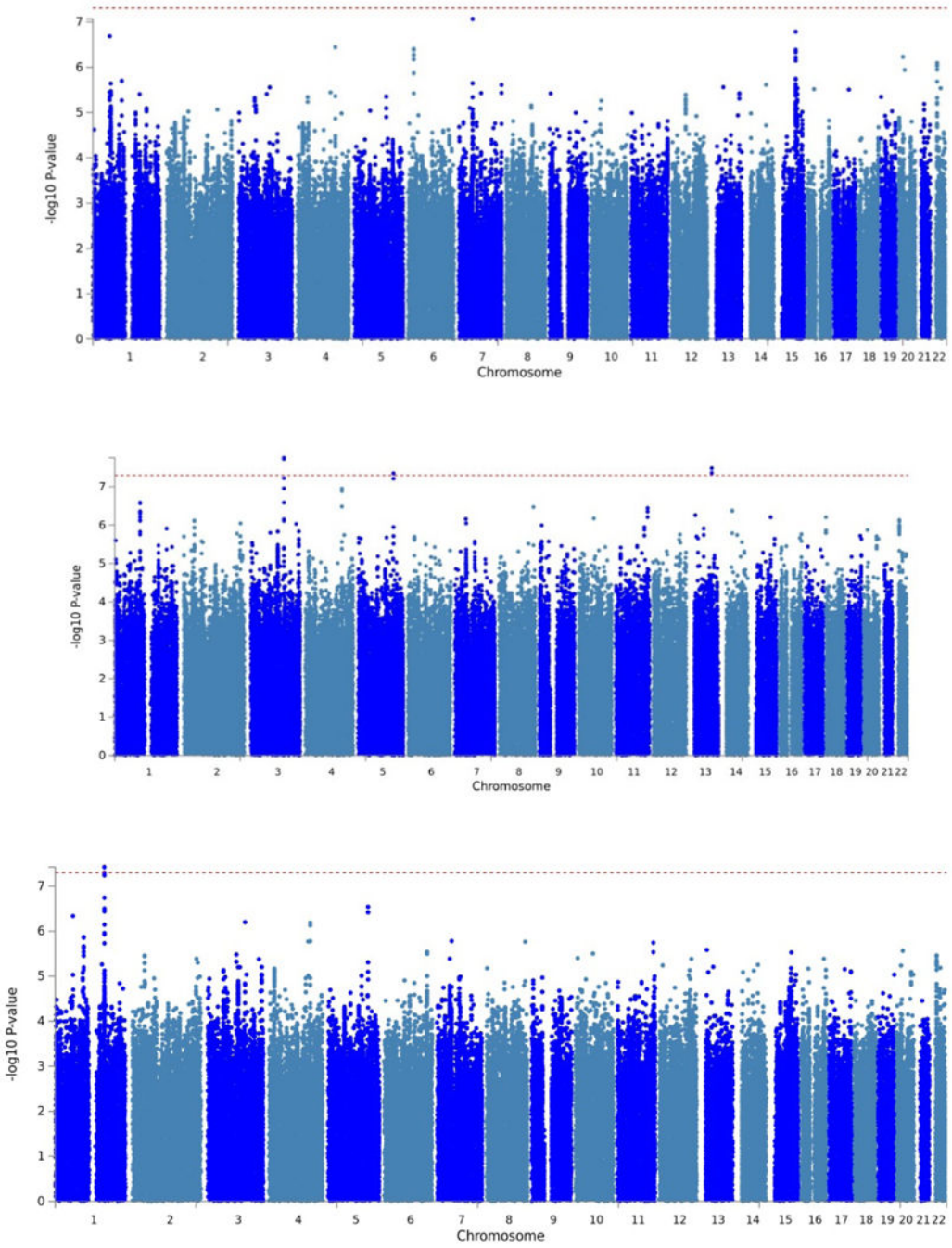
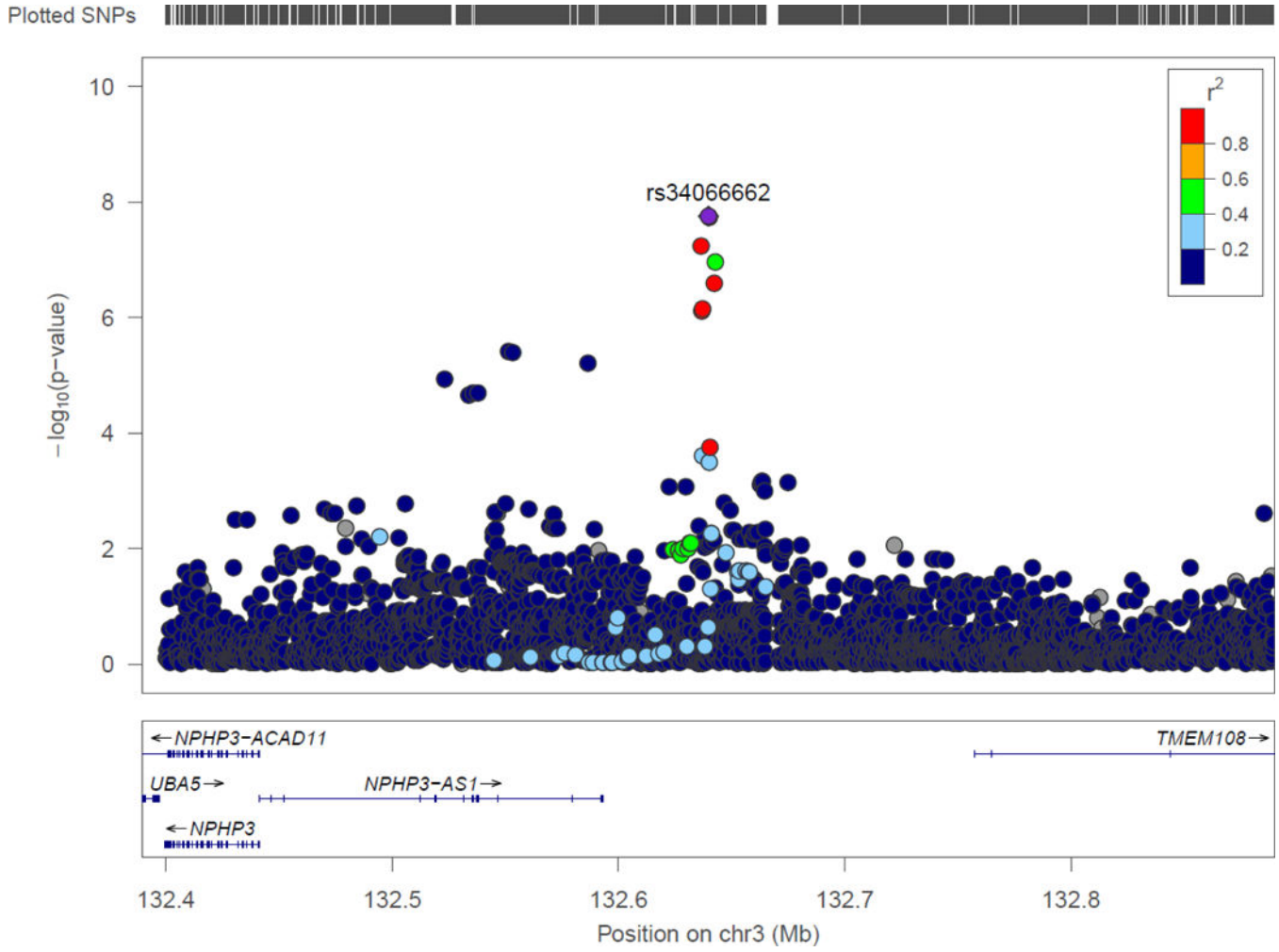


Figure 1:
Manhattan plots for the (PANEL **a**) European-American (EA), (Panel **b**) African-American (AA), and (PANEL **c**) trans-ancestral (EA+AA).



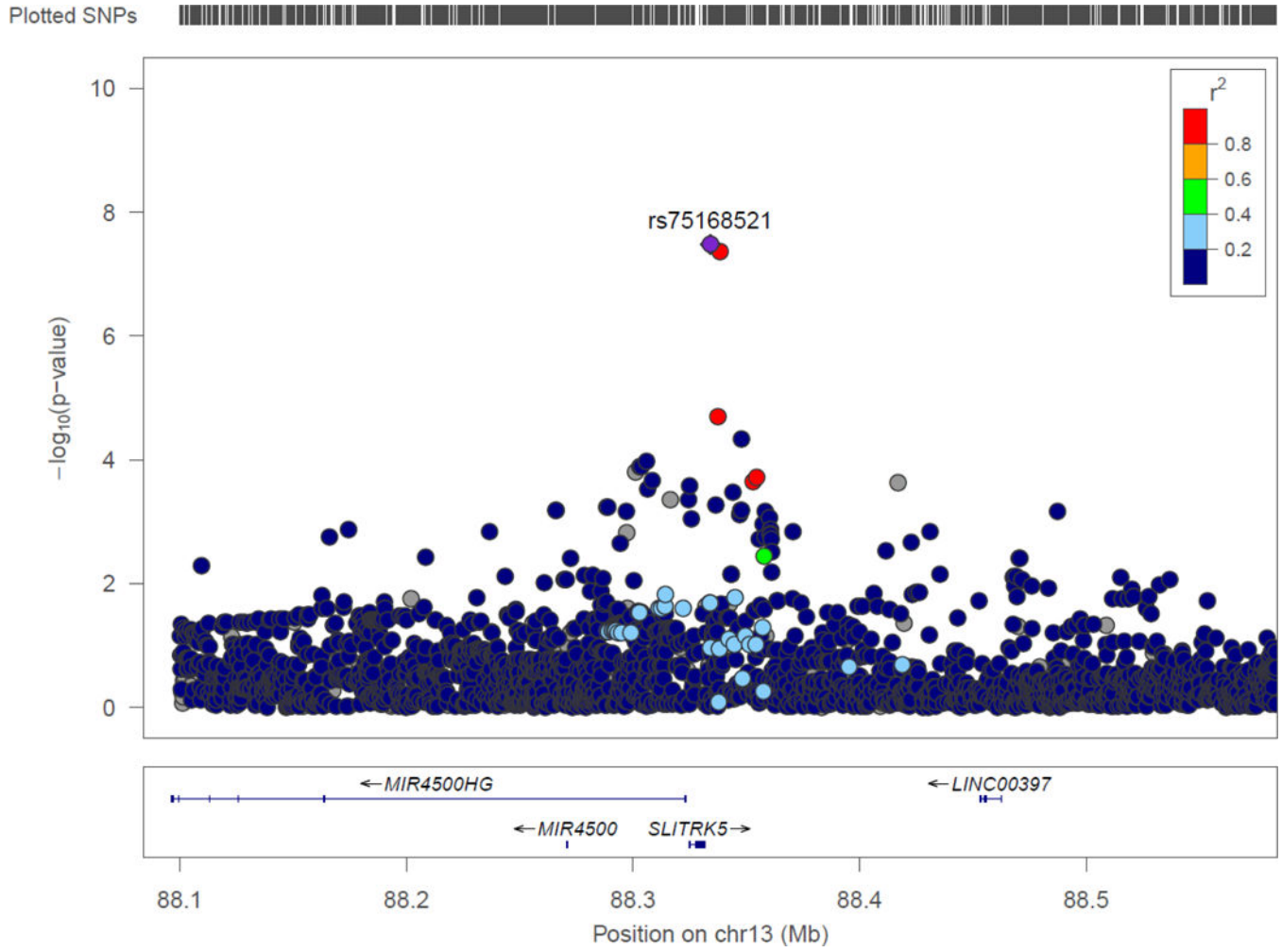
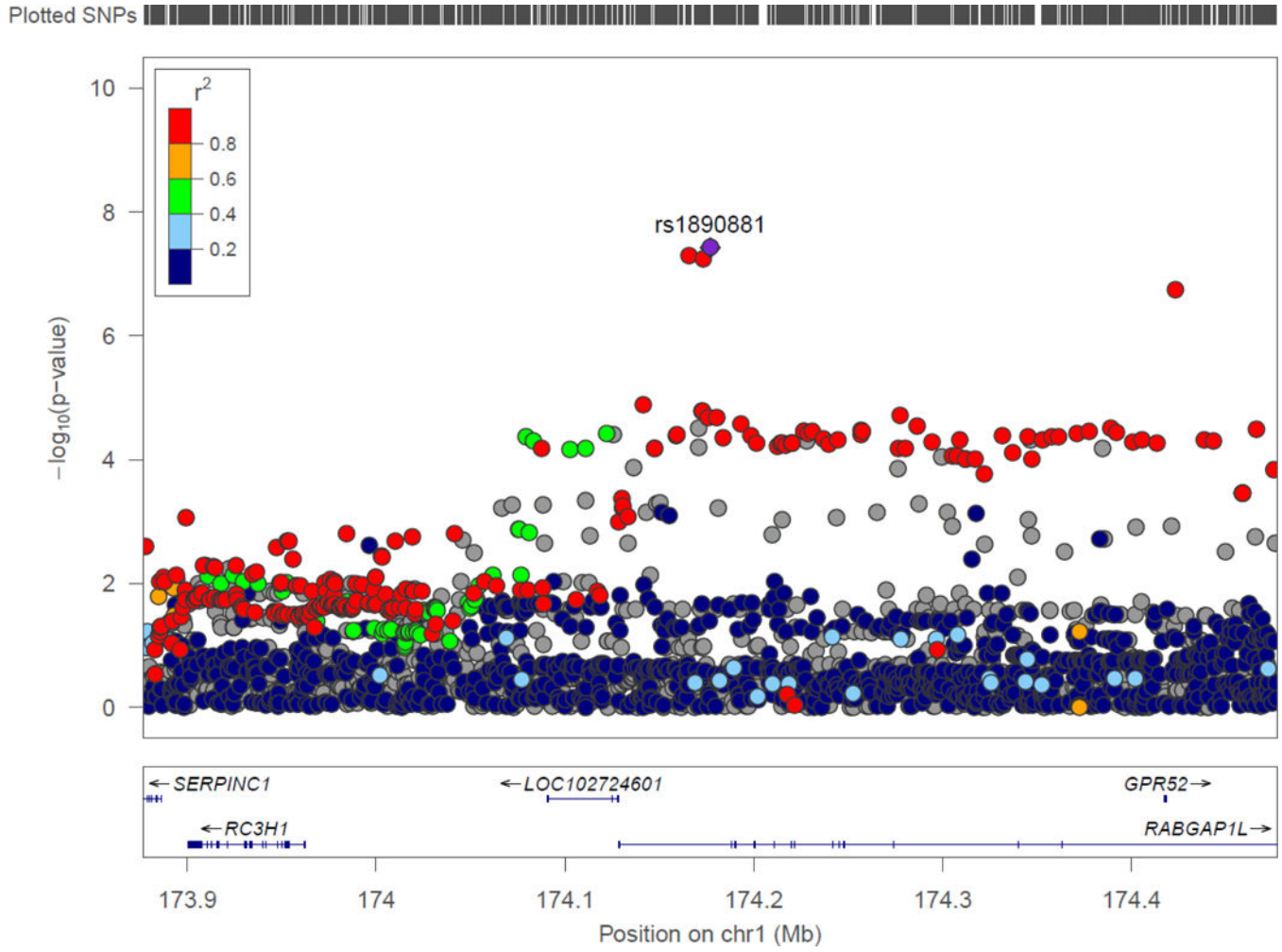


