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Polygenic Risk Score Prediction of Alcohol Dependence Symptoms Across Population-Based and Clinically Ascertained Samples

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Abstract

Background—Despite consistent evidence of the heritability of alcohol use disorders (AUDs), few specific genes with an etiological role have been identified. It is likely that AUDs are highly polygenic; however, the etiological pathways and genetic variants involved may differ between populations. The aim of this study was thus to evaluate whether aggregate genetic risk for AUDs differed between clinically ascertained and population-based epidemiological samples.

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Methods—Four independent samples were obtained: two from unselected birth cohorts (ALSPAC, *N*=4304; FinnTwin12, *N*=1135) and two from families densely affected with AUDs, identified from treatment-seeking patients (COGA, *N*=2097; IASPSAD, *N*=706). AUD symptoms were assessed with clinical interviews, and participants of European ancestry were genotyped. Genome-wide association (GWA) was conducted separately in each sample and the resulting association weights were used to create polygenic risk scores in each of the other samples (12 total discovery-validation pairs), and from meta-analyses within sample type. We then tested how well these aggregate genetic scores predicted AUD outcomes within and across sample types.

Results—Polygenic scores derived from one population-based sample (ALSPAC) significantly predicted AUD symptoms in another population-based sample (FinnTwin12), but not in either clinically ascertained sample. Trend level associations (uncorrected $p < .05$) were found for polygenic score predictions within sample types but no or negative predictions across sample types. Polygenic scores accounted for 0-1% of the variance in AUD symptoms.

Conclusions—Though preliminary, these results provide suggestive evidence of differences in the genetic etiology of AUDs based on sample characteristics such as treatment-seeking status, which may index other important clinical or demographic factors that moderate genetic influences. Although the variance accounted for by genome-wide polygenic scores remains low, future studies could improve gene identification efforts by amassing very large samples, or reducing genetic heterogeneity by informing analyses with other phenotypic information such as sample characteristics. Multiple complementary approaches may be needed to make progress in gene identification for this complex disorder.

Keywords

ALSPAC; COGA; FinnTwin; IASPSAD; genetic heterogeneity

Introduction

Alcohol use problems, including pathological alcohol use disorders (AUDs), are a leading contributor to the global burden of disease, responsible for substantially increasing risk for premature death, disability, and preventable illness (World Health Organization, 2009). Extensive work in genetic epidemiology using twin and adoption studies has shown that AUDs are heritable, with genetic factors accounting for about half of the inter-individual variation in risk (Verhulst et al., 2015). However, the transition from statistical studies of latent genetic risk to molecular genetic studies identifying the actual genes involved in the etiology of alcohol problems has had limited success.

Current evidence indicates that AUDs have a complex, highly polygenic architecture driven by the aggregation of hundreds or thousands of common genetic variants of very small individual effect (Sullivan et al., 2012). Heritability estimates using measured genotypes of common variants (23-33%: Yang et al., 2014; Mbarek et al., 2015), while somewhat lower than those from twin studies, confirm that current genotyping platforms harbor genetic variants of importance to AUDs, but few of these have been identified despite considerable research efforts. Candidate gene studies and the more stringent, atheoretical genome-wide association studies (GWAS) have found only a few replicable genetic variants underlying

alcohol use and alcohol problems, and even the most robust of these account for a minimal proportion of the heritability (Hart and Kranzler, 2015). Polygenic risk score methods that sum genetic liability across many variants at nominal thresholds of association (i.e. The International Schizophrenia Consortium, 2009) have had some success in predicting individual risk for AUDs (Hart and Kranzler, 2015), but as of yet the variance accounted for by such scores is generally less than 3%, well below clinical prediction utility.

The slow progress in gene identification at both the individual variant level and the aggregate polygenic level is likely due in part to the heterogeneous pathways by which many different individuals develop alcohol problems, even though alcohol problems are typically classified in research by a single binary case-control diagnostic status for AUD (Hart and Kranzler, 2015). AUDs have numerous and heterogeneous demographic predictors (Grant et al., 2015), and a long history in the literature suggests that there are multiple “types” of alcoholism that display different epidemiological patterns and may represent divergent syndromes despite their shared symptoms (Leggio et al., 2009; Babor et al., 1992; Cloninger et al., 1988). An AUD diagnosis is itself a constellation of many possible subsets of symptoms that fall under both the physical (e.g. tolerance; withdrawal) and psychosocial (e.g. impairments in work and social functioning) domains, and some evidence suggests that distinct genetic factors underlie different symptom clusters (Kendler et al., 2012). The development of AUDs may thus be driven in different groups of individuals by distinct physiological and/or psychological factors whose genetic etiologies are not perfectly overlapping (Hines et al., 2005).

