ORIGINAL ARTICLE

Genetic markers associated with abstinence length in alcohol-dependent subjects treated with acamprosate

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Acamprosate supports abstinence in some alcohol-dependent subjects, yet predictors of response are unknown. To identify response biomarkers, we investigated associations of abstinence length with polymorphisms in candidate genes in glycine and glutamate neurotransmission pathways and genes previously implicated in acamprosate response. Association analyses were conducted in the discovery sample of 225 alcohol-dependent subjects treated with acamprosate for 3 months in community-based treatment programs in the United States. Data from 110 alcohol-dependent males treated with acamprosate in the study PREDICT were used for replication of the top association findings. Statistical models were adjusted for relevant covariates, including recruitment site and baseline clinical variables associated with response. In the discovery sample, shorter abstinence was associated with increased intensity of alcohol craving and lower number of days between the last drink and initiation of acamprosate treatment. After adjustment for covariates, length of abstinence was associated with the *GRIN2B* rs2058878 ($P = 4.6 \times 10^{-5}$). In the replication sample, shorter abstinence was associated with increased craving, increased depressive mood score and higher alcohol consumption. Association of abstinence length with *GRIN2B* rs2058878 was marginally significant ($P = 0.0675$); as in the discovery sample, the minor A allele was associated with longer abstinence. Furthermore, rs2300272, which is in strong linkage disequilibrium with rs2058878, was also associated with abstinence length ($P = 0.049$). This is the first report of a replicated association of genetic markers with the length of abstinence in acamprosate-treated alcoholics. Investigation of the underlying mechanisms of this association and its usefulness for individualized treatment selection should follow.

Translational Psychiatry (2014) **4**, e462; doi:10.1038/tp.2014.103; published online 7 October 2014

INTRODUCTION

The economic costs associated with alcohol use and misuse are staggering, and evidence-based treatment strategies are needed.[1,2] Meta-analyses favor acamprosate for its ability to support abstinence,[3–5] which is the most stable type of remission in alcohol-dependent subjects.[6] Only a fraction of those treated respond to acamprosate.[7] Clinical characteristics, including the number of sober days before initiation of acamprosate treatment, were shown to be associated with abstinence length.[8–10] However, no biomarkers allowing reliable identification of potential responders to acamprosate treatment are currently known. It is expected that a pharmacogenomic approach may lead to the discovery of such biomarkers, enabling individualized recommendations for treatment selection and improved treatment outcomes.[11,12]

Acamprosate shares structural similarities with glycine and glutamate[13] and is thought to work by counteracting the 'relief craving' associated with increased glutamate levels in alcoholics with a history of withdrawal.[14,15] Several genes, including *GATA4*, *PER2* and *SLC29A1*, were associated with response to acamprosate treatment in human and animal studies.[16–18] Recent findings also

indicate that glycine, which activates N-methyl-D-aspartate (NMDA) and glycine receptors,[19,20] may have an important role in alcohol use disorders and treatment response.[21–25] Therefore, in an effort to identify genetic markers associated with abstinence in alcohol-dependent human subjects receiving acamprosate treatment, we investigated sequence variation in genes involved in composition of the glycine and NMDA receptors, glycine and glutamate reuptake, synthesis and metabolism, along with sequence variation in candidate genes previously reported to be associated with acamprosate response in human and animal studies.

To make study findings practically meaningful, we considered several potentially important issues. First, we conducted our study in samples reflecting the population where results will be applied, that is, alcohol-dependent subjects treated in community-based programs. Second, we monitored non-genetic (that is, clinical and demographic) patient characteristics and accounted for relevant covariates and potential confounders in the genetic analyses. Third, to ensure that findings were relevant and readily translated into clinical practice, we utilized diagnostic tools and outcome measures used in community-based treatment programs. In

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Received 25 July 2014; accepted 24 August 2014

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accordance with these considerations, we conducted an open label, naturalistic prospective study investigating genetic markers associated with abstinence in alcohol-dependent subjects treated with acamprosate in community-based programs. Replication analyses were performed using data from a previously conducted study of acamprosate (PREDICT[26]).

We identified genetic markers associated with the length of sobriety during acamprosate treatment. This is an important step toward the development of personalized treatment recommendations for patients with alcohol use disorders, as genetic markers may be used for selection of patients who have the highest probability of responding to acamprosate treatment. We think that with the limited number of antidipsotropic medications and lack of uniformity in treatment response, the development of such recommendations is of special importance for improvement of treatment outcomes in patients with alcohol use disorders.

MATERIALS AND METHODS

Discovery study
Subjects and recruitment sites. This study was approved by the Institutional Review Board of the Mayo Clinic Rochester and Mayo Clinic Health System, and was conducted according to the Code of Ethics of the World Medical Association (Declaration of Helsinki). All participants signed informed consent approved by the Mayo Clinic Institutional Review Board.

Both men and women between the ages of 18 and 80 with a primary diagnosis of current alcohol dependence based on DSM-IV-TR criteria with the last drink 5 or more days before enrollment were included in the study. We excluded subjects unable to provide informed consent; those unable to speak English; those with psychotic disorders or unstable psychiatric or medical conditions (see Supplementary Materials); women who were pregnant, lactating or planning to become pregnant; subjects taking disulfiram; and those allergic to acamprosate.

Participants were recruited from community-based residential and outpatient treatment programs affiliated with Mayo Clinic in Rochester, Minnesota, and the Mayo Clinic Health System sites in Austin, Minnesota, Albert Lea, Minnesota, and La Crosse, Wisconsin (Supplementary Table S1). In addition, self-referred participants residing in communities adjacent to referral sites not enrolled in treatment programs but interested in taking acamprosate were recruited and included in the analyses as a separate 'study site.' A description of programs and the number of subjects recruited at each site is presented in Supplementary Materials.

Assessment. A detailed description of assessments is presented in Supplementary Table S2 and summarized below. The lifetime presence of alcohol dependence and comorbid disorders was assessed using the semistructured interview PRISM.[27] Time Line Follow-Back[28] was used to assess alcohol consumption before and during treatment. Craving intensity was assessed using Penn Alcohol Craving Scale (PACS).[29,30] Association of alcohol use with positive or negative emotional states was assessed using the Inventory of Drug Taking Situations.[31,32] The intensity of depressive symptoms and anxiety were assessed using a depression scale from the patient health questionnaire,[33] and the Generalized Anxiety Disorder Assessment.[34] Alcoholic Anonymous attendance monitoring[35] was used to estimate utilization of support networks.

Treatment outcomes including abstinence (defined as time between initiation of acamprosate treatment and first alcohol use) or alcohol use were assessed with self-report (Timeline Follow-Back) and from available medical records during follow-up visits. Gamma-glutamyl transpeptidase measurements were used to assess the accuracy of self-reported sobriety. Compliance with acamprosate was assessed by pill count. The use of other medications was also monitored and considered as potential covariates in the analyses.

Selection of candidate genetic targets in the discovery study. Candidate genes (Supplementary Table S3) were selected based on the following considerations. First, we used a pathway-based approach to systematically investigate genes encoding enzymes involved in glycine metabolism, glycine transporters and subunits of glycine receptors and the NMDA receptor, which is known to be involved in glycine effects.[19,36] Second, because acamprosate action is thought to be associated with increased brain glutamate levels,[14,15] we included genes associated with glutamate

reuptake, synthesis and degradation. Third, we included candidate genes reported to be associated with acamprosate treatment outcomes in human or animal studies[16–18] and genes associated with alcoholism treatment response.[37–39]

We used SNPPicker[40] for selection of tag single-nucleotide polymorphisms (SNPs) to attain comprehensive coverage of each candidate gene. In addition, 28 SNPs were selected from a panel of ancestry informative markers[41] to verify self-reported ancestry. The list of 548 candidate SNPs included in the study is presented in Supplementary Table S4.

Genotyping and quality control. Genotyping was conducted using Illumina Golden Gate custom panel of 576 single-nucleotide variants.[42] Of the 576 SNPs included in the genotyping panel (548 candidate SNPs, 28 ancestry informative SNPs), 19 failed and 11 had minor allele frequencies $< 2\%$ and were excluded from analysis. Of the 433 subjects who started acamprosate treatment and were genotyped, four were excluded from analyses because of low call rates ($< 90\%$). As part of genotype quality control, 18 subjects were genotyped in duplicate and a CEPH trio was genotyped eight times. No discordant genotype calls were observed for these replicated samples. Hardy–Weinberg equilibrium was evaluated; however, no SNPs were removed (Supplementary Table S4). To verify self-reported race, STRUCTURE v2.3.3 (Stanford University, Stanford, CA, USA) was used to estimate ancestry of individuals based on the 28 ancestry informative markers. Four self-reported European Americans were removed as they appeared to have $> 50\%$ non-European ancestry.

Data analyses. Time-to-event (first alcohol use) survival analysis methods were used to examine the association of clinical and genetic markers with treatment outcomes. Demographic and baseline clinical characteristics were first evaluated for associations with length of abstinence using univariate Cox proportional hazard models. As we found an association of treatment outcome with recruitment site, likely reflecting differences in populational characteristics and non-pharmacological treatment components across sites, we controlled for recruitment site as a covariate in all subsequent analyses. Backward stepwise variable selection with covariates that were univariately related to response ($P < 0.05$) was performed to identify other relevant covariates and potential confounders for the pharmacogenetic analyses. On the basis of the results of this selection process, all genetic analyses were adjusted for enrollment site, days since last drink at baseline and baseline PACS.

Replication study
Study sample. To replicate top association findings in the discovery sample, we conducted similar analyses in a subset of participants from the study PREDICT, a double-blind randomized controlled trial that compared treatment outcomes including length of abstinence among alcohol-dependent subjects of German descent recruited from inpatient facilities and treated with acamprosate, naltrexone or placebo for 3 months.[26] Clinical and genetic data from 110 males treated with acamprosate who were also included in a genome-wide association study of alcohol dependence[43] and, thus, had genotype data available, were included in the replication analyses. The diagnostic assessments and instruments used in the study PREDICT were described previously.[26]

Genotyping, selection of candidate genetic targets and data analysis in the replication sample. A subset of PREDICT study participants was genotyped using Illumina Human-Hap 550, Illumina Human 610 and Illumina Human 660w quad BeadChips (Illuminia, San Diego, CA, USA) for a genome-wide association study of alcohol dependence.[43,44] We selected four top SNPs associated with treatment outcome in the discovery sample ($P < 0.001$) for replication. Three of those SNPs were not genotyped in the replication sample. Therefore, we imputed those SNPs using the ShapeIT tool, v1 (Christchurch, New Zealand) for phasing and IMPUTE (v2.3, Oxford, UK) for imputation.[45,46] The European sample from 1000 genomes data was used as the reference panel, and the three imputed SNPs had dosage $R^2 > 0.99$.

Analysis of our Replication Set data followed the same approach as the Discovery Set analysis. Specifically, Cox proportional hazard models were used to examine the association of length of abstinence with clinical variables and candidate SNPs. Backward stepwise variable selection was again used to identify covariates and potential confounders for the genetic analyses. Genetic association analyses were then performed for SNP genotypes coded as the minor allele count.

RESULTS

Clinical and demographic variables associated with abstinence in the discovery sample

Of the 443 subjects who started acamprosate, 225 European American subjects with available 3-month outcome data (any alcohol use $N = 93$; abstainers $N = 132$) were included in the analyses. The length of abstinence was significantly associated with the recruitment site as well as several clinical variables (Table 1). Increased craving and depression scores (measured by PACS and patient health questionnaire 9, respectively) were associated with shorter abstinence. Longer abstinence was associated with increased number of days since last drink before initiation of acamprosate, increased attendance of Alcoholics Anonymous meetings, having an Alcoholics Anonymous sponsor and attendance at counseling sessions. However, after adjustment for study site, only the baseline PACS score and the number of days between the last drink and initiation of acamprosate treatment remained strongly associated with treatment outcome ($P < 0.0001$ and $P = 0.0002$, respectively). Using a backward stepwise variable selection process, covariates (including study site, PACS score and the number of days between last drink and initiation of acamprosate treatment) were selected for adjustment in pharmacogenomic analyses.

The observed changes in plasma gamma-glutamyl transpeptidase levels between baseline and 3-month follow-up were consistent with self-reported abstinence and alcohol use. As shown in Supplementary Figure S1, gamma-glutamyl transpeptidase levels were elevated at baseline and decreased markedly during treatment in abstainers and non-abstainers. At the 3-month follow-up, average gamma-glutamyl transpeptidase levels were significantly lower and closer to the normative range in abstainers compared with non-abstainers ($P < 0.001$).

Single SNP association with treatment outcomes in the discovery sample

A complete list of candidate SNPs and their association with the length of abstinence is presented in Supplementary Table S4, and summarized in Figure 1. The top results ($P < 0.001$) are also presented in Table 2. The strongest association finding, minor GRIN2B rs2058878 A allele, remains significantly associated with longer abstinence after Bonferroni correction for the number of SNPs included in the analyses ($P = 4.6 \times 10^{-5}$, corrected $P = 0.024$). As the replication sample included only alcohol-dependent males, we also explored the association of the top four SNPs with treatment outcome in a subsample of males. As shown in Table 2, the evidence for association with treatment outcome was stronger for minor GLRB rs17035723 A allele in the male subset (hazard ratio = 2.88, $P = 0.98 \times 10^{-5}$). The hazard ratios of the three GRIN2B SNPs, including rs2058878, were also higher in males; however, P-values reflecting their association with treatment outcome were less significant, possibly because of the decreased sample size in the subsample of males.

Clinical and demographic variables associated with abstinence in the replication sample

As shown in Table 3, the replication sample included only alcohol-dependent males and was characterized by higher alcohol consumption compared with the discovery sample. Similar to the discovery sample, increased craving (measured by the Obsessive Compulsive Drinking Scale) was associated with shorter abstinence. Shorter abstinence was also associated with higher alcohol consumption, increased depressive symptoms (measured by the Beck Depression Inventory) and younger age at onset of alcohol dependence. A final multivariable model of potentially relevant covariates (selected using the same process as in the discovery data set) included Beck Depression Inventory score, number of drinks per drinking day and age of onset of alcoholism; all SNP association analyses were adjusted for these covariates.

Table 1. Demographic and clinical measures associated with the length of abstinence during first 3 months of acamprosate treatment in 225 alcoholics in the discovery sample

Parameter	Mean ± s.d. or N (%)	Association with Relapse Risk			
		Unadjusted		Adjusted for site	
		HR (95% CI)	P-value	HR (95% CI)	P-value
Study site			< **0.0001**	—	—
Age at evaluation	44.8 ± 11.7	0.99 (0.97, 1.01)	0.35	0.99 (0.98, 1.01)	0.51
Gender (male)	148 (65.8%)	0.67 (0.45, 1.02)	0.060	0.72 (0.47, 1.09)	0.12
AA meetings/month	11.8 ± 12.8	0.96 (0.93, 0.98)	**0.0008**	0.98 (0.96, 0.99)	**0.043**
Counseling sessions/month	11.7 ± 14.4	0.98 (0.96, 1.00)	**0.017**	0.99 (0.97, 1.01)	0.27
AA sponsor (yes or no)	109 (54.0%)	0.52 (0.33, 0.81)	**0.0043**	0.62 (0.39, 0.97)	**0.036**
IDTS-negative score	56.6 ± 22.4	1.00 (0.99, 1.01)	0.51	0.99 (0.99, 1.01)	0.85
IDTS-positive score	56.3 ± 25.3	1.00 (0.99, 1.01)	0.41	1.00 (0.99, 1.01)	0.57
IDTS temptation score	48.7 ± 23.4	1.01 (1.00, 1.02)	0.083	1.00 (0.99, 1.01)	0.52
Baseline PHQ-9	9.4 ± 6.3	1.04 (1.01, 1.08)	**0.0073**	1.02 (0.99, 1.06)	0.16
Baseline GAD-7	8.8 ± 5.9	1.03 (0.99, 1.07)	0.092	1.01 (0.97, 1.04)	0.65
Baseline PACS	13.8 ± 8.5	1.09 (1.06, 1.12)	< **0.0001**	1.06 (1.03, 1.10)	< **0.0001**
Maxi drinks per day	16.4 ± 11.4	0.99 (0.98, 1.01)	0.56	1.00 (0.98, 1.01)	0.70
Average drinks per drinking day	11.3 ± 7.4	0.99 (0.96, 1.02)	0.53	1.00 (0.97, 1.03)	0.82
Antipsychotic use	6 (2.7%)	2.13 (0.78, 5.80)	0.14	1.03 (0.37, 2.89)	0.96
Benzodiazepine use	35 (15.6%)	0.87 (0.48, 1.56)	0.63	0.93 (0.56, 1.55)	0.71
Mood stabilizer use	7 (3.1%)	1.71 (0.63, 4.66)	0.29	1.45 (0.70, 2.99)	0.22
Antidepressant use	87 (38.7%)	1.02 (0.70, 1.61)	0.78	0.99 (0.79, 1.22)	0.50
Number of days between last drink and acamprosate treatment	21.8 ± 14.9	0.95 (0.93, 0.97)	< **0.0001**	0.96 (0.94, 0.98)	**0.0002**

Abbreviations: AA, Alcoholics Anonymous; CI, confidence interval; GAD-7, Generalized Anxiety Disorder assessment scale; HR, hazard ratio; IDTS, Inventory of Drug Taking situations; PACS, Penn Alcohol Craving Scale; PHQ-9, nine item depression scale from the patient health questionnaire; TLFB, The Alcohol Timeline Follow-Back. P-values below 0.05 are marked in bold.
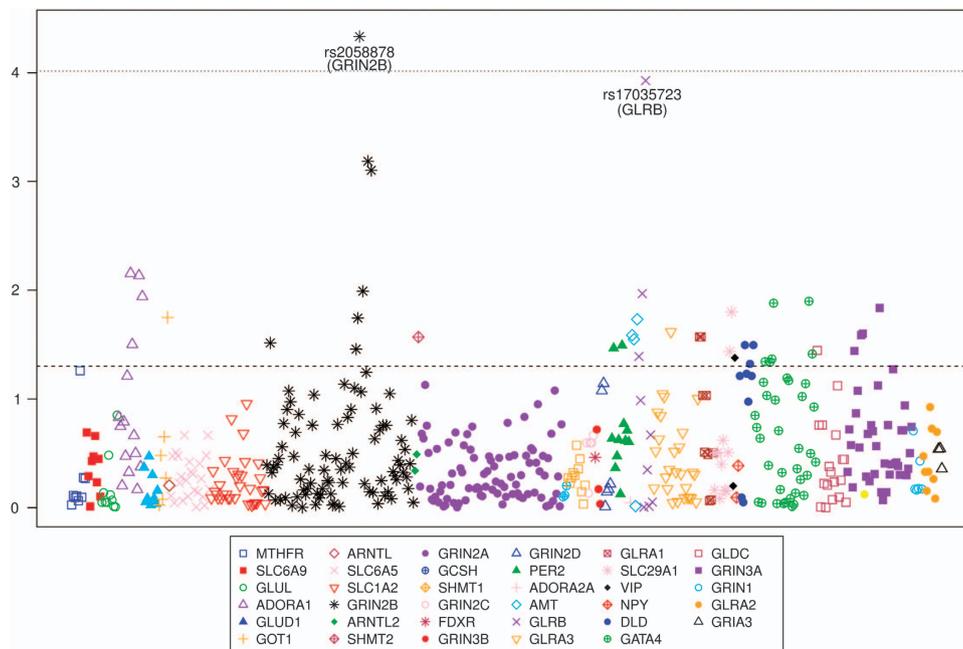
Figure 1. Single-nucleotide polymorphism (SNP) association with length of abstinence in the Discovery Sample. Results adjusted for recruitment site, Penn Alcohol Craving Scale and number of days between the last drink and initiation of acamprosate treatment. Dotted line represents the Bonferroni-corrected alpha level of $0.05/518 = 9.65E-05$. Dashed line represents nominal significance of $P = 0.05$.

Table 2. Candidate SNPs associated with length of abstinence in the discovery sample ($P < 0.001$)

SNP	Gene	Minor allele	MAF	Total sample (N = 225)		Males (N = 148)	
				HR	P-value	HR	P-value
rs2058878	GRIN2B	A	0.493	0.49	0.000046	0.41	0.00021
rs17035723	GLRB	A	0.139	2.14	0.00012	2.88	0.000098
rs2160733	GRIN2B	C	0.198	1.78	0.00065	1.85	0.0051
rs2160734	GRIN2B	G	0.488	0.57	0.00079	0.53	0.0061

Abbreviations: HR, hazard ratio; MAF, minor allele frequency. Results are adjusted for recruitment site, Penn Alcohol Craving Scale and number of between last drink and initiation of acamprostate treatment. Results for the entire sample are shown along with results for the male subset

Table 3. Demographic and clinical measures associated with the length of abstinence during first 3 months of acamprosate treatment in the replication sample of 110 male alcoholics participating in study PREDICT

Parameter	Mean ± s.d.	HR (95% CI)	P-value
Age at evaluation	45.1 ± 8.4	0.98 (0.95, 1.01)	0.13
Age of onset	31.7 ± 10.1	0.95 (0.95, 0.99)	**0.036**
Average drinks per heavy drinking day	19.2 ± 10.4	1.03 (1.01, 1.06)	**0.0087**
Average drinks per drinking day	18.8 ± 10.6	1.03 (1.01, 1.05)	**0.0084**
BDI score	6.1 ± 5.0	1.06 (1.01, 1.11)	**0.012**
OCDS total score	13.8 ± 6.6	1.02 (0.98, 1.06)	0.30
Percent drinking days	62.2 ± 24.3	1.00 (0.99, 1.01)	0.48
Percent heavy drinking days	60.6 ± 24.6	1.00 (0.99, 1.01)	0.53
Days since last drink	22.0 ± 4.2	1.03 (0.96, 1.09)	0.43

Abbreviations: BDI, Beck Depression Inventory; CI, confidence interval; HR, hazard ratio; OCDS, Obsessive Compulsive Drinking Scale.

SNP associations with treatment outcomes in the replication sample

As presented in Table 4, the association of the imputed rs2058878 genotypes with abstinence length in the replication sample was marginally significant (hazard ratio = 0.72, $P = 0.068$). As in the discovery sample, the minor A allele was associated with lower risk of relapse (longer abstinence). To gain additional insights into this association signal, we used the available genome-wide SNP data from the replication sample to search for nearby SNPs that were in high linkage disequilibrium ($\geqslant 0.9$) with rs2058878. We identified the GRIN2B SNP rs2300272 (minor allele frequency = 0.48, linkage disequilibrium = 0.9 with rs2058878), which was genotyped in the replication sample. Analysis of this SNP revealed association of the minor rs2300272 G allele with shorter abstinence in the replication sample (hazard ratio = 1.43, $P = 0.049$).

The association of minor alleles of GRIN2B rs2160734 and rs11612353 (proxy for rs2160733) SNPs with abstinence length was in a reverse direction compared with the discovery sample.

The minor GLRB rs11100096 G allele (proxy for rs17035723 A) showed association with treatment outcome in the same direction as the discovery sample; however, the association was not statistically significant.

DISCUSSION

Our findings indicate that the minor GRIN2B rs2058878 A allele is associated with longer abstinence during the first 3 months of acamprosate treatment. This association was replicated in an independent sample with marginally significant evidence of association. Furthermore, the replication sample provided significant evidence for association of abstinence length with rs2300272, which is in high linkage disequilibrium with

SNP	Gene	Minor allele	MAF	O/I	HR	P-value
rs2058878	GRIN2B	A	0.48	I	0.72	0.068
rs17035723	GLRB	T	0.18	I	0.82	0.48
rs2160733	GRIN2B	C	0.23	I	0.76	0.24
rs2160734	GRIN2B	C	0.47	O	1.31	0.13

Table 4. Replication analysis using the PREDICT sample (N = 110 acamprosate-treated subjects)

Abbreviations: HR, hazard ratio; MAF, minor allele frequency; O/I, indicator of observed or imputed SNP; SNP, single-nucleotide polymorphism. Results are adjusted for the Beck Depression Inventory score, age of onset of alcoholism and the number of drinks per drinking day.

rs2058878. This is the first report showing the association of these genetic markers with the length of abstinence in alcohol-dependent subjects. It provides a compelling reason to investigate these markers as potential pharmacogenetic predictors of acamprosate treatment response.

GRIN2B rs2058878 was selected as a tag SNP to investigate the association of treatment outcome with corresponding gene segments. It is possible that other rarer variants located in the gene segment 'tagged' by rs2058878 and rs2300272 are responsible for physiological mechanisms contributing to the reported associations and a search for such functional variants is necessary. It is also possible that rs2058878 or rs2300272 may have a functional role associated with the expression and/or function of GRIN2B. Indeed, rs2058878 is located in an intronic region where enrichment of histone 3 lysine 4 mono-methylation and histone 3 lysine 27 acetylation was found in several data sets of the UCSC ENCODE database.[47] These marks indicate the presence of transcriptional enhancers,[37,48] suggesting a role for the region around rs2058878 in the regulation of GRIN2B expression. Interestingly, rs2058878 A allele is part of a canonical E-box sequence identified as a binding site for the transcription factors NeuroD and NeuroG in mouse and Xenopus laevis.[49] Review of the Human Brain Transcriptome database (http://hbatlas.org/pages/hbtd) indicates that expression of the GRIN2B gene in human brain areas seems to inversely correlate with expression of the NeuroD and NeuroG transcription factors. These factors are known to have an active role during neural development in humans[50] and to act both as transcriptional activators as well as repressors.[51,52] However, the predicted consensus site is abolished in the rs2058878 T allele (which is associated with shorter abstinence). Thus, altered transcription factor binding to the rs2058878-containing region and consequent changes in GRIN2B expression could contribute to the association between rs2058878 and duration of abstinence.

The GRIN2B gene encodes the GluN2B subunit of NMDA receptor. Our finding of association between GRIN2B variants and the length of abstinence in alcohol-dependent human subjects treated with acamprosate is in line with experimental evidence associating increased GluN2B subunit expression with chronic ethanol treatment and development of physical dependence.[53] Moreover, recent findings indicate that the effects of acamprosate may be related to the Ca^{2+} ion.[54] Providing that Ca^{2+} influx in neurons is controlled by NMDA receptor, it is of special interest to investigate the effects of rs2058878 or rs2300272 on Ca^{2+} influx.

Evidence also indicates that GluN2B-containing NMDA receptors are activated by glycine[19,20] and are primarily responsible for its intracellular effects[36,55] and related behavioral phenotypes, including alcohol intake.[56] Moreover, GluN2B-containing NMDA receptors are functionally important for long-term depression.[20] Consequently, altered production of GluN2B-containing NMDA receptors may disrupt the balance between long-term depression and long-term potentiation, which are fundamental in brain physiology. Therefore, understanding the effects of rs2058878 and rs2300272 in human alcohol use disorders and animal models of alcohol-related phenotypes is of special interest, especially in the context of experimental evidence suggesting that glycine may have an important role in alcohol dependence and acamprosate response.[21,22,57]

In addition to investigating genetic variant effects, clinical and demographic variations between study subjects and recruitment sites were investigated as potential predictors of treatment outcomes in our study. Several clinical variables (for example, craving intensity, depressive symptoms and the length of sobriety before initiation of acamprosate treatment) and non-pharmacological treatment components (for example, attendance at Alcoholics Anonymous meetings and having an Alcoholics Anonymous sponsor) were found to be associated with abstinence length. The predictive role of these factors should be accounted for in the pharmacogenomic studies and further investigated in prospective clinical trials. Our findings indicate that tools routinely used for assessment of patients treated in community-based programs, including PACS and Timeline Follow-Back, can be useful for these purposes.

Results of this study should be considered in the context of the following limitations. We have not used a placebo arm in the discovery sample, which limits our ability to differentiate association findings related to acamprosate effects from those associated with sobriety independent from acamprosate effects. Prospective placebo controlled studies are needed to validate the role of our top SNPs as potential pharmacogenomic markers of abstinence length associated with or independent from acamprosate effects.

Analyses presented here are limited to the first 3 months of acamprosate treatment and, therefore, may be relevant only during early stages of abstinence. It is possible that genetic and clinical markers associated with early relapse may be different from those associated with later relapse. This possibility will be investigated in the ongoing analyses focused on genetic and clinical predictors of sobriety during the subsequent 3 months of acamprosate treatment in our study cohort.

Finally, both discovery and replication samples were relatively small, which limited power to discover all meaningful associations. However, analyses resulted in potentially important association findings.

In conclusion, our findings indicate that GRIN2B rs2058878 and rs2300272 SNPs are associated with abstinence length during the first 3 months of acamprosate treatment. These findings support experimental evidence implicating NMDA receptors in the treatment effects of acamprosate. Future studies should prospectively investigate the potential role of these SNPs as biomarkers of abstinence length in treatment-seeking alcoholics and determine the physiological and molecular mechanisms underlying these association findings.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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DISCLAIMER

The contents of this study are solely the responsibility of the authors and do not necessarily represent the official views of the NIH.

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Supplementary Information accompanies the paper on the Translational Psychiatry website (http://www.nature.com/tp)