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Epigenetic moderators of naltrexone efficacy in reducing heavy drinking in Alcohol Use Disorder: A randomized trial

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Abstract

Polymorphisms in genes associated with opioid signaling and dopamine reuptake and inactivation may moderate naltrexone efficacy in Alcohol Use Disorder (AUD), but the effects of epigenetic modification of these genes on naltrexone response are largely unexplored. This study tested interactions between methylation in the μ-opioid receptor (*OPRM1*), dopamine transporter (*SLC6A3*), and catechol-O-methyltransferase (*COMT*) genes as predictors of naltrexone effects on heavy drinking in a 16-week randomized, placebo-controlled trial among 145 treatment-seeking AUD patients. *OPRM1* methylation interacted with both *SLC6A3* and *COMT* methylation to moderate naltrexone efficacy, such that naltrexone-treated individuals with lower methylation of the *OPRM1* promoter and the *SLC6A3* promoter (p=.006), *COMT* promoter (p=.005), or *SLC6A3* 3' untranslated region (p=.004), relative to placebo and to those with higher *OPRM1* and *SLC6A3* or *COMT* methylation, had significantly fewer heavy drinking days. Epigenetic modification of opioid- and dopamine-related genes may represent a novel pharmacoepigenetic predictor of naltrexone efficacy in AUD.

Introduction

The opioid antagonist naltrexone reduces heavy drinking among individuals with Alcohol Use Disorder (AUD) (1), but is not effective for everyone. Genomic factors might identify subgroups with superior response (2). Naltrexone is believed to reduce drinking through opioid-mediated effects on alcohol-induced dopamine release (3, 4), so genes associated

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Author Contributions

Dr. Schacht conceived the study, conducted the data analysis and interpretation, and drafted the paper. Drs. Hoffman and Chen assisted with data analysis and interpretation. Dr. Anton conceived and designed the study and assisted with data interpretation. All authors revised the paper critically for important intellectual content, approved of the final version, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

with opioid signaling and dopamine reuptake and inactivation are logical targets. We previously reported epistatic interactions between functional polymorphisms in the μ-opioid receptor (MOR), dopamine transporter (DAT), and catechol-O-methyltransferase (COMT) genes (*OPRM1, SLC6A3*, and *COMT)* that predicted naltrexone response (5–7). We focused on single nucleotide polymorphisms (SNPs) in *OPRM1* (rs1799971) and *COMT* (rs4680) and a variable number tandem repeat (VNTR) polymorphism in the *SLC6A3* 3' untranslated region (rs28363170). Rs1799971 encodes an A-to-G substitution associated with increased MOR binding affinity for β-endorphin (8) that may also increase MOR affinity for naltrexone (9), potentiating naltrexone effects. The *SLC6A3* 10-repeat (10R) and *COMT* val alleles have been associated with relatively greater DAT expression (10) and COMT efficacy, respectively (11, 12). Since the DAT and COMT are the primary methods of dopamine inactivation in the striatum (13) and prefrontal cortex (PFC) (14), respectively, these alleles likely reduce synaptic dopamine accumulation in these areas.

We initially reported that, among non-treatment-seeking AUD individuals, naltrexone, relative to placebo, reduced alcohol self-administration and cue-elicited ventral striatal activation most among *OPRM1* G-allele carriers who were also *SLC6A3* 10R homozygotes (5, 6). We subsequently replicated and extended this finding in a randomized controlled trial (RCT) among treatment-seeking AUD patients, in which naltrexone, relative to placebo, most effectively reduced heavy drinking among *OPRM1* G-allele carriers who were also *SLC6A3* 10R or *COMT* val-allele homozygotes (7). Taken together, these data suggested that a combination of genetically predisposed enhanced MOR function and reduced striatal or cortical synaptic dopamine accumulation was associated with superior naltrexone response.