Failure to account for such heterogeneity, when it exists, decreases power to detect clear genetic associations in samples where such groups are combined. Differential genetic effects have already been identified as a function of certain sample characteristics such as age of AUD onset and patterns of associated personality traits and comorbid disorders (Ali et al., 2015; Cloninger et al., 1988; Dick et al., 2007; Kuo et al., 2008). There are many additional lines along which such etiological differences may split, given the complex biological, psychological, and social influences impacting alcohol problems. One such possibility is the source of the population, which in research studies is usually either clinical samples of treatment-seeking patients or unselected population-based cohorts. Ascertained samples have, by definition, a higher prevalence of alcohol problems than the general population and a different distribution of AUDs and alcohol-associated traits. The burden of comorbid illness (beyond AUDs) is known to be higher in clinical samples (Kaufmann et al., 2014) and they differ on a number of demographic and disease-related characteristics (Blanco et al., 2015). It is also plausible that the mechanisms underlying alcohol problems in treatment-seeking individuals differ from those driving alcohol problems in the general population, particularly when clinical samples are obtained based on family history of AUD, which can represent both different genetic predispositions and contextual risk factors associated with the familial environment. Proposed typologies of alcoholism (e.g. Cloninger et al., 1988) also suggest that individuals with a prominent family history of AUDs have a more strongly genetically influenced form of the disorder, although it is not known whether the genetic influences may also differ qualitatively from those involved in sporadic onset cases.

The question of genetic heterogeneity as a function of sample ascertainment has not been explicitly tested, but the emerging literature in this area suggests an imperfect correspondence between the genetic influences on alcohol use behaviors across populations. For example, while alcohol metabolism genes show reliable associations across multiple alcohol-related phenotypes in multiple samples, other genes/genetic variants have not replicated across clinical case-control samples (Hart and Kranzler, 2015) and population-based studies of alcohol consumption (Schumann et al., 2016) and problems (Sanchez-Roige et al., 2017). Hansell et al. (2009) have previously found evidence for differences in genetic associations for alcohol outcomes between unselected samples versus participants ascertained for a family history of AUDs. Further, the genetic correlations between alcohol consumption and problems, and between alcohol phenotypes and other psychiatric traits, show different patterns in general population samples (Sanchez-Roige et al., 2017) and in ascertained case-control studies like the Psychiatric Genomics Consortium (Walters et al., in preparation). The aim of the present study was thus to investigate whether there may be differences in the genetic liability for alcohol problems across two sample types: unselected, population-based epidemiological cohorts and clinically ascertained families densely affected with AUDs. To test this hypothesis, we use polygenic risk scoring methods to predict aggregate genetic risk for AUD symptoms across four independent birth cohort or clinically ascertained samples with in-depth clinical assessments of AUDs.

Materials and Methods

Participants

The present study includes secondary analysis of data from four ongoing studies with measures of AUDs: two population-based samples drawn from unselected regional birth cohorts and two clinically ascertained samples recruited from alcohol treatment programs and hospitals. Study procedures for collecting all samples were approved by the relevant institutional review boards, including the ALSPAC Ethics and Law Committee and the Local Research Ethics Committees, Ethics Committee of the Hospital District of Helsinki and Uusimaa and university IRBs from each study site. All participants provided informed consent. These studies have been described in detail elsewhere; a summary of each can be found below and in Table 1.

Population-based samples

ALSPAC—The Avon Longitudinal Study of Parents and Children (ALSPAC) sample (Golding et al., 2001; Boyd et al., 2013) includes children born in Avon, UK in 1991-1992. ALSPAC recruited a total of 15,458 children from a birth cohort of 15,247 pregnancies, most of whom have been followed longitudinally since birth and assessed at multiple waves. Please note that the study website contains details of all the data that is available through a fully searchable data dictionary at http://www.bris.ac.uk/alspac/researchers/data-access/data-dictionary. The present study uses data from the follow-up assessment at age 18 (M = 17.9, range = 16.3 – 20.0), which included an in-person, computer-assisted clinic assessment of alcohol problems, supplemented by imputed data from a similar assessment at age 16.5 for those with missing data (Edwards et al., 2015). Included in the present study were 4,304

individuals (57.0% female) of European ancestry who had both phenotypic and genotypic data available for analysis.

FinnTwin12—The FinnTwin12 (FT12) sample includes five birth cohorts of twins born in Finland from 1983 to 1987, identified from national birth records (Kaprio, 2006; Kaprio et al., 2002). These twins have been assessed longitudinally with self-report surveys from age 12 through approximately age 22, and a subset of the epidemiological sample was selected for "intensive" study, receiving in-person clinical assessments at ages 14 and 22. Included in the present study are 1,135 individuals from 652 families in this intensive subset who completed clinical assessments of AUDs at age 22 (M = 22.4, range = 20-26), and who also provided a DNA sample for genotyping. The sample was 53.6% female, with 39.4% monozygotic (MZ) twins, 30.5% same-sex dizygotic (DZ) twins, 29.8% opposite-sex DZ twins, and 0.3% single (unpaired) twins of unconfirmed zygosity.

Clinically ascertained samples

COGA—The Collaborative Study on the Genetics of Alcoholism (COGA) sample (Begleiter and Reich, 1995) was recruited from treatment programs around six research sites in the United States, with probands and their extended families undergoing structured clinical interviews about alcohol use behaviors at the initial assessment and in multiple waves of follow up assessments. A subset of 118 families of European ancestry was selected for genotyping, as described by Wang et al. (2013). The maximum number of AUD symptoms across waves/ages was used for analysis, and this data was available for 2097 individuals (53.6% female), with a mean age at maximum AUD symptoms of 35.1 (range: 13-88). For more direct comparison with the younger population-based samples, we also replicate some analyses (see Data Analysis section below) in a subset of 879 young adults from the COGA families (M age = 20.9, range: 15-26).

IASPSAD—The Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD) sample (Prescott et al., 2005) included alcoholic probands recruited from hospitals and community alcohol treatment facilities in Ireland and Northern Ireland. Eligible probands of European ancestry underwent a clinical interview for AUDs and provided information on potentially affected siblings, who were also assessed with a clinical interview. This sample included 591 probands and 610 affected siblings, all of whom met DSM-IV criteria for an alcohol dependence diagnosis. Phenotypic and genotypic data were available for a subset of this sample, resulting in a total of 706 participants from 430 families (34.3% female; M age = 41.8, range = 17 – 71) included in the present analyses.

Measures

Alcohol Problems Phenotypes—In the ALSPAC sample, the measure of alcohol problems (in the past year) was a factor score derived from items assessed in a computer-assisted clinic interview: the 10-item Alcohol Use Disorders Identification Test (AUDIT; Babor et al., 2001), the seven DSM-IV alcohol dependence items (American Psychiatric Association, 2000), and three additional alcohol consequences items, as previously described (Salvatore et al., 2014; Edwards et al., 2015). Values for the alcohol problems factor score ranged from −0.45 to 4.18. In FT12, COGA, and IASPSAD, the measure of

alcohol problems was a sum score of lifetime DSM-IV alcohol dependence symptoms (range: 0-7) assessed with a personal interview using the Semi-Structured Assessment for the Genetics of Alcoholism (Bucholz et al., 1994). The mean (*SD*) numbers of alcohol dependence symptoms for FT12, COGA (all ages/under age 27), and IASPSAD were 1.1 (1.4), 2.2 (2.3)/1.7 (1.8), and 6.4 (1.0), respectively. Across all studies, participants who had not initiated alcohol use at the time of assessment were coded as missing for the AUD measures, and were not included in the genetic analyses or the sample sizes reported above.

Genotypes—Blood or saliva samples were collected from participants in all four samples for DNA extraction and genotyping on a microarray chip. Each study employed a different genotyping platform, but used the genotyped polymorphisms to impute to a common set of variants in the 1000 Genomes Phase 1 reference panel (Abecasis et al., 2012) after applying standard quality control procedures. A summary of the methods for genotyping, quality control filtering, and imputation procedures across samples can be found in Table 1. All samples were assessed for population stratification using multidimensional scaling modeling, and only participants of European ancestry were included for analysis.

Data Analysis

The analyses conducted in this study involved testing whether the genetic variants underlying alcohol problems in one sample type (e.g. population-based or ascertained) were associated with alcohol problems to the same degree in independent samples from the same versus different sample types. We used polygenic risk score methods to take information about which genetic variants, or single nucleotide polymorphisms (SNPs), were associated with alcohol problems in one sample and applied this to create individual-level genetic risk scores (aggregated across genomic loci) in each of the other samples. Our analytic plan thus included two steps: first, obtaining SNP weights from a GWAS of alcohol problems in each "discovery" sample, and second, creating polygenic scores and testing how well they predicted alcohol problem risk in each independent "validation" sample. As an additional attempt to improve discovery power, we also conducted a meta-analysis of the individual GWAS within each sample type (ALSPAC+FT12 and COGA+IASPSAD) and used these meta-analysis summary statistics to create additional polygenic scores in the two samples of the other type. Each of the four study samples was therefore used once as a discovery GWAS sample and four times as a validation sample for testing the value of the polygenic scores in predicting alcohol problems.

Discovery GWAS—In ALSPAC and IASPSAD, GWAS of the alcohol problems phenotypes described above have previously been reported (Edwards et al., 2015; Adkins et al., 2017). Covariates included in these analyses were sex (both studies) and age (IASPSAD; ALSPAC was age-standardized). We use SNP-level summary statistics from those reports to create polygenic risk scores. In FT12 and COGA, we conducted a GWAS of DSM-IV alcohol dependence symptom counts, using residuals of these symptom counts after the effects of relevant covariates were regressed out. These covariates included sex for the age-standardized FT12 sample, and sex, age at maximum AUD symptoms, and the first two ancestry principal components for COGA. These analyses were run in the GenABEL package (Aulchenko et al., 2007) for R version 3.2.1 (R Core Team, 2015), using the

polygenic (Thompson and Shaw, 1990) and *mmscore* (Chen and Abecasis, 2007) procedures to control for the non-independence of related subjects, in parallel to the methods used in the IASPSAD GWAS. In FT12, one twin from MZ pairs was excluded from the GWAS for a total discovery $n = 905$. In both FT12 and COGA, GWAS of DSM-IV alcohol dependence symptoms have previously been conducted (see Wang et al., 2013; Meyers, 2012); however, we repeat the analyses here because the datasets have been imputed to the 1000 Genomes reference panel since the original reports and additional follow-up assessments have been collected for some participants. No new genome-wide significant SNP associations were found in these analyses. Meta-analyses of the GWAS summary statistics were also conducted for the population-based (ALSPAC+FT12) and ascertained (COGA+IASPSAD) sample results using METAL (Willer et al., 2010), with a sample size-weighted scheme. Additional meta-analyses were run for each pair of samples, within and between sample type, to test for heterogeneity in SNP association statistics between them using a random effects meta-analysis model.

A potential confounding factor in comparing the population-based and clinically ascertained samples is the difference in age between sample types. The ALSPAC and FT12 samples are young adults, aged approximately 17 to 26, while the COGA and IASPSAD samples, being initially recruited from treatment-seeking patients, include many more middle-aged and older adults. There are known differences in the heritability of alcohol use between adolescence and adulthood (Edwards and Kendler, 2013; Bergen et al., 2007), which may affect cross-sample replication. To reduce this possibility, we also conducted the same GWAS procedure in a subset of the COGA families of corresponding age to ALSPAC/FT12 (age < 27, $n = 879$) who were mostly ascertained as children or extended family members of the recruited probands. We use these to supplement polygenic risk score analyses in the larger sample. There were too few individuals of comparable age in IASPSAD to perform complementary analyses.

Polygenic score creation—To create comparable polygenic risk scores across samples, we selected a set of SNPs that were available in all four samples with a minor allele frequency (MAF) > 5% and an imputation quality INFO/R^2 score > .90 (n = 3,116,334). These SNPs were then used to create polygenic scores with the software package PRSice (Euesden et al., 2015), which automates the implementation of the *score* procedure in PLINK v. 1.9 (Chang et al., 2015) to optimize selection of the most informative pairwise polygenic score. Best-guess genotypes with a certainty above 0.9 were used in creating the polygenic scores for all samples.

Using PRSice, the list of common SNPs was pruned based on linkage disequilibrium (LD) to obtain a set of autosomal SNPs in approximate linkage equilibrium ($R^2 < .10$) for each discovery-validation pair with a sliding 250kb window. PLINK's *clump* procedure was used to prioritize the selection of SNPs with stronger association signals in the discovery GWAS to index these LD blocks, in order to enhance the predictive ability of the scores. PLINK's *score* method then summed the total number of minor alleles from the set of score SNPs for each individual in the validation sample, weighting each score SNP by the magnitude and sign of its GWAS association statistic (c.f. The International Schizophrenia Consortium, 2009). For each discovery-validation sample pair, this list of score SNPs was further filtered

based on association *p* value thresholds in the discovery sample to create a series of SNP sets with decreasing stringency of nominal GWAS association (thresholds of $p < .001$ to $p < .50$). PRSice implements this in a high-resolution fashion in *p* value increments of 0.01 and selects the threshold yielding scores with the strongest phenotypic prediction in the validation sample.

Polygenic score prediction—After creating polygenic scores in each of the validation samples, we used these scores to predict the alcohol problems outcomes by fitting a series of linear models, using the *lme4* package (Bates et al., 2015) in R version 3.2.1 (R Core Team, 2015). Scores from the best *p* value threshold selected by PRSice in each discovery-validation pair were entered into a regression model, along with the same covariates as in the respective discovery GWAS, with the alcohol problems measure as the dependent variable. All polygenic scores were approximately normally distributed with a mean of zero and a range between 0.0003 and 0.126 units. To account for non-independent observations in family-based samples, we used a linear mixed model framework with the family unit as a random effect. We used a generalized linear model (*lm*/*lmer* functions) to predict the alcohol problems factor score in ALSPAC and the alcohol dependence symptom counts in FT12, COGA, and IASPSAD (log+1 transformed to adjust for the zero-inflated distributions). To account for multiple testing, we performed a Bonferroni *p*-value correction by dividing $\alpha = .05$ by the number of discovery-validation pairs of analyses (23 pairs, $\alpha_{adj} = .0022$), given that scores from multiple *p* value thresholds within a discovery-validation set are necessarily nested within each other and not independent tests. We calculated the variance explained by the polygenic scores by comparing the R^2 change between the full model and a model in which the score was dropped and only the covariates remained (Nagelkerke's pseudo-R^2 for the generalized linear mixed models, as implemented in the R package *MuMIn* (Bartoń, 2016)). We conducted power analyses using the *pwr* package in R (Champely, 2015) in order to estimate our ability to find significant effects of the polygenic risk at levels detected in studies with similar methodology.

Results

Heterogeneity in genetic effects

We tested for heterogeneity in the SNP association statistics between individual sample GWAS using random-effects meta-analysis. The proportion of SNPs meeting a nominal heterogeneity test *p* value (<.05) in each pair of samples ranged from 0.0417 to 0.0512, on par with the expected chance proportion of 0.05, and the patterns did not systematically differ between pairs of the same versus different sample types (full results available upon request).

Polygenic score prediction

Table 2 displays the results of the linear models assessing how well the polygenic risk scores predict alcohol problems in each validation sample. Each "block" of the table represents a discovery-validation set, with the discovery GWAS sample in the row and the validation sample in the column. Within the population-based samples of ALSPAC and FT12 (upper blocks of Table 2), polygenic scores estimated based on weights from the ALSPAC GWAS

were highly significant, accounting for 1.03% of the variance in AUD symptoms in FT12. In the reciprocal analyses, scores based on GWAS weights from the smaller FT12 sample were positively but not significantly associated (p =.006) with alcohol problems in the ALSPAC validation sample. Within the clinically ascertained samples of COGA and IASPSAD (lower blocks of Table 2), polygenic scores weighted in one sample did not significantly predict AUDs in the other, although there was a nominal trend (p = .03 to p = .07) in the expected direction.

Looking across ascertained and population-based sample types (shaded grey blocks), there were no significant associations between polygenic scores created in one sample type and AUDs predicted in the other (i.e., population-based to ascertained and vice versa). We note that there was a suggestive trend in which all cross-sample predictions between different sample types, save one (ALSPAC to IASPSAD), were in a negative direction with a nominal level of statistical significance (p < .05) but would not be considered significant after correction for multiple testing. Similar patterns were observed for polygenic scores based on the meta-analyses of each sample type, with non-significant levels of prediction for all discovery-validation pairs.

Replication in young adults

We repeated these polygenic risk score analyses in a subset of adolescents/young adults from the COGA sample. Results from this replication are shown in Table 3, with the top panel indicating the set of analyses in which the COGA young adults made up the validation sample for GWAS and score weighting, and the bottom panel indicating the analyses in which they were the discovery sample for score prediction. There were no statistically significant associations between polygenic scores and AUDs in this set of analyses. The pattern of association (negative across sample type, positive within sample type, at nominal significance levels) was the same as in the full COGA sample.

Power

Table 4 presents calculations of the expected power each validation sample had to detect associations for polygenic scores accounting for 0.2% to 5% of the phenotypic variance. All samples had adequate power to detect associations accounting for 2% or more of the trait variance, which is comparable to or less than what others have found using the same genome-wide polygenic risk scoring method (The International Schizophrenia Consortium, 2009; Salvatore et al., 2014; Hart and Kranzler, 2015).

Discussion

Using four independent samples with clinical assessments of problem alcohol use behaviors, we found a differential prediction of polygenic scores for alcohol problems across clinically ascertained samples and population-based epidemiological samples. We found significant cross-prediction within our two population-based birth cohort samples, with aggregate genetic variants identified in ALSPAC predicting AUDs in FT12, albeit only contributing to a small identifiable proportion of the variance. However, similar scores derived from ALSPAC did not predict risk for AUDs in either of two clinically ascertained samples

(COGA; IASPSAD), despite COGA having a sample size nearly double that of FT12. Cross-sample polygenic risk prediction based on the other three discovery GWAS weights were less conclusive, with no significant risk prediction found within the two clinically ascertained samples or between any pair of samples from different types. We observed a consistent trend towards positive cross-prediction within sample types but null or negative prediction from population-based samples to clinically ascertained samples and vice versa, but this effect requires replication given the lack of robust statistical support. The consistency in the pattern of results in two samples from each type encourages confidence.

As with most other existing GWAS of alcohol phenotypes, we are limited in power by the sample sizes for estimating genetic risk associations and thus consider our results preliminary. However, these findings provide suggestive evidence that genetic heterogeneity in the construct of AUDs/alcohol problems may exist between, and even within, different types of populations. Previous studies have identified phenotypic and genetic heterogeneity underlying alcohol phenotypes attributable to other sample-specific factors such as age of onset, patterns of symptom endorsements, and psychiatric comorbidities (Kuo et al., 2008; Dick et al., 2007; Cloninger et al., 1988; Edwards and Kendler, 2013), and so it seems that heterogeneity may be the rule rather than the exception for alcohol use disorders and other complex psychiatric and behavioral traits. This phenomenon is important to consider in light of the knowledge that heterogeneity can have major consequences on power for gene identification studies (Manchia et al., 2013), so even smaller studies such as this can provide insight into potential challenges to consider when designing large scale efforts in the future. Indeed, though gene identification efforts for alcohol-related outcomes are still in their infancy compared to some other complex traits, our results fit into the emerging pattern of results demonstrating that different genes and genetically correlated traits appear to be linked to alcohol problems in ascertained and population studies (Hart and Kranzler, 2015; Sanchez-Roige et al., 2017; Schumann et al., 2016: Walters et al., in preparation). Such studies should consider testing for heterogeneity as a function of population type (in addition to several other sample characteristics) when meta-analyzing across multiple samples to confirm or refute the suggestive results found here.

It is not immediately clear what might be the driving force behind differences in genetic influences on AUD symptoms/problems between ascertained and epidemiological samples. Heritability estimates calculated either by GCTA (Yang et al., 2011) or GenABEL differed between samples, but not systematically across sample types (ALSPAC: 0.06, FinnTwin12: 0.32, COGA: 0.35, COGA young adults: 0.25, IASPSAD: 0.04). Post-hoc sample comparisons did not indicate obvious systematic differences between the sample types based on characteristics such as internalizing/externalizing disorder symptoms (e.g. major depression, anti-social personality disorder) or related traits and behaviors (e.g. personality, other substance use), although differences in the measures collected between studies preclude a direct statistical comparison of many such characteristics (details available upon request). However, the polygenic risk scores based on GWAS weights for alcohol problems did not show a differential pattern of predicting these traits across population-based and ascertained samples either, indicating that the differential prediction of alcohol problems outcomes between sample types seen in our results was not simply driven by sample

differences in internalizing/externalizing traits or comorbidities, as suggested by some AUD typologies (e.g. Cloninger et al., 1988).

We speculate that the genetic influences on alcohol problems in clinically ascertained populations may differ, in quantity or quality, from those within the general population. Symptom profiles or motives for alcohol use could, for example, define mechanisms by which different individuals develop the same AUD outcome, and these might differ systematically between populations. Clinical samples of substance dependent individuals are often not representative of the broader population (Blanco et al., 2008), and treatment-seeking individuals often have more severe illness and additional comorbidities (Kaufmann et al., 2014). Overall symptom endorsement rates are higher in clinical samples, but there could also be differences in endorsement profiles, since the syndromic criteria for AUD allows for many possible combinations of symptom subsets. Some evidence indicates unique genetic influences between symptom clusters (Kendler et al., 2012). There is also evidence that drinking for coping motives is more strongly tied to psychological dependence, while drinking for social or sensation-seeking motives are associated with heavy/frequent use (Kuntsche et al., 2005), but despite different underlying mechanisms both could lead to AUD symptoms/diagnoses. If any one of these pathways is overrepresented in clinical samples (due perhaps to ascertainment bias from greater severity or medical/psychiatric comorbidities that increase treatment-seeking), the etiological overlap with AUD phenotypes in the general population would be reduced.

It could also be that the environments of individuals growing up in families densely affected with alcoholism differ substantially from other subsets of the population, and thus a set of genes enriched for interaction effects with certain environmental factors might be influential to AUDs only in this subgroup. Gene-environment interaction has a strong influence on alcohol use and misuse, and a variety of socially restrictive environments including parental monitoring, marital status, and legal barriers to alcohol access have been shown to moderate genetic risk for AUDs (Dick and Kendler, 2012; Young-Wolff et al., 2011). Differences in either the types of genetic/environmental risk factors experienced or their degree of prevalence between populations contributes to the persistent difficulty in identifying a robust, coherent set of influences involved in the etiology of alcohol problems.

