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Exome Chip Meta-analysis Fine Maps Causal Variants and Elucidates the Genetic Architecture of Rare Coding Variants in Smoking and Alcohol Use

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ABSTRACT

BACKGROUND: Smoking and alcohol use have been associated with common genetic variants in multiple loci. Rare variants within these loci hold promise in the identification of biological mechanisms in substance use. Exome arrays and genotype imputation can now efficiently genotype rare nonsynonymous and loss of function variants. Such variants are expected to have deleterious functional consequences and to contribute to disease risk.

METHODS: We analyzed ~250,000 rare variants from 16 independent studies genotyped with exome arrays and augmented this dataset with imputed data from the UK Biobank. Associations were tested for five phenotypes: cigarettes per day, pack-years, smoking initiation, age of smoking initiation, and alcoholic drinks per week. We conducted stratified heritability analyses, single-variant tests, and gene-based burden tests of nonsynonymous/loss-of-function coding variants. We performed a novel fine-mapping analysis to winnow the number of putative causal variants within associated loci.

RESULTS: Meta-analytic sample sizes ranged from 152,348 to 433,216, depending on the phenotype. Rare coding variation explained 1.1% to 2.2% of phenotypic variance, reflecting 11% to 18% of the total single nucleotide polymorphism heritability of these phenotypes. We identified 171 genome-wide associated loci across all phenotypes. Fine mapping identified putative causal variants with double base-pair resolution at 24 of these loci, and between three and 10 variants for 65 loci. Twenty loci contained rare coding variants in the 95% credible intervals.

CONCLUSIONS: Rare coding variation significantly contributes to the heritability of smoking and alcohol use. Fine-mapping genome-wide association study loci identifies specific variants contributing to the biological etiology of substance use behavior.

Keywords: Alcohol, Behavioral genetics, GWAS, Heritability, Nicotine, Tobacco

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Tobacco and alcohol use together account for more morbidity and mortality in Western society than any other single risk factor or health condition (1). These preventable and modifiable behaviors are heritable (2), but previous human and model organism research, including genome-wide association studies (GWASs) of common variants, have resulted in few associated genetic variants, which most prominently feature genes involved in alcohol/nicotine metabolism and nicotinic receptors (3–7).

Advances in sequencing, genotyping, and genotype imputation now allow cost-effective investigation of rare and low frequency variants. Compared with common variants (minor allele frequency [MAF] >1%) most commonly used in GWASs, rare variants have greater potential to elucidate biological mechanisms of complex traits, including substance use and addiction (8,9). In particular, nonsynonymous and loss-of-function coding variants, which result in the loss of normal function of a protein, may have greater phenotypic impact and more direct mechanistic interpretation than other variants that do not have obvious biological consequences (10,11).

No large-scale genome- or exome-wide study of rare variation has been conducted to date. The vast majority of existing addiction-related rare variant studies have used targeted sequencing of putative addiction-associated loci to discover and test for association in relatively small samples. Existing research has led to intriguing leads, including rare variant associations in loci that span nicotinic receptor gene clusters (12–21) and alcohol metabolism genes (22–24) for nicotine and alcohol dependence, respectively. This strategy has also produced rare variant associations in novel loci. In one case, gene-level association tests were used to find an association with rare variants in *SERINC2* (24). In another case, a burden test across *PTP4A1*, *PHF3*, and *EYS* showed association with alcohol dependence (25). Unfortunately, these genes are not obviously involved in etiological processes related to addiction, and replications have not been reported to date.

Previous studies have also attempted to leverage information about predicted functional consequences of rare mutations to improve association analyses. One study of nicotine dependence found significant rare single-variant associations in *CHRNB4*, but only when variants were weighted by their predicted effect on the cellular response to nicotine and acetylcholine (26). Such positive findings could benefit from replication, which has not always been straightforward. For example, all rare variant associations in addiction are, to our knowledge, candidate gene analyses with type I error thresholds based only on the number of tests within that region. Historically, such analyses have produced overly optimistic estimates of the number of associated loci (27). Genome-wide analyses with more conservative type I error thresholds have reported null rare variant findings across an array of phenotypes relevant to addiction (28–30). Precisely because genome-wide analyses are conducted on many variants across the genome, they are in principle able to discover novel rare variant associations within new or known loci. One way to improve power in genome-wide analyses is through genetic association meta-analysis, which entails the aggregation of results across many studies to achieve large sample sizes.

Here, we attempted to expand on these previous discoveries by conducting the largest meta-analytic investigation of exonic rare variants to date. We conducted an exome-wide association meta-analysis of nicotine and alcohol use across 16 studies genotyped on the exome array, which genotypes low-frequency nonsynonymous and putative loss-of-function exonic variants. We combined these data with the UK Biobank, which includes approximately 400,000 individuals of European ancestry with genotype imputation to the Haplotype Reference Consortium (31) imputation reference panel and relevant smoking/drinking phenotypes. Sample sizes for well-imputed variants were thus enlarged, and the availability of noncoding variants from UK Biobank enabled comprehensive analysis of genetic architecture (32) and fine mapping (33).

We conducted single-variant and gene-based tests of association with five smoking and drinking phenotypes. We applied a novel fine-mapping analysis to prioritize causal variants using statistical and functional information. We also evaluated the contribution of rare exonic variants to the heritability of these phenotypes. Family studies, as well as studies of the aggregate effects of common variants, have found both alcohol use and tobacco use to be heritable behaviors (30,34–38). Research on the aggregate contribution of rare variants, however, has been scarce, with previous work on related phenotypes in smaller samples failing to detect aggregate effects for smoking and alcohol consumption (28). We used meta-analytic summary statistics to quantify the contribution to heritability of variants in various functional categories and frequency bins.

METHODS AND MATERIALS

Seventeen studies contributed summary statistics for meta-analysis. These studies, their sample sizes, and available phenotypes are listed in Tables S1 and S2 in Supplement 1. We augmented our 16 exome chip cohorts with the UK Biobank, in which imputation to the Haplotype Reference Consortium panel was used in lieu of an exome chip array. All individuals were of European ancestry, as determined by genetic principal components.

Phenotypes

Phenotypes were selected to represent multiple stages of smoking. These included initiation, heaviness of use among smokers, and a measure of total lifetime exposure to tobacco. For alcohol use only, a measure of amount of alcohol use was systematically available across studies. The selected phenotypes are relevant to prior GWASs of smoking and alcohol use; are commonly available in psychological, medical, and epidemiological datasets; and are known to be correlated with measures of substance dependence (4,39–41).

1. Cigarettes per day: the average number of cigarettes smoked in a day among current and former smokers. Studies with binned responses used their existing bins. Studies that recorded an integer value binned responses into one of four categories: 1 = 1 to 10, 2 = 11 to 20, 3 = 21 to 30, 4 = 31 or more. Anyone reporting zero cigarettes per day was coded as missing. This phenotype is a component of commonly used measures of nicotine dependence such as the Fagerström Test for Nicotine Dependence.

2. Pack-years: defined in the same way as cigarettes per day but not necessarily binned, divided by 20 (cigarettes in a pack), and multiplied by number of years smoking. This yielded a measure of total overall exposure to tobacco and is relevant to disease outcomes for which smoking is a risk factor, such as cancer and chronic obstructive pulmonary disease risk.
3. Age of initiation of smoking: a measure of early cigarette use. Defined as the age at which a participant first started smoking regularly.
4. Smoking initiation: a binary variable of whether the individual had ever been a regular smoker (1) or not (0), and often defined as having smoked at least 100 cigarettes during his or her lifetime.
5. Drinks per week: a measure of drinking frequency/quantity. The average number of drinks per week in current or former drinkers.

Genotypes

Fourteen of the 17 studies were genotyped with the Illumina HumanExome BeadChip (Illumina, San Diego, CA), which contains ~250,000 low-frequency nonsynonymous variants, variants from the GWAS catalog, and a small number of variants selected for other purposes. Two studies were genotyped on the Illumina Human Core Exome, which includes an additional ~250,000 tag single nucleotide polymorphisms (SNPs). The remaining study, the UK Biobank, was imputed using Haplotype Reference Consortium panel (31,42), as well as the reference panel by UK 10K and 1000 Genomes Project. An integrated callset was released by the UK Biobank team (42). Our UK Biobank genetic association analyses were conducted based on the integrated callset with additional quality control.

Generation of Summary Association Statistics

Seventeen independent studies (see Table S1 in Supplement 1) with smoking and drinking phenotypes were included in the discovery phase. Individual studies conducted association analysis accounting for age, sex, any study-specific covariates, and ancestry principal components (see Table S2 in Supplement 1 for genomic control values), and they submitted summary statistics for meta-analysis. For studies with related individuals (see Table S1 in Supplement 1), relatedness was accounted for in linear mixed models using empirically estimated kinships from common SNPs (43). Residuals were inverse-normalized to help ensure well-behaved test statistics for rare variant tests.

Quality control of per-study summary statistics included evaluation and correction of strand flips and allele flips through systematic comparison of alleles and allele frequencies against the reference datasets ExAC v2.0, 1000 Genomes Phase 3, and dbSNP. Variants with call rates <0.9 or Hardy-Weinberg $p < 1 \times 10^{-7}$ were also removed. The latter filter was meant to avoid findings that could not be more broadly replicated across the 17 studies.

Meta-analysis

Association testing was done in stages. First, we conducted genome-wide association meta-analysis. Variants with p values less than the genome-wide significance threshold of 5×10^{-8} were deemed statistically significant. Loci were defined as 1 million base-pair windows surrounding a "sentinel" (most significant) variant in the locus. Overlapping or adjacent loci were combined into a single locus. Conditional analysis and fine mapping was then performed within each locus. We attempted to replicate one very rare variant (rs36015615 in *STARD3* associated with cigarettes per day) (see Results and Table 1) that was available in two other exome chip consortia: the CHD Exome+ Consortium (N = 17,789) and the Consortium for Genetics of Smoking Behaviour (N = 28,583). Both consortia defined their phenotypes, including cigarettes per day, similarly, as the usual number of cigarettes smoked in a day corrected for sex, age, and principal components (and/or genetic relatedness, as appropriate), and both consortia inverse-normalized the data before association analysis.

We also conducted gene-level association tests grouping nonsynonymous, stop-gain, stop-loss, and splice variants within each gene, using rareMETALS version 6.0 (44). Variant annotation was conducted using SEQMINER with RefSeq 1.9 (45). Two complementary gene-level association tests were performed: the sequence kernel association test (46,47) with an MAF cutoff of 1%, and a simple burden test (48) that summed the number of rare alleles within a given gene, again with a maximum MAF of 1%. We chose variants with MAF ≤1% because we were interested in the contribution of variants with a frequency lower than that which has been reliably imputed and tested in past GWAS meta-analyses. We considered a gene association to be significant if the p value surpassed a Bonferroni correction for the number of genes tested for a given phenotype and test, assuming approximately 20,000 genes in the genome ($.05/20{,}000 = 2.5 \times 10^{-6}$).

We performed iterative conditional analysis using a partial correlation–based score (PCBS) statistic (49), which can perform proper conditional analysis for meta-analysis that combines datasets measured using different arrays. PCBS takes GWAS meta-analysis summary statistics and linkage disequilibrium (LD) estimated from the Haplotype Reference Consortium panel as input.

As a key step to evaluate the contribution of variants within a genome-wide significant locus (33), we used our PCBS framework to apply two complementary fine-mapping techniques to identify putatively causal genetic variants. The first technique was a Bayesian approach described previously (50) that estimates the posterior probability of association based on the statistical strength of the association for variants in each locus. We also applied a version of fgwas (51) modified to work within the PCBS, which assumes that variants in different functional categories have potentially different prior probability of association. For loci with a single association signal, effect sizes and variance from single-SNP analyses were used. If a locus contained multiple signals, we used effect sizes and variance from conditional analysis adjusting for all other index variants in this region.

Finally, we attempted to replicate previous rare variant associations referenced in the introduction and listed in Table S4 in Supplement 2. We attempted replication in our phenotypes for any single variant when that variant was directly genotyped or imputed. We applied a liberal threshold that corrected only

Table 1. All Nonsynonymous/Loss-of-Function Variants With Posterior Probability of Association >.80 From One of the Two Fine-Mapping Methods

SNP	REF/ALT	n^a	ALT AF	GWAS p Value	B	SE	Direction	Annotation	Posterior Probability of Association		Number SNPs (Low-Frequency Coding SNPs) in 95% Credible Interval	
									Without Functional Prior	With Functional Prior (fgwas)	Without Functional Prior	With Functional Prior (fgwas)
Cigarettes per Day												
rs36015615[b]	G/A	69,951	.0002	3.2×10^{-8}	1.2	0.210	==+=++=+=X=X+X+++	Nonsynonymous [*STARD3*]	.82	.62	8997 (6211)	11,302 (6232)
rs16969968	G/A	153,918	.34	2.5×10^{-139}	0.096	0.0038	+−++−+++++−++++++	Nonsynonymous [*CHRNA5*]	.84	.92	2(0)	2 (0)
Drinks per Week												
rs1260326	T/C	357,854	.61	4.6×10^{-40}	0.032	0.0024	+++++++++−−++++++	Nonsynonymous [*GCKR*]	1.0	1.0	1 (0)	1 (0)
rs1229984	T/C	334,588	.98	2.3×10^{-173}	0.25	0.0088	=+−XXXX+XXXX=++++	Nonsynonymous [*ADH1B*]	1.0	1.0	1 (1)	1 (1)
rs28929474	C/T	357,854	.02	2.2×10^{-11}	−0.057	0.0085	−−++−−−−−++++−−−−+	Nonsynonymous [*SERPINA1*]	.95	1.0	1 (1)	1 (1)
rs1800566	G/A	357,854	.18	2.00×10^{-8}	0.017	0.0031	++++++++++++−−++++−	Nonsynonymous [*NQO1*]	.32	.97	103 (0)	1 (0)
Smoking Initiation												
rs2232423	A/G	433,216	.11	1.40×10^{-8}	−0.019	0.0034	−+−+−−−+−−−−−−	Nonsynonymous [*ZSCAN12*]	.84	.64	502 (0)	2 (0)
rs35891966	G/A	433,216	.07	1.30×10^{-8}	−0.024	0.0042	−−−+−+−−−++−−−	Nonsynonymous [*NAV2*]	.98	1.0	1 (0)	1 (0)
rs147052174	G/T	433,216	.02	1.2×10^{-7}	0.043	0.0080	+++++++++−++++	Nonsynonymous [*FAM163A*]	.81	1.0	2432 (66)	1 (0)
rs6265	C/T	433,216	.19	1.9×10^{-10}	−0.017	0.0030	++−++−++−−+−−−	Nonsynonymous [*BDNF*]	.32	.83	25 (0)	2 (0)
rs61754158	C/T	433,216	.01	1.4×10^{-6}	−0.055	0.0114	−−+−+−−−=+=−+−	Nonsynonymous [*HEATR5A*]	.39	.87	9742 (195)	9742 (195)
rs34967813	A/G	433,216	.31	8.1×10^{-7}	−0.011	0.0023	−−−−+−−+−−+−−−	Nonsynonymous [*RYR2*]	.14	.98	7413 (56)	1 (0)

A variant is considered "rare" if minor allele frequency (MAF) < 0.01, and low frequency if $0.01 \leq$ MAF < 0.05. In the Direction column, each symbol represents the contribution of one of the studies. A plus sign (+) indicates that the ALT allele had a positive effect in that study, a minus (−) indicates a negative effect, an equals sign (=) indicates that the variant was monomorphic, and an X indicates that it was absent in that study. The order of studies for cigarettes per day and drinks per week was ARIC, UKB, COGA, FINNTWIN, FUSION, GECCO, HRS, ID1000, MEC, METSIM, MHI, MCTFR, NAGOZALC, NESCOG, SardiNIA, TwinsUK, and WHI. For smoking initiation, the order is the same, except COGA and MCTFR were not available. See Table S1 in Supplement 1 for study acronym explanations.

ALT, alternate allele; ALT AF, allele frequency of the alternate allele estimated in the meta-analysis; GWAS, genome-wide association study; REF, reference allele on GRCh37; SNP, single nucleotide polymorphism.

[a]Across all studies that genotyped the variant.

[b]rs36015615 did not replicate in two additional datasets. See Results section.

for the number of tests conducted for this replication exercise (.05/46 = .001).

Genetic Architecture

We performed heritability and genetic correlation analyses using LD score regression (52). The method calculates LD scores from the Haplotype Reference Consortium, and the estimation of heritability with these LD scores then follows established methods (53,54). Heritability was estimated for each trait and partitioned by annotation category and frequency bins. First, we annotated variants on the exome chip based on gene definitions in RefSeq 1.9, using SEQMINER version 6.0 (55). A variant is classified as coding if it belongs to either one of the following categories: nonsynonymous, stop gain, stop loss, and splice. Seven functional categories were considered in the model, including intergenic, intron, common coding (MAF >0.01), rare coding (MAF <0.01), synonymous, and 3′/5′ untranslated regions. We fitted the baseline model with seven categories and estimated phenotypic variance explained by each category.

RESULTS

GWAS analyses behaved well, with genomic control values for the GWAS across exome chip and UK Biobank imputed variants between 1.05 and 1.3. The intercept for LD score regression ranged between 0.99 and 1.1, indicating absent or minimal effects of population stratification (per-study genomic control values can be found in Table S2 in Supplement 1). A total of 171 loci were identified under the genome-wide significance threshold ($p < 5 \times 10^{-8}$), including 3, 11, 17, 93, and 47 loci for age of initiation of smoking, cigarettes per day, pack-years, smoking initiation, and drinks per week, respectively. A list of all sentinel variants within each locus is shown in Table S5 in Supplement 2. QQ plots and Manhattan plots are available in Figures S1 and S2 in Supplement 1 (additional exploratory GWAS meta-analyses of individuals with significant African ancestry are provided in Supplement 1 [including up to 8974 individuals from three studies]) (see also Table S3 and Figures S3 and S4 in Supplement 1). The genome-wide significant association results included known loci associated with smoking and alcohol use phenotypes. These included associations between smoking phenotypes and variants within the *CHRNA5-CHRNA3-CHRNB4* nicotinic receptor cluster, nicotine metabolism gene *CYP2A6,* and a locus near dopamine receptor *DRD2*. We also replicated previous associations between nonsynonymous variant rs1229984 in *ADH1B* and drinks per week. Only one very rare variant was associated with any of our five phenotypes. This was rs36015615 (MAF = 0.0002), a nonsynonymous variant in *STARD3*, associated with cigarettes per day ($p = 3.2 \times 10^{-8}$). This novel variant did not replicate in either of two replication consortium datasets, the CHD Exome+ Consortium ($N = 17{,}789$; $B = -0.01$, $p = .94$) or the Consortium for Genetics of Smoking Behaviour ($N = 28{,}583$; $B = 0.056$, $p = .84$). Based on the estimated genetic effects in the discovery sample ($\beta = 1.2$), the power for replication is >99%. However, if we assume that the observed effect sizes in the replication datasets are correct, there is 5% power for replication based on this estimated effect. The pattern of results may be due to winner's curse, or the discovered variant may be a false positive finding. Additional studies are required to narrow the possible interpretations.

The fine-mapping analysis of all 171 GWAS loci pinpointed putatively causal variants with high resolution in some cases. The 95% credible interval for 34% of the loci had <10 SNPs and 24 loci had double base-pair resolution, including several instances in which the sole putative causal variant was nonsynonymous and of lower frequency, although in only one case with MAF <1%. The resolution increased somewhat when functional information was used to inform the prior probability, with double base-pair resolution at 32 loci, and 44% of loci having <10 SNPs in the 95% credible interval. Table 1 includes all nonsynonymous or loss-of-function variants within the genome-wide significant loci that had a posterior probability of association >.80 from at least one of the fine-mapping methods. Additional results from the fine-mapping analysis are available in Tables S6 and S7 in Supplement 2. Several known functional variants were identified through this method, including rs16969968 (56), a nonsynonymous variant in nicotinic receptor gene *CHRNA5* associated with cigarettes per day (posterior probability of association [PPA] = .92 and .84 from the fine-mapping analysis with and without functional priors, respectively); rs1229984 (57), a nonsynonymous variant in alcohol metabolism gene *ADH1B* associated with drinks per week (PPA = 1.0 and 1.0); and, although with somewhat weaker evidence, rs6265 (58), a nonsynonymous variant in brain-derived neurotrophic factor *BDNF* associated with smoking initiation (MAF = 0.19; PPA = .83 and .32).

Novel variants in novel genes were also prioritized at high resolution. To take the most statistically compelling examples in Table 1, we found that rs28929474, a low-frequency nonsynonymous variant in *SERPINA1*, was associated with drinks per week (MAF = 0.02; PPA = 1.0 and .95). When homozygous, the alternate T (allele frequency = 0.02; frequency of TT genotype under Hardy-Weinberg = 4 in 10,000) allele is a leading cause of α_1-antitrypsin deficiency. Here, we find that the same risk allele, the T allele, is associated with approximately a 0.05-SD decrease in drinks per week. We also discovered that rs35891966, a variant in *NAV2*, was associated with smoking initiation (MAF = 0.07; PPA = 1.0 and .98) at single base-pair resolution. *NAV2* is involved in neuronal development and was previously shown to be differentially expressed between smokers and nonsmokers, but not previously implicated in GWASs (59).

Results of gene-based tests are provided in Table 2. A novel gene, rho guanine nucleotide exchange factor 37 (*ARHGEF37*), was associated with age of initiation of smoking ($p = 1.9 \times 10^{-6}$). *ARHGEF37* has not been widely studied and its function in not well known. Another novel gene without an immediate biological interpretation, was HEAT repeat-containing protein 5A (*HEATR5A*), associated with smoking initiation ($p = 1.4 \times 10^{-8}$). We also discovered a significant gene-based association between known alcohol metabolism gene *ADH1C* and drinks per week ($p = 1.4 \times 10^{-27}$ and $p = 1.9 \times 10^{-40}$ from the burden and sequence kernel association test tests, respectively). Finally, even with relaxed p value thresholds, we failed to replicate genes identified in previous rare variant association studies referenced in the introduction (Table S4 in Supplement 2), with the exception of *ADH1C* and *CHRNA5*, two loci long known to be associated with alcohol use and smoking, respectively.

Table 2. Significant Gene-Based Test Results, Assuming a Bonferroni Threshold of .05/20,000 = 2.5×10^{-6}

Phenotype	Gene	*n*	Variants	*B*	SE	*p* Value	Method
Age of Initiation of Smoking	*ARHGEF37*	147,010	17	0.08	0.017	1.9×10^{-6}	Burden
Smoking Initiation	*HEATR5A*	427,262	41	–0.02	0.009	1.4×10^{-8}	SKAT
Drinks per Week	*ADH1C*	353,265	4	–0.15	0.014	1.8×10^{-27}	Burden
Drinks per Week	*ADH1C*	353,265	4	–0.15	0.014	1.9×10^{-40}	SKAT

No significant genes were identified for the other two phenotypes.
SKAT, sequence kernel association test.

The estimated total SNP heritability for age of initiation of smoking, cigarettes per day, pack-years, smoking initiation, and drinks per week was 6%, 9%, 10%, 14%, and 16%, respectively. Significant phenotypic variance was explained by rare nonsynonymous variants for all traits, ranging from 1.0% to 2.2% (Table 3). As a fraction of the SNP heritability, rare nonsynonymous variants accounted for 11% to 18%. Results for all seven functional categories are listed in Table S8 in Supplement 2; appreciable heritability was accounted for by common and rare coding variants, and intergenic variants. Variants in the untranslated regions and intronic regions contributed less. Almost all pairs of phenotypes were genetically correlated (Table 4), and the directions of the genetic correlations were in the expected direction. For instance, cigarettes per day was positively correlated with drinks per week (0.2 $\pm$ 0.09), consistent with the observation that increased alcohol consumption is correlated with increased tobacco consumption. Age of initiation of smoking has a negative correlation with all other traits, which is consistent with the observation that an earlier age of smoking initiation is correlated with increased tobacco and alcohol consumption in adulthood. The patterns and magnitudes of correlation are highly similar when considering only rare nonsynonymous variants (Table 4).

Table 3. Estimation of Heritability Explained by Variants on Exome Array

		Heritability Estimates		
Annotation	Phenotype	$\hat{h}^2$	$se(\hat{h}^2)$	*p* Value
All Variants	Age of initiation of smoking	.06	.0049	7.7×10^{-35}
	Cigarettes per day	.09	.0019	$<2.2 \times 10^{-303}$
	Pack-years	.10	.0022	$<2.2 \times 10^{-303}$
	Smoking initiation	.14	.0007	$<2.2 \times 10^{-303}$
	Drinks per week	.16	.0089	7.3×10^{-73}
Rare Coding Variants (MAF < 0.01)	Age of initiation of smoking	.011	.0015	2.8×10^{-2}
	Cigarettes per day	.010	.0006	1.7×10^{-2}
	Pack-years	.018	.0007	8.5×10^{-6}
	Smoking initiation	.022	.0002	3.9×10^{-16}
	Drinks per week	.020	.0013	1.8×10^{-7}

We estimate the heritability based on a baseline model with seven different functional categories. The reported heritability $\hat{h}^2$ is based on the cumulative value from the functional categories with significant heritabilities. We also report the SD $(se(\hat{h}^2))$ and *p* values, estimated using jackknife resampling.
MAF, minor allele frequency.

DISCUSSION

With a maximum sample size ranging from 152,348 to 433,216, the present study is the largest study to date of low-frequency nonsynonymous and loss-of-function variants in smoking and alcohol use. Our meta-analytic study design combined studies genotyped on the exome array with imputed genotypes in the UK Biobank and allowed us to comprehensively evaluate the contribution of rare and low-frequency variants to the etiology of tobacco and alcohol use. All told, we identified 171 genome-wide significant loci for the five phenotypes.

We showed that the rare variants (MAF $\leq$1%) together explain 1.0% to 2.2% of the phenotypic variance for the five traits, amounting to 11% to 18% of the total SNP heritability. A number of putatively causal low-frequency nonsynonymous variants in novel genes were identified through two complementary fine-mapping techniques. These include a variant known to affect α_1-antitrypsin deficiency in *SERPINA1*. The effect of the risk allele resulted in a decrease in drinks per week. One interpretation is that this variant leads to impaired liver function through α_1-antitrypsin deficiency, which, in turn, reduces alcohol consumption. Interestingly, neither this particular variant nor the locus surrounding it was associated with smoking phenotypes, even though α_1-antitrypsin deficiency also affects lung function over time. Other mechanisms by which *SERPINA1* exerts its effect on alcohol consumption are certainly possible. Another novel nonsynonymous variant was in neuron navigator 2 (*NAV2*), associated with smoking initiation. *NAV2* has not previously been associated with substance use or addiction. Given its suspected involvement in neuronal growth and migration, a putatively causal nonsynonymous variant is a strong candidate for functional follow up experiments. Other genes implicated in the fine-mapping analysis have less direct interpretations (e.g., *HEATR5A*), and such results will benefit from replication and/or follow-up experiments. In general, fine-mapping studies narrowed the credible set of likely causal variants to single or double base-pair resolution for 24 loci (Table S6 in Supplement 2). Some loci were not amenable to fine mapping, with credible intervals containing thousands of SNPs in some cases. Given the high cost in money and time of conducting functional experiments at the cellular or organismal level, fine-mapping likely causal variants can be extremely useful in predicting functional consequences and prioritizing variants for further work.

Gene-based tests identified a small number of associated genes, including an expected association with *ADH1C* and drinks per week. The other two associated genes, *ARHGEF37* and *HEATR5A*, do not lend themselves to ready biological interpretations.

Table 4. Estimation of Genetic Correlation Between Smoking and Drinking Traits

Trait 1	Trait 2	Genetic Correlation		
		$\hat{r}_g$	$se(\hat{r}_g)$	p Value
Aggregated Genetic Correlation Induced by All Variants on the Exome Array				
Drinks per Week	Smoking initiation	0.43	0.06	1.7×10^{-11}
Drinks per Week	Age of initiation of smoking	0.01	0.13	9.3×10^{-1}
Drinks per Week	Pack-years	0.22	0.10	2.6×10^{-2}
Drinks per Week	Cigarettes per day	0.20	0.09	3.1×10^{-2}
Smoking Initiation	Age of initiation of smoking	−0.64	0.11	1.1×10^{-8}
Smoking Initiation	Pack-years	0.45	0.08	4.9×10^{-8}
Smoking Initiation	Cigarettes per day	0.10	0.07	1.5×10^{-1}
Age of Initiation of Smoking	Pack-years	−0.63	0.17	2.1×10^{-4}
Age of Initiation of Smoking	Cigarettes per day	−0.26	0.16	9.9×10^{-2}
Pack-years	Cigarettes per day	0.77	0.13	2.2×10^{-9}
Genetic Correlation Induced by Rare (MAF <1%) Nonsynonymous Variants				
Drinks per Week	Smoking initiation	0.49	0.08	1.2×10^{-10}
Drinks per Week	Age of initiation of smoking	−0.04	0.30	8.9×10^{-1}
Drinks per Week	Pack-years	0.08	0.02	2.7×10^{-4}
Drinks per Week	Cigarettes per day	0.09	0.02	5.2×10^{-5}
Smoking Initiation	Age of initiation of smoking	−1.10	0.21	1.3×10^{-7}
Smoking Initiation	Pack-years	0.63	0.08	1.5×10^{-14}
Smoking Initiation	Cigarettes per day	0.23	0.08	3.3×10^{-3}
Age of Initiation of Smoking	Pack-years	−1.10	0.33	1.5×10^{-3}
Age of Initiation of Smoking	Cigarettes per day	−0.69	0.32	3.2×10^{-2}
Pack-years	Cigarettes per day	0.87	0.14	1.4×10^{-9}

We estimate genetic correlations between five smoking and drinking traits. For genetic correlation estimates ($\hat{r}_g$), their SD ($se(\hat{r}_g)$) and p values are reported.

MAF, minor allele frequency.

We showed that rare coding variants available on the exome chip or imputable by the Haplotype Reference Consortium, with frequency <1%, explain significant proportions of phenotypic variance and a substantial proportion of the total SNP heritability. The exome chip was designed to genotype coding variants uncovered in ~12,000 sequenced exomes. By design, the exome chip comprehensively ascertained high-confidence rare nonsynonymous, splice, and stop variants within those sequences, and it only sparsely genotypes in other classes of variation, including common variants. The Haplotype Reference Consortium panel imputed data also have limited accuracy when the underlying genetic variants are rare. Therefore, our current investigation did not fully explore the genetic architecture of very rare variants (i.e., with MAF <0.1%). With the development of larger imputation reference panels, and the availability of large-scale deep whole-genome sequences (e.g., the TOPMed [Trans-Omics for Precision Medicine Study] study), we expect to be able to conduct an even more comprehensive analysis of the genetic architecture for variants with ever lower frequencies. Ultimately, the discovery of low frequency variants with small effects will require even larger sample sizes. For example, for rare variants with MAF of 0.1% and effects of 0.2, 0.15, and 0.1 SDs on the phenotype, to identify associations at $\alpha = 5 \times 10^{-8}$ with 80% of power, sample sizes of 500,000, 890,000, and 1,990,000 are required. While such numbers seemed astronomical just a few years ago, they will indeed be attainable in the next few years with the availability of large biobank datasets and ever-improving imputation. Another limitation of the present study is the limited samples sizes from non-European ancestries, in which only exploratory analyses were possible. Substantial improvements can be made to the resolution of fine-mapping analysis by leveraging disparate LD information across samples with diverse ancestry (33). Future researchers will do well to include individuals of diverse ancestry.

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