Figure 2:

Regional association plots for genome-wide significant loci on (**a**) chromosome 3 and (**b**) chromosome 13 in analyses of AA families



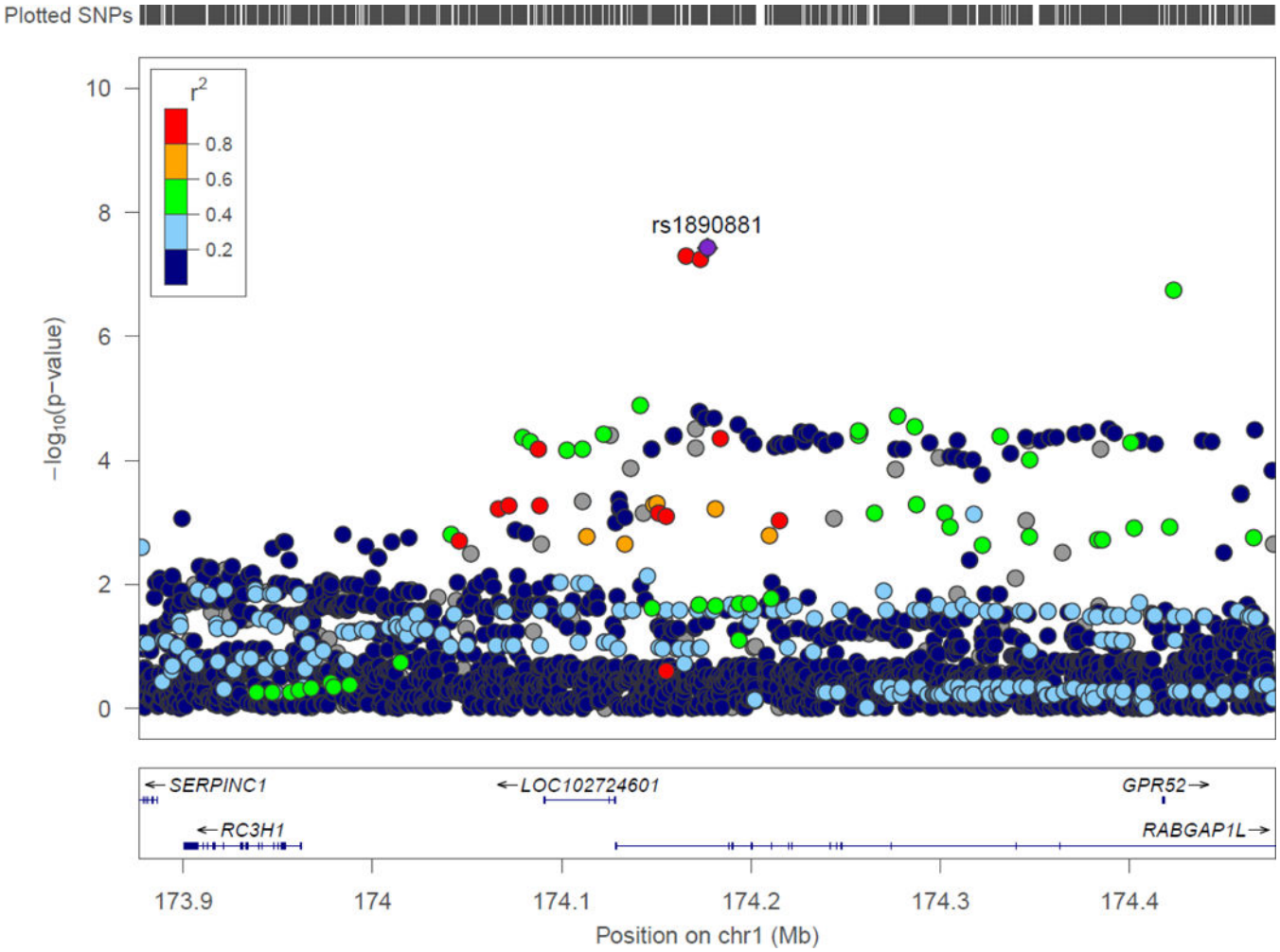


Figure 3:

Regional association plots for genome-wide significant loci on chromosome 1 in the trans-ancestral (EA+AA) analysis shown with (**a**) LD based on CEU population, and (**b**) LD based on AFR population

Table 1.

Sample size for primary analysis of dependence on alcohol or any illicit drug (ANYDEP), as well as for post-hoc analyses of top loci for alcohol and individual drug phenotypes.

	European-American (EA)		African-American (AA)	
	Cases (#)	Controls (#)	Cases (#)	Controls (#)
ANYDEP	2,927	4,364	1,315	1,817
Alcohol	2,351	4,364	901	1.817
Cannabis	1,228	4,364	667	1,817
Cocaine	765	4,364	581	1,817
Sedatives	267	4,364	31	1,817
Stimulants	530	4,364	53	1,817
Opioids	334	4,364	142	1,817
drug_noalc	563	4,364	422	1,817

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Table 2:

Results for most significant SNPs associated with *ANYDEP*.

	Chr:bp	Effect allele: alternate allele	Effec tallele frequency (EA:AA)	EA: beta, SE, p-value	AA beta, SE, p-value	Trans-ancestral (EA+AA) beta, SE, p-value
rs34066662	3:132639776	T:C	0.06:0.19	0.094 (0.082), 0.249	−0.398 (0.071), 1.77E-08	−0.181 (0.056), 1.33E-03
rs58801820	3:132640091	T:G	0.06:0.19	0.094 (0.070), 0.249	−0.393 (0.082), 1.89E-08	−0.180 (0.056), 1.28E-03
rs75168521	13:88334193	T:C	0.91:0.85	−0.037 (0.073), 0.615	−0.428 (0.078), 3.31E-08	−0.217 (0.056), 1.11E-04
rs78886294	13:88338399	T:C	0.91:0.85	−0.035 (0.073), 0.626	−0.426 (0.078), 4.38E-08	−0.212 (0.056), 1.41E-04
rs527904740	5:141988181	GAA:GAAA	NA:0.95	-	−0.694 (0.127), 4.48E-08	-
rs1890881	1:174176923	T:C	0.93:0.85	0.327 (0.083), 8.95E-05	0.350 (0.082), 1.94E-05	0.339 (0.062), 3.77E-08
rs74611272	7:51850533	T:C	0.96:0.99	−0.520 (0.097), 8.6E-08	0.118 (0.292), 0.687	−0.46 (0.096), 1.65E-06

Legend: Covariates included sex, birth cohorts, ancestral principal components, array;

Chr = chromosome; Bp = base pair; EA = European American; AA = African American; SE = standard error;

Table 3.

Results for individual drugs for top variants associated with *ANYDEP*.

	Chr:bp	Alcohol Beta, SE, p-value	Cannabis beta, SE, p-value	Cocaine beta, SE, p-value	Stimulant beta, SE, p-value	Sedative beta, SE, p-value	Opioid beta, SE, p-value
				In African American (AA) GWAS			
rs34066662	3:132639776	−0.396 (0.08), 1.70E-06	−0.347 (0.087), 8.05E-05	−0.305 (0.091), 8.24E-04	−0.612 (0.313), 0.051	−1.40 (0.534), 0.009	−0.733 (0.227), 0.0013
rs58801820	3:132640091	−0.393 (0.08), 1.74E-06	−0.343 (0.087), 8.19E-05	−0.303 (0.091), 8.55E-04	−0.586 (0.313), 0.061	−1.36 (0.535), 0.011	−0.734 (0.228), 0.0013
rs527904740	5: 141988181	−0.730 (0.144), 4.24E-07	−0.645 (0.154), 2.90E-05	−0.507 (0.184), 5.88E-03	−0.852 (0.414), 0.04	−0.759 (0.558), 0.17	−0.516 (0.321), 0.11
rs75168521	13:88334193	−0.403 (0.09), 4.08E-06	−0.370 (0.091), 4.49E-05	−0.281 (0.112), 0.012	−0.593 (0.254), 0.019	−0.562 (0.313), 0.07	−0.154 (0.208), 0.459
rs78886294	13:88338399	−0.402 (0.09), 4.57E-06	−0.368 (0.090), 4.71E-05	−0.287 (0.110), 0.009	−0.581 (0.252),0.021	−0.547 (0.313), 0.08	−0.180 (0.204), 0.380
rs1890881	1:174176923	0.438 (0.09), 7.22E-06	0.171 (0.111), 0.125	0.330 (0.105), 1.6E-03	−0.450 (0.245), 0.065	0.167 (0.342), 0.626	0.161 (0.189), 0.393
				In European American (EA) GWAS			
rs1890881	1:174176923	0.366 (0.09), 5.36E-06	0.245 (0.110), 0.026	0.344 (0.130), 0.008	0.197 (0.140), 0.159	0.222 (0.185),0.231	0.100 (0.169), 0.550
rs74611272	7:51850533	−0.487 (0.102), 1.79E-06	−0.597 (0.122), 9.59E-07	−0.492 (0.152), 1.24E-03	−0.411 (0.167), 0.014	−0.463 (0.258), 0.07	−0.513 (0.223), 0.022