Although germline genetic variation may have utility in predicting naltrexone response, the rs1799971 G allele is relatively infrequent (15), and epistatic interactions render the genotype combinations associated with superior response rarer still. A more common mechanism by which genomic factors might affect naltrexone response is epigenetic modification. DNA methylation at cytosine residues in CpG (cytosine, followed by guanine) dinucleotides, which are disproportionately clustered into islands in gene promoter regions, inhibits transcription factor binding and recruits histone deacetylase complexes that compact chromatin, thereby decreasing gene expression (16). Given naltrexone's neurochemical mechanism of action, *OPRM1, SLC6A3*, and *COMT* methylation could moderate naltrexone response. Methylation of each gene's promoter has been associated with downstream effects on its expression and the function of the protein it encodes. Greater *OPRM1* promoter methylation in a neural-derived cell line was associated with less MOR expression (17); greater *SLC6A3* promoter methylation in blood, with less striatal DAT availability (18); and greater *COMT* promoter methylation in a human cell line, with less *COMT* expression (19). *OPRM1* and *SLC6A3* methylation have also been associated with AUD and drinking. *OPRM1* (20) and *SLC6A3* (21, 22) promoter methylation were greater among AUD individuals, relative to controls, as was methylation of CpG sites in the *SLC6A3* body (23). Methylation of several *OPRM1* CpG sites predicted drinking relapse during in a naltrexone RCT, although *OPRM1* methylation did not independently moderate naltrexone effects on drinking (24). Importantly, *SLC6A3* promoter methylation in blood and substantia nigra correlated highly (18), as did *COMT* promoter methylation in

peripheral leukocytes and a variety of brain regions, including PFC (25, 26), suggesting that peripheral *SLC6A3* and *COMT* methylation may be biomarkers of neural methylation.

Using genomic DNA extracted from peripheral leukocytes from participants in our previous naltrexone RCT (27), the current study tested whether interactions between *OPRM1, SLC6A3* and *COMT* promoter methylation predicted naltrexone effects on drinking. We hypothesized that methylation of these regions would interact in their effects on naltrexone response in a manner similar to, but not attributable to, the epistatic interactions that predicted response in that RCT, such that naltrexone-treated individuals with lower *OPRM1* methylation (and potentially greater MOR expression) and either lower *SLC6A3* or *COMT* methylation (and potentially greater DAT or COMT expression, engendering less striatal or cortical dopamine accumulation) would demonstrate the least heavy drinking, relative to placebo and to individuals with other combinations of methylation of these regions. The interaction between methylation of CpG sites in the *SLC6A3* VNTR and *OPRM1* promoter methylation was also explored.

Methods

Overview.

Detailed methods for the parent RCT (ClinicalTrials.gov identifier: NCT00920829) have been reported previously (7, 27). The Medical University of South Carolina Institutional Review Board approved all procedures, and all participants provided informed consent before participation. Data were collected between June 2009 and December 2015. The study comprised an initial assessment visit, a baseline visit, and nine follow-up visits over a 16-week treatment period. Briefly, community-dwelling participants seeking AUD treatment were assessed for inclusion/exclusion criteria and genotyped for rs1799971. One aim of the parent RCT was to test whether rs1799971 genotype independently predicted naltrexone efficacy, so participants who carried the minor (G) allele were over-selected (n=73) to comprise 50% of the 146 evaluable participants (Supplemental Figure 1).

Participants.

Participants were required to be ages 18–70; report heavy drinking (at least 4/5 standard drinks per day for women/men) on at least 50% of days in the 90 days before assessment; and meet *DSM-IV* (*Diagnostic and Statistical Manual of Mental Disorders, revised 4th edition*) diagnostic criteria for Alcohol Dependence, as assessed by the Structured Clinical Interview for *DSM-IV* (28). Participants were also required to self-identify as Caucasian or Asian, secondary to low rs1799971 G-allele frequency among individuals of African descent; we previously reported that analysis of population allele frequencies for 50 SNPs included on the methylation assay used here indicated a high degree of correspondence between self-reported and SNP-identified ancestry (7). Participants who reported cocaine or marijuana use in the 90 days before assessment were included, as long as they did not meet *DSM-IV* criteria for dependence on either substance or any other except nicotine and had a negative urine drug screen upon medication randomