Genetic Contributions to Alcohol Use Disorder Treatment Outcomes: A Genome-wide Pharmacogenomics Study

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ABSTRACT:

Naltrexone can aid in reducing alcohol consumption, while acamprosate supports abstinence; however, not all patients with alcohol use disorder (AUD) benefit from these treatments. Here we present the first genome-wide association study of AUD treatment outcomes based on data from the COMBINE and PREDICT studies of acamprosate and naltrexone, and the Mayo Clinic CITA study of acamprosate. Primary analyses focused on treatment outcomes regardless of pharmacological intervention and were followed by drug-stratified analyses to identify treatment-specific pharmacogenomic predictors of acamprosate and naltrexone response. Treatment outcomes were defined as: (1) time until relapse to any drinking (TR) and (2) time until relapse to heavy drinking (THR; ≥5 drinks for men, ≥4 drinks for women in a day), during the first three months of treatment. Analyses were performed within each dataset, followed by meta-analysis across the studies (N=1090 European ancestry participants). Single nucleotide polymorphisms (SNPs) in the BRE gene were associated with THR (min p=1.6E-08) in the entire sample, while two intergenic SNPs were associated with medication-specific outcomes (naltrexone THR: rs12749274, p=3.9E-08; acamprosate TR: rs77583603, p=3.1E-09). The top association signals for TR (p=7.7E-08) and second strongest signal in the THR (p=6.1E-08) analysis of the naltrexone-treated subset map to PTPRD, a gene previously implicated in addiction phenotypes in human and animal studies. Leave-one-out polygenic risk score analyses showed significant associations with TR (p=3.7E-04) and THR (p=2.6E-04). This study provides the first evidence of a polygenic effect on AUD treatment response, and identifies genetic variants associated with potentially medication-specific effects on AUD treatment response.
INTRODUCTION

Alcohol use disorder (AUD) is highly prevalent, and presents a significant health burden worldwide [1]. Several medications are approved for treatment of AUD by the U.S. Food and Drug Administration, but they are underutilized and pharmacological treatment of AUD remains a major challenge [2]. Recent reviews have highlighted the need for a precision medicine approach in the context of AUD treatment in order to increase the utility and safety of available medications [3, 4].

Treatment efficacy of acamprosate and naltrexone is supported by large systematic meta-analyses of randomized controlled trials [5, 6], which suggest that naltrexone helps people refrain from excessive drinking while acamprosate is effective in supporting abstinence [6-12]. Despite these benefits, a considerable proportion of AUD patients fail to benefit from these drugs. The number needed to treat has been estimated to be 7-12 for acamprosate and 12-20 for naltrexone, reflecting variable response rates across studies [7, 8, 13, 14]. Garbutt et al. performed a systematic review to identify moderators of naltrexone response and concluded that available data were insufficient to guide clinical treatment [15]. Similarly, limited success was achieved in studies searching for clinical moderators of acamprosate response [16]. Although secondary analyses including application of machine learning approaches have identified potential moderators of naltrexone and acamprosate response [17], further efforts are needed to identify predictors for personalized medicine. Therefore, discovery of biomarkers of treatment response is a high priority in AUD research [18].

Genetic variation contributes to inter-individual differences in drug response [19-22], and it is expected that genomic factors can aid in prediction of response to AUD treatment [23, 24]. Moreover, discovery of genetic variation and biological pathways involved in treatment response may reveal important information about the mechanisms of drug action, accelerating further drug discovery efforts. Candidate gene studies identified several genetic variations that may influence treatment outcomes in AUD [25-28]. However, the contribution of candidate gene studies to the understanding of genetic effects on AUD treatment response has been limited, highlighting the need for genome-wide pharmacogenomic studies. Yet, to date, no genome-wide association studies (GWASs) of acamprosate or naltrexone treatment response have been published.