Regardless of the reason, if genetic heterogeneity between samples is indeed at play, this study's findings have implications both for the field's understanding of AUDs and for the design of research protocols. The results suggest, first, that sample characteristics (including, but also beyond the typical demographic contenders like sex and age) may play a critical role in defining distinctive genetic and/or phenotypic etiologies. The poor polygenic prediction across sample types in this study, and even now in some consortia, is evidence of a massive degree of heterogeneity across populations that must be better confronted. Second, these findings suggest caution in research methods that combine participants across different populations, e.g. in large-scale meta-analyses and replications. Combining across genetically heterogeneous populations can undermine a study's power to detect or replicate true effects. Although a brute force approach with large sample sizes can certainly be effective (e.g. Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014), it may be less tractable for disorders like AUDs that have stronger environmental and gene-

environment interaction effects. Smaller studies can take complementary approaches to increase power by conducting more in-depth phenotypic assessments and identifying homogeneous subgroups or homogenous etiological pathways and thereby advance the understanding of complex disorders like AUDs. The examination of intermediate traits (endophenotypes) that link the biology to the disorder, such as alcohol metabolism, is an additional complementary tactic to improve gene identification success.

This study has several limitations that should be considered, most notably the lack of harmonization across phenotypic, genotypic, and analytic methods. Because the data was taken from multiple ongoing studies designed and implemented independently, it is not feasible for measures and analytic protocols to be identical. Importantly, all sample genotypes were imputed to a common reference panel and screened to select a set of common markers meeting common quality control thresholds, and the phenotypes in all studies included a common set of DSM-IV alcohol dependence symptoms. We note specifically that the ALSPAC phenotype differed from the others in order to take advantage of existing GWAS results; however, the past-year factor score measure is also a more appropriate for this younger sample given that AUD symptoms are quite rare in this age range, when most initiation has only recently occurred and frequent consumption is still a deviant behavior (Substance Abuse and Mental Health Services Administration, 2014). Age difference between samples is also an important limitation, although we note that previous studies have found that the same genetic factors influence AUDs in both adolescence and adulthood (Palmer et al., 2013), and the replication in the young adult subset of COGA demonstrated consistency of our results.

Lack of cross-study harmonization is evident across several aspects of the methods, but is not systematic across one group of samples or another, so we would expect this to make our results more conservative rather than to induce spuriousness. This may have reduced our ability to detect true associations of the polygenic risk scores, especially given the small effect sizes that were found. However, we were well powered to detect effects accounting for >2% of the variance in AUDs, which would not be expected of a single gene but is reasonable for an aggregate genome-wide score. A combination of methods to improve gene identification (e.g. larger and less heterogeneous discovery samples, more precise phenotyping, integration of bioinformatics information) could be helpful in creating more accurate polygenic risk scores in the future, in addition to potentially identifying individually important genetic variants.

Despite these limitations, this study provided modest but consistent evidence across multiple samples and multiple sets of polygenic scores that suggests a genetic architecture underlying alcohol use disorders and problems that does not wholly overlap between population-based versus clinically ascertained samples. Our findings indicate that these and, likely, other sample characteristics need to be taken into account in the design and implementation of research studies, and in our efforts to understand the genetic and environmental etiology of the costly and challenging public health problem of alcohol use disorders.

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Table 1

Sample descriptives and genotyping procedures in four studies of alcohol use disorders.

	ALSPAC	FT12	COGA	IASPSAD
N	4,304	1,135	2,097	706
% Female	57.0	53.6	53.6	34.3
Mean age (SD)	17.9	22.4	35.1	41.8
Recruitment strategy	Birth cohort	Twin birth cohort	Families of treatment-seeking patients	Siblings of treatment-seeking patients
Phenotype	Factor score of DSM-IV and AUDIT AD symptoms	DSM-IV AD symptoms	DSM-IV AD symptoms	DSM-IV AD symptoms
Genotyping platform	Illumina Human Hap550 Quad[a]	Illumina Human670-QuadCustom BeadChip[a]	Illumina Human OmniExpress array 12.VI[a]; Illumina Human 1M-Duo BeadChip[a]	Affymetrix Human SNP Array 6.0[b]
Quality control filtering	MAF < 1%, call rate < 95%, HWE $p < 5 \times 10^{-7}$, missingness > 3%, heterozygosity outliers, gender mismatch, cryptic relatedness (IBD > 10%)	MAF < 1%, call rate < 95%, HWE $p < 10^{-6}$, missingness > 5%, heterozygosity outliers, gender mismatch, cryptic relatedness (outside of known families)	MAF < 5%, GenCall score < .15, HWE $p < 10^{-6}$, inconsistent calls between Omni and 1M arrays, Mendelian errors, cryptic relatedness (outside of known families)	MAF < 1%, call rate < 97%, HWE $p < 10^{-6}$, missingness > 3%, cryptic relatedness (IBD > 5% outside of known families)
1000 Genomes version	Phase 1 v3, March 2012	Phase 1 v3, March 2012	Phase 1 v2, December 2010 (EUR)	Phase 1 v3, March 2012
Reference genome build	GRCh37	GRCh37	GRCh37	GRCh37
Imputation software	MACH/Minimac[c]	ShapeIT[e]; IMPUTE2[f]	BEAGLE 3.3.1[d]	ShapeIT[e]; IMPUTE2[f]
Number of imputed SNPs	8,458,542 (MAF>.01)	6,729,635 (MAF>.01)	4,150,783 (MAF>.05)	8,347,617 (MAF>.01)
Reference for full genotyping details	Edwards et al. (2015); Fatemifar et al. (2013)	Salvatore et al. (2014); Konttinen et al. (2015)	Kapoor et al. (2014); Wang et al. (2013)	Adkins et al. (2017)

AD = alcohol dependence, MAF = minor allele frequency, HWE = Hardy-Weinberg equilibrium, IBD = identity-by-descent, SNP = single nucleotide polymorphism.