Legend: Covariates included sex, birth cohorts, ancestral principal components, array; for some SNPs and individual drugs, statistical convergence issues arose when including birth cohorts, and thus, age was substituted for birth cohort.

Chr = chromosome; Bp = base pair; SE = standard error; GWAS = genome-wide association study; SNP = single nucleotide polymorphism

Table 4:

Results for alcohol dependence and for *drug_noalc* (alcohol dependent individuals excluded from cases and controls) for top SNPs.

	Chr:bp	Effect allele: alternate allele	Alcohol dependence (Lai et al*)	*drug_noalc*
		African-American (AA) GWAS		
rs34066662	3:132639776	T:C	−0.454 (0.092), 8.92E-07	−0.396 (0.110), 3.14E-04
rs58801820	3:132640091	T:G	−0.451 (0.092), 9.00E-07	−0.393 (0.109), 3.01E-04
rs527904740	5:141988181	GAA: GAAA	−0.767 (0.182), 2.65E-05	−0.628 (0.169), 2.06E-04
rs75168521	13:88334193	T:C	−0.486 (0.112), 1.56E-05	−0.371 (0.107), 4.88E-04
rs78886294	13:88338399	T:C	−0.484 (0.113), 1.77E-05	−0.368 (0.106), 5.04E-04
rs1890881	1:174176923	T:C	0.554 (0.121), 4.65E-06	0.235 (0.116), 0.04
		European-American (EA) GWAS		
rs1890881	1:174176923	T:C	0.471 (0.108), 1.29E-05	0.012 (0.014), 0.42
rs74611272	7:51850533	T:C	−0.442 (0.121), 2.61E-04	−0.073 (0.018), 5.10E-05

*Refers to accompanying paper by Lai et al which examined alcohol dependent cases (DSM-IV dependent) and controls (drank at least one drink of alcohol and endorsed at most 1 criterion for alcohol or any drug dependence).

Chr = chromosome; Bp = base pair; SNP = single nucleotide polymorphism

Table 5.

Associations between response of the ventral striatum to positive > negative feedback and genotype in the Duke Neurogenetic Sample

		African-Americans (AA): beta [95% C.I.], p-value		European-Americans (AA): beta [95% C.I.], p-value	
SNP	Effect allele	**RIGHT VS**	**LEFT VS**	**RIGHT VS**	**LEFT VS**
rs34066662	T	−0.073 [−0.164 – 0.019], 0.103	−0.062 [−0.143 – 0.018], 0.132	0.002 [−0.053 – 0.057], 0.932	−0.008 [−0.057 – 0.043], 0.762
rs75168521	C	−0.111 [−0.19 - −0.036], 0.004	−0.065 [−0.153 - −0.24], 0.143	0.025 [−0.017 – 0.068], 0.247	0.011 [−0.027 – 0.048], 0.565
rs1890881	C	−0.098 [−0.175 - −0.020], 0.013	−0.134 [−0.213 - −0.057], 0.001	−0.004 [−0.039 – 0.030], 0.801	−0.036 [−0.068 - −0.004], 0.029

SNP = single nucleotide polymorphism; chr5:141988181 was not available. Original article reported the T allele as the effect allele for rs1890881.