Recent GWASs of AUD and other alcohol use related traits have identified a growing number of loci associated with these phenotypes. Moreover, various downstream analyses, including gene and pathway-based analyses and polygenic risk score (PRS) analyses have provided further
insights into the genetic architecture of AUD and related traits, including genetic overlap with other psychiatric and behavioral traits [29-32]. Analyses of quantitative measures of problem drinking and alcohol consumption in large samples have identified more genetic variations contributing to these phenotypes, while identifying loci contributing to AUD has proven more challenging due to the complexity of AUD and the difficulty of compiling large samples of patients evaluated for this phenotype and receiving similar treatments [29-32]. Despite its clinical relevance, AUD treatment response has not yet been studied with the GWAS approach. Investigating the genetics of AUD treatment response is expected to not only reveal genetic variation potentially useful in treatment selection but may also contribute to our understanding of AUD risk and prognosis.

GWAS of treatment outcomes have been performed in the context of other addictions, most notably nicotine dependence. Large studies of smoking behavior phenotypes, including smoking cessation, have demonstrated both shared and unique genetic contributions across these phenotypes, nicotine use disorder, and other diseases [33-35]. Genetic studies of nicotine clearance and nicotine metabolism biomarkers have provided other important insights regarding the genetic contributions to complex smoking related phenotypes [36, 37]. However, similar to AUD, little progress has been made in understanding pharmacogenetic factors that contribute to response to specific smoking cessation treatments.

Here we present the first GWAS of AUD treatment response based on data from three of the largest studies of acamprosate and naltrexone completed to date, with a total sample of more than 1,000 patients treated for AUD: The US-based COMBINE study [38], the German-based PREDICT study [39], and the CITA study [27]. While COMBINE and PREDICT were randomized, placebo-controlled studies designed to evaluate response to acamprosate and naltrexone, the CITA study was an open-label study of acamprosate designed for pharmacogenomics analyses. Using data from these three studies, we performed GWAS analyses of AUD treatment outcomes (across treatment options) as well as pharmacogenomics GWAS of acamprosate and naltrexone response, separately. Post-GWAS analyses were performed including gene-level tests, gene-set and tissue enrichment analyses, and polygenic risk score (PRS) analyses to evaluate polygenic predictors of treatment outcomes across datasets. Our results provide the first evidence of a polygenic signal for AUD treatment outcomes across studies.

METHODS
**Samples**

This study used a new genomic dataset derived from three previously completed studies of acamprosate and/or naltrexone treatment of AUD: the COMBINE, PREDICT, and CITA studies. The key characteristics of these three studies are summarized in Supplemental Table S1. The COMBINE study was a randomized placebo-controlled trial with 1383 alcohol dependent participants, comparing outcomes after 16 weeks of naltrexone or acamprosate, or both, with or without a combined behavioral intervention [38]. DNA for genetic studies was obtained from a subset of COMBINE study participants. PREDICT was a double-blind randomized trial comparing outcomes after three months of acamprosate, naltrexone, or placebo treatment in 426 AUD patients [39]. The CITA study was a single-arm pharmacogenomics study designed to investigate associations of acamprosate treatment outcomes with genetic polymorphisms in genes and pathways previously implicated in acamprosate response (N=443) [27]. All subjects included in our analyses provided consent allowing use of their clinical data and DNA for genetic studies of AUD and response to its treatment, and this study was approved by the Mayo Clinic Institutional Review Board.

**Genotyping, quality control and imputation**

Male participants from the PREDICT study (N=266) were previously genotyped using three different Illumina® (Illumina, San Diego, CA, USA) genome-wide single nucleotide polymorphisms (SNP) platforms as part of a GWAS of alcohol dependence [40]. In CITA, samples were genotyped using Illumina® HumanCore (Illumina, San Diego, CA, USA) arrays and/or Infinium® OmniExpressExome-8 BeadChips (Illumina, San Diego, CA, USA). Of the 1383 subjects enrolled in the COMBINE study, DNA from 758 subjects was genotyped using Infinium® OmniExpressExome-8 BeadChips (Illumina, San Diego, CA, USA). All genetic data underwent quality control followed by imputation. Details of the genotyping, quality control, and imputation are included in the Supplemental Methods. After quality control, data from 498 COMBINE participants, 266 PREDICT participants and 319 CITA participants of European ancestry with treatment outcome data were available for analysis. Following imputation, 5.6 million SNPs with minor allele frequency (MAF) >0.05 in at least one of the studies were analyzed and included in the meta-analyses.

**Assessment of Treatment Outcomes**
Detailed descriptions of study procedures and assessments in the COMBINE, PREDICT and CITA studies are presented elsewhere [27, 38, 39]. In brief, baseline patient characteristics were collected in each study, and outcomes were assessed during three or more months of treatment. Baseline measures included demographic data (age, sex) as well as clinical information including alcohol consumption prior to treatment initiation. Treatment outcomes were derived from timeline follow back (TLFB) data collected at different time points during a three-month period after treatment initiation. For this study, the primary treatment outcome measures were: (1) time until relapse to any drinking during the first three months of treatment, and (2) time until relapse to heavy drinking (≥5 drinks for men, ≥4 drinks for women in a day) during the first three months of treatment. Outcomes for patients that were lost to follow-up in the first three months prior to relapse (or heavy relapse) were treated as censored observations in the survival analysis. Although the two outcomes we analyzed are highly correlated because for many patients first relapse was an episode of heavy drinking, we considered both outcomes, as acamprosate is believed to be effective in preventing relapse to any drinking while naltrexone has been reported to be more effective in preventing relapse to heavy drinking [6, 8, 9].

**Genome-Wide Association Analyses**

The primary analyses included patients treated with acamprosate, naltrexone, or placebo to identify predictors of treatment outcomes regardless of pharmacological intervention. Drug-stratified analyses were then run to identify treatment-specific (i.e. pharmacogenomic) predictors of acamprosate and naltrexone response. All GWAS were run in the three datasets separately, followed by fixed-effects meta-analysis. In each dataset, allelic associations with time until relapse (TR) and time until heavy relapse (THR) were assessed using Cox proportional hazards models. All analyses were adjusted for genetic principal components (PCs), if needed, to control for remaining population stratification in the European ancestry samples. The Cox proportional hazards analyses were run using the survival package in R (version 3.6.2), with SNP effect sizes estimated using hazard ratios (HR). Meta-analyses of GWAS summary statistics were performed using METAL [41].

**Gene Level Associations and Gene-Set and Tissue Enrichment Analyses**

Gene-level tests were performed using Multi-marker Analysis of GenoMic Annotation (MAGMA) [42] as implemented in functional mapping and annotation of genetic associations (FUMA) [43],
which uses Brown’s method to combine SNP p-values across a gene, while accounting for linkage disequilibrium (LD) between SNPs (i.e. $r^2$). Competitive gene-set analyses accounting for potential confounders such as gene size and LD were also performed using MAGMA in FUMA. MAGMA implementation in FUMA was further used to perform tissue enrichment analyses that test whether association results are enriched for genes expressed in particular tissue types based on Genotype-Tissue Expression (GTEx) data [44].

**Leave-one-out Polygenic Risk Score Analyses**

Leave-one-out PRS analyses were used to test for a reproducible polygenic predictor of treatment outcomes between datasets. Specifically, leave-one-out PRSs for TR and THR were generated for each target study based on the results of a discovery GWAS meta-analysis where the target cohort was left out. The PRSs were constructed using PRSice2 [45] to prune ($r^2 >0.1$ within a 500 kb window) and restrict SNPs to a given p-value threshold ($p=0.0001, 0.001, 0.01, 0.05, 0.1, 0.2, 1$), with SNP alleles weighted by their log (HR) estimates. The PRSs for TR and THR were tested for association with the respective treatment outcome using Cox proportional hazards models. Analyses of the COMBINE study data were adjusted for the first four PCs to account for the heterogeneity observed in this dataset. Finally, the results from the leave-one-out PRS analyses of the three datasets were meta-analyzed to assess overall PRS prediction of treatment response. Nagelkerke’s pseudo-$R^2$ was calculated to estimate the variance explained in TR or THR by the PRS in each dataset, and weighted average $R^2$ values were calculated across the three datasets using the effective N for each cohort.

**RESULTS**

Descriptive statistics for the three datasets are shown in Table 1. In total, 639 (59%) of the study participants relapsed to any drinking, while 564 (52%) relapsed to heavy drinking.

**Genome-wide association study**

Manhattan plots for the two GWAS meta-analyses in the full cohort (all treatments) are shown in Figure 1; the corresponding Quantile/Quantile (QQ) plots are shown in Supplemental Figure S1. In the meta-analysis across the three studies no individual SNPs were significantly associated with TR; the SNP with the strongest evidence for association is located in an intron of *KCNQ4*
(rs1078110, P=6.2E-07). In the full cohort analysis of THR, significant associations were observed for a set of SNPs in BRE (14 genome-wide significant SNPs, top SNP rs56951679, minor allele HR=1.5, p=1.6E-08). The effect estimate for the top SNP at this locus (rs56951679) suggests that carrying one additional copy of the minor allele (C) is associated with 1.5 times higher risk of relapse to heavy drinking. A regional association plot for this locus is shown in Supplemental Figure S2.

In drug-stratified meta-analyses of outcomes in patients treated with naltrexone (Supplemental Figure S3), an intergenic SNP located between the long intergenic non-coding RNA RP4-710M16.2 and the PPAP2B gene was associated with THR (rs12749274, HR=2.90, p=3.9E-08). While not achieving genome-wide significance, the strongest evidence for association with TR and second strongest for THR was observed with a SNP in an intron of PTPRD (rs62533259: TR minor allele HR=2.2, p=7.7E-08; THR minor allele HR=2.4, p=6.1E-08). PTPRD has been implicated in multiple addiction related phenotypes in humans and other species[46].

One SNP was significantly associated with TR during acamprosate treatment; this intergenic SNP is located between a non-coding RNA gene and the ribosomal protein L29 pseudogene (rs77583603; p=3.1E-09; Supplemental Figure S4). The top signal in the analyses of THR in acamprosate-treated patients was rs34797278 in a non-coding RNA (p=5.4E-08). All loci with suggestive evidence of association (p<5*10^-6) with one of the outcome measures are shown in Supplementary Tables S1 (all treatments), S2 (naltrexone), and S3 (acamprosate).

Supplementary Figure S5 shows scatterplots of results (p-values) from analyses of TR vs. THR, and for analyses of acamprosate treatment outcomes vs. naltrexone treatment outcomes. As expected, TR GWAS signals were highly correlated with THR signals (panels A, B, and C of Supplementary Figure S5), but results for acamprosate treatment outcomes showed little correlation with signals for naltrexone treatment outcomes (panels D and E of Supplementary Figure S5). Although TR signals were generally highly correlated with THR signal, the top association from the THR analysis of the full cohort (the BRE top SNP p=1.6E-08) showed much weaker evidence for association with TR (p=1E-04). On the other hand, the PTPRD rs62533259 association in the naltrexone-treated subset was a top signal in both the TR and THR analyses (shown in panel B of Supplemental Figure S5). However, the same PTPRD variants were not associated with TR or THR in the acamprosate-treated patient subset (noted in panels D and E of Supplemental Figure S5).
Gene-level, Gene-Set, and Tissue Enrichment Analyses