[a] Illumina, Inc., San Diego, CA, USA,

[b] Affymetrix, Santa Clara, CA, USA,

[c] Li et al., 2010,

[d] Browning & Browning, 2007,

[e] Delaneau et al., 2012,

[f] Howie et al., 2009

Table 2

Polygenic score association results predicting alcohol use disorder-related phenotypes across four independent samples.

	VALIDATION SAMPLE			
	ALSPAC			
DISCOVERY SAMPLE	***Score P threshold***	***Beta***	***p***	***R^2***
ALSPAC	–	–	–	–
FT12	<0.030	16.7	0.006	0.18%
COGA	<0.036	−7.1	0.229	0.04%
IASPSAD	<0.001	−2.1	0. 031	0.11%
Meta (COGA/IASPSAD)	<0.010	−0.4	0.161	0.05%
	FT12			
DISCOVERY SAMPLE	*Score P threshold*	*Beta*	*p*	*R^2*
ALSPAC	**<0.004**	**252.2**	**5.4E−04**	**1.03%**
FT12	**–**	**–**	**–**	**–**
COGA	<0.001	−6.1	0.049	0.28%
IASPSAD	<0.010	−29.7	0.011	0.47%
Meta (COGA/IASPSAD)	<0.011	−0.6	0.297	0.03%
	COGA			
DISCOVERY SAMPLE	*Score P threshold*	*Beta*	*p*	*R^2*
ALSPAC	<0.130	−604.3	0.067	0.09%
FT12	<0.116	−81.5	0.020	0.21%
Meta (ALSPAC/FT12)	<0.023	2.3	0.067	0.09%
COGA	–	–	–	–
IASPSAD	<0.115	61.7	0.070	0.11%
	IASPSAD			
DISCOVERY SAMPLE	*Score P threshold*	*Beta*	*p*	*R^2*
ALSPAC	<0.014	88.0	0.042	0.41%
FT12	<0.006	−7.1	0.026	0.53%
Meta (ALSPAC/FT12)	<0.067	−1.7	0.048	0.39%
COGA	<0.015	9.8	0.026	0.53%
IASPSAD	–	–	–	–

Shaded boxes indicate prediction across different sample types (ascertained to population-based or vice versa). Bolded values are significant ($p < .05$) after correcting for multiple testing. “Score P threshold” indicates the GWAS P-value threshold for selecting which SNPs were included in the polygenic score based on best cross-sample prediction.

Table 3

Polygenic score association results predicting alcohol use disorder-related phenotypes between the young adult (age 16-26) subset of the COGA sample and three independent samples.

		VALIDATION SAMPLE: COGA young adults		
DISCOVERY SAMPLES:	***P threshold***	***Beta***	***p***	***R^2***
ALSPAC	<0.006	−242.4	0.027	0.42%
FT12	<0.062	−91.9	0.013	0.59%
Meta (ALSPAC/FT12)	<0.129	−10.9	0.016	0.50%
IASPSAD	<0.203	111.8	0.094	0.20%

	DISCOVERY SAMPLE: COGA young adults			
VALIDATION SAMPLES:	*P threshold*	*Beta*	*p*	*R^2*
ALSPAC	<0.021	−8.2	0.020	0.13%
FT12	<0.305	−48.1	0.177	0.07%
IASPSAD	<0.013	4.1	0.135	0.17%

Shaded boxes indicate prediction across different sample types (ascertained to population-based or vice versa). Bolded values are significant ($p < .05$) after correcting for multiple testing. “Score P threshold” indicates the GWAS P-value threshold for selecting which SNPs were included in the polygenic score based on best cross-sample prediction.

Table 4

Power to detect polygenic score associations accounting for specified proportions of the variance in alcohol use disorder-related phenotypes across four samples.

R^2	ALSPAC	FT12	COGA	COGA young adults	IASPSAD
0.2%	0.684	0.219	0.255	0.122	0.132
0.5%	0.982	0.507	0.638	0.270	0.286
1.0%	1	0.832	0.947	0.543	0.550
1.5%	1	0.956	0.996	0.759	0.753
2.0%	1	0.991	1	0.890	0.878
5.0%	1	1	1	1	1