There were no significant gene-level associations based on MAGMA analyses (see Methods for details) of the GWAS results presented above, although the BRE gene association with THR in the full sample approached significance (p=4.0E-6, Bonferroni-corrected p=0.079). In MAGMA gene-set association analyses of THR in naltrexone-treated patients, the strongest association was with a curated gene set named “boyault_liver_cancer_subclass_g56_up” representing up-regulated genes in hepatocellular carcinoma subclass G56, defined by unsupervised clustering; the 11 genes in this gene set showed enrichment for association with THR in the naltrexone-treated patients (p=1.0E-06, Bonferroni corrected p=0.016; most strongly associated genes in this gene set were DPP4, SMYD2, and TBX3). Also, in the analyses of naltrexone treatment outcomes, the top tissues based on expression of genes enriched for association signals (at p<0.05) were brain tissues, including the hippocampus, putamen, basal ganglia, cortex, and amygdala (Supplemental Fig S6), but these enrichment results were not statistically significant after multiple testing correction.

Leave-one-out Polygenic Prediction

Leave-one-out analyses provided evidence of a polygenic effect on treatment response in AUD. Specifically, for both TR and THR, PRSs derived from SNP effects observed in any two of the studies (using p-value threshold of 0.05) explained a statistically significant proportion of variance in the same outcome in the left-out dataset (Nagelkerke’s R² = 1.3%; p=3.7E-04 for time until relapse, Nagelkerke’s R²=1.3%; p=2.6E-04 for time until heavy relapse; Figure 2).

DISCUSSION

Using data from three studies of AUD treatment outcomes, we performed the first GWAS for AUD treatment response, including drug-stratified pharmacogenomic analyses of acamprosate and naltrexone treatment outcomes, and we identified several genome-wide significant associations. We also performed PRS analyses to assess if a polygenic signal captured by this GWAS is associated with AUD treatment outcomes in independent samples. Results of these leave-one-out PRS analyses provided the first evidence of association between AUD treatment outcomes and PRSs, reflecting combined effect of variation across the genome on response to AUD treatment. While the proportion of variation in the treatment response explained by the PRSs is small, as expected for relatively small studies of complex traits, the significant
association demonstrates that polygenic effects contribute to AUD treatment outcomes motivating further research into identifying contributing genetic factors.

Significant evidence of association was observed between THR and SNPs in the brain and reproductive organ-expressed protein (BRE) gene (aliases include BABAM2, BRCC4 and BRCC45), which encodes BRISC and BRACA1 A complex member 2. BRE is ubiquitously expressed in human tissues, but most prominently in the zona glomerulosa of the adrenal cortex, where mineralocorticoids (e.g., aldosterone) are synthesized and secreted, as well as in glia and neurons [47]. Although the exact role of BRE in mineralocorticoid synthesis and secretion is unclear, BRE expression is altered in adrenal abnormalities, suggesting BRE involvement in adrenal function [47]. Chronic alcohol consumption has been shown to increase blood aldosterone levels in humans and other species, and increased aldosterone level was correlated with higher alcohol consumption, craving and anxiety levels in AUD patients [48]. Aldosterone may regulate alcohol use behaviors and anxiety via mineralocorticoid receptors expressed in limbic regions [49], including regions involved in regulating anxiety, stress-induced alcohol consumption, craving, and inhibitory control. Thus, our finding of association of BRE SNPs with THR may be related to regulation of stress response and alcohol craving.

Furthermore, BRE has shown genome-wide significant associations with a range of phenotypes from different domains (e.g. metabolic, immunological, cardiovascular) [50]. With respect to psychiatric traits, genetic variation in BRE has been associated with alcohol intake frequency and drinks per week in the general population [33]. However, it is also worth noting that according to GTEx, BRE rs56951679 (and other top SNPs in our analysis) is a splicing qualitative trait locus (sQTL) for FNDC4 exon 7 in human cortex, hippocampus and nucleus accumbens. FNDC4, a secreted factor highly expressed in liver and brain, was implicated in sex-specific neurotoxic consequences of chronic alcohol withdrawal [51]. The mechanisms of action through which variants in the BRE region may impact response to AUD treatment need to be further explored.

The one SNP with genome-wide significant evidence of association with acamprosate treatment response (rs77583603; p=3.1E-09 for TR) is located between a non-coding RNA gene and the ribosomal protein L29 pseudogene and is not itself annotated as functionally significant. Analyses of data from patients treated with naltrexone identified a SNP near PPAP2B that was significantly associated with THR. PPAP2B (a.k.a. PLPP3, LPP3) encodes phospholipid phosphatase 3. Changes in PPAP2B gene expression have been observed in nucleus accumbens and amygdala of rodents after voluntary ethanol consumption [52, 53], and PPAP2B


has been implicated in alcoholic fatty liver in humans [54]. These genes/variants identified in the medication-specific analyses have not been previously implicated in AUD or other addiction-related traits in humans, and their potential role in AUD treatment response needs further investigation.

An intronic SNP of PTPRD, while not quite genome-wide significant, was the strongest association signal for TR (p=7.7E-08) and the second strongest association for THR (p=6.1E-08) in the naltrexone analysis. This finding is notable because PTPRD (protein tyrosine phosphatase receptor type D) has been implicated in multiple addiction related phenotypes in humans and in animal studies [55, 56]. Protein tyrosine phosphatases are signaling molecules that regulate a variety of cellular processes, and PTPRD likely plays a role in neuronal cell adhesion. Uhl and Martinez [46] provided a comprehensive review of PTPRD genetics and neurobiology, and discussed its potential role as a pharmacological target with effects on brain phenotypes. Of note, the PTPRD effect we observed appears to be a pharmacogenomic effect specific to naltrexone, as our study provided no evidence of association of the same PTPRD variant with acamprosate treatment response. The impact of PTPRD on naltrexone treatment outcomes is, therefore, intriguing and warrants further investigation.

We performed GWAS of treatment outcomes in the full cohort of patients enrolled in the COMBINE, PREDICT and CITA studies, as well as drug-specific analyses of treatment outcomes for acamprosate and naltrexone. The drug-stratified analyses aim to identify genetic variants that impact response to a given drug. The analyses of the full sample may also identify pharmacogenomic effects, but have greater power to identify genetic variants that impact risk of relapse regardless of specific treatment – these variants may include markers of severity of AUD or AUD subtypes that confer differential risk of relapse following treatment. Two genes implicated in this study, BRE and PTPRD, were previously associated with AUD or related phenotypes. Several known AUD risk genes, particularly ADH1B, that have been most consistently associated with AUD in large studies [32], were not significant predictors of treatment response in our analyses. The lack of such findings may be partly due to a relatively small contribution of AUD risk genes to treatment response, which would be consistent with findings in other psychiatric disorders including major depressive disorder [57, 58]. Additionally, low-frequency variants that are protective against AUD could have very low frequencies in the studied samples of AUD patients, reducing the power to detect their contribution to treatment response. Indeed, this may be the case with ADH1B variants that are protective against AUD but have low allele frequencies in populations of European ancestry. For example, the well-
established AUD-associated SNP rs1229984 in ADH1B has a frequency of ~5% in European populations [59]. In the COMBINE dataset this SNP had a frequency of only ~2%, whereas in CITA it had a frequency of ~1%, and in PREDICT its frequency was <1%. Because of its low frequency and poor imputation quality in the CITA and PREDICT samples, this SNP was not analyzed in these datasets. However, analysis of the COMBINE dataset provided marginal evidence of association for rs1229984 with TR (HR=0.32, p=0.016) and THR (HR=0.27, p=0.0053); the effect estimates suggest the minor allele at rs1229984, which is protective against AUD, may have a strong protective effect against relapse during treatment, reducing the risk of relapse by about 70%. Because its MAF was below 0.05 in all of the samples, this SNP was excluded from the meta-analyses. As evidence accumulates regarding other genetic variants that contribute to AUD and related phenotypes, and as larger studies allow the computation of more predictive polygenic risk scores for AUD and related traits, it will be important to further examine the association of those variants and polygenic risks with AUD treatment outcomes.

This study made use of data from three prior studies of AUD treatment. These three studies have important similarities allowing for combined analysis of treatment outcomes, including using the TLFB to assess drinking outcomes. However, the three studies also differed in relevant patient characteristics. The available PREDICT study data were only from men, but the COMBINE and CITA study samples included 31% and 36% women, respectively. Reported pre-treatment baseline alcohol consumption also differed, with PREDICT participants having the highest (and CITA participants having the lowest) alcohol consumption at baseline and participants in the COMBINE study having the shortest duration of abstinence prior to treatment initiation. The studies also differed in rates of treatment outcomes, with the CITA sample having the lowest percentage of any-drinking relapse and heavy-drinking relapse. While this heterogeneity may have limited the replicability of findings between the three datasets and reduced power of the meta-analysis, the differences between the studies also mean that identified genetic associations are likely robust genetic predictors of treatment outcomes that apply to a broad range of patients.

In addition to sample heterogeneity that may have reduced the power of this study, other limitations need to be noted. The sample size of this study is smaller than what is typically required for well-powered GWAS of complex traits, particularly when the goal is identification of specific genetic risk variants. However, this sample size of approximately 1,000 was expected to be adequate to detect polygenic effects, as supported by our PRS results. Other limitations of
this study include a lack of thorough investigation of the role of intermediate or potentially confounding factors such as demographic and clinical variables or comorbidities (e.g. age, sex, smoking) as well as measures of AUD severity or alcohol consumptions. Because each of the contributing studies collected different data at baseline, harmonization of these variables and investigation of their role in the context of pharmacogenomics effects on AUD treatment response was beyond the scope of this study, but should be further investigated in the future. Finally, because of the limited ancestral diversity in the contributing studies, the analysis was limited to patients of European ancestry, limiting the generalizability of the findings to other populations, and reducing the power to identify effects of variants that are rare in European ancestry populations such as the ADH1B variant discussed above, or the ALDH2 Glu504Lys polymorphism (rs671) that generates extraordinarily significant association to AUD (e.g. \( p = 10^{-4740} \)), but only in populations where the locus is actually polymorphic. Studies of the genetics of AUD treatment response in samples with other ancestries are needed.

In conclusion, these first GWAS analyses of AUD treatment outcomes had limited power for discovery of specific genetic variants associated with response to AUD treatment. Nevertheless, they provided important insights, including the first demonstration of polygenic effects on AUD treatment outcomes, and identification of genetic variants potentially associated with AUD treatment response. These findings motivate further investigation of the genetic contribution to AUD outcomes and study of the biological mechanisms underlying response to medications used in the treatment of AUD.

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**Conflicts of Interest**

Dr. Weinshilboum is a co-founder of and stockholder in OneOme LLC, a pharmacogenomics decision-support company. Dr. O’Malley reports non-financial support from Amygdala, AstraZeneca, and Novartis, personal fees from Emmes Corporation (DSMB member NIDA Clinical
Trials Network), Alkermes, Dicerna, Opiant, and from the American Society of Clinical Psychopharmacology Alcohol Clinical Trials Initiative supported by Alkermes, Amygdala Neurosciences, Arbor Pharmaceuticals, Dicerna, Ethypharm, Indivior, Lundbeck, Mitsubishi and Otsuka, grants from National Institute on Alcohol Abuse and Addiction, and grants from National Institute on Drug Abuse outside the submitted work. In the past 3 years, Dr. Anton has been a consultant for Alkermes, Allergan, Dicerna, Insys, Laboratorio Farmaceutico C.T., Foxo Bioscience. He also received grant funding from Laboratorio Farmaceutico C.T. He is a chair and participant in the Alcohol Clinical Trials Initiative (ACTIVE) that has received support (in the past or currently) from Abbvie, Alkermes, Amygdala, Arbor, Dicerna, Ethypharm, Glaxo Smith Kline, Indivior, Janssen, Eli Lilly, Lundbeck, Mitsubishi, Otsuka, Pfizer, and Schering. All other authors have no conflicts of interest to declare.

**Figure Legends**

**Figure 1**: Manhattan Plots for (A) Time until relapse to any drinking and (B) Time until relapse to heavy drinking, in all subjects.

**Figure 2**: Leave-one-out PRS analysis of (A) time until relapse to any drinking and (B) time until relapse to heavy drinking. In each of the three studies (CITA, COMBINE and PREDICT) PRSs were constructed based on a discovery GWAS in the remaining two samples across a range of p-value thresholds (\(p_T\) denoted using different colors described in the legend) to select SNPs for inclusion in the PRS. The selected SNPs (after LD pruning) were used to compute PRSs and the association of the PRSs with the outcome (TR or THR) was tested. The plots show the \(-\log_{10}(p\text{-values})\) for these association tests (on the y-axis) in each sample, as well as the meta-analysis of the leave-one-out PRS associations across the studies. The PRS association meta-analyses provided significant results for both time until relapse (\(p=3.7E-04, \text{Nagelkerke's } R^2 = 1.3\% \text{ at } p_T = 0.05\)) and time until heavy relapse (\(p=2.6E-04, \text{Nagelkerke's } R^2 = 1.3\% \text{ at } p_T = 0.10\)).
Table 1: Demographic and Clinical Characteristics of the COMBINE, PREDICT and CITA participants included in the pharmacogenomics GWAS.

| Sample sizes | COMBINE | PREDICT | CITA |
|--------------|---------|---------|------|
| Total N      | 498     | 266     | 319  |
| Acamprosate N| 223     | 110     | 319  |
| Naltrexone N | 199     | 102     | -    |
| Placebo only or no pills N | 177 | 54 |      |

| Age, mean(SD) | COMBINE | PREDICT | CITA |
|---------------|---------|---------|------|
| Age, mean(SD) | 45.3 (10.6) | 45.2 (8.5) | 43.4 (11.6) |
| Sex, male N(%) | 343 (68.9%) | 266 (100%) | 204 (64.0%) |
| Age of onset | 30.5 (11.9) | 30.2 (10.2) | 29.6 (12.0) |

| Baseline alcohol consumption⁶, mean (sd) | COMBINE | PREDICT | CITA |
|-----------------------------------------|---------|---------|------|
| Days since last drinking day | 8.0 (5.4) | 22.0 (4.4) | 23.4 (15.6) |
| Average number of drinks per drinking day | 12.7 (7.5) | 21.0 (12.7) | 11.9 (7.4) |
| % Drinking days⁶ | 71.8 (24.8) | 82.0 (26.8) | 52.2 (29.8) |
| % heavy drinking days | 63.9 (24.8) | 79.4 (27.9) | 47.6 (30.4) |

| Treatment Outcomes: | COMBINE | PREDICT | CITA |
|---------------------|---------|---------|------|
| Relapse, N (%)      | 380 (76.3%) | 158 (59.4%) | 101 (31.7%) |
| Relapse in acamprosate subset, N (%) | 167 (74.9%) | 62 (56.4%) | 101 (31.7%) |
| Relapse in naltrexone subset, N (%) | 146 (73.4%) | 65 (63.7%) | - |
| Heavy Relapse, N (%) | 338 (67.9%) | 142 (53.4%) | 84 (26.3%) |
| Heavy relapse in acamprosate subset, N (%) | 144 (64.6%) | 56 (50.9%) | 84 (26.3%) |
| Heavy relapse in naltrexone subset, N (%) | 127 (63.8%) | 57 (55.9%) | - |

*Baseline alcohol consumption measures are based on 90 days before start of treatment in COMBINE and CITA, and 30 days before start of treatment in PREDICT.

¹% drinking days = 100 - % days abstinent in the same time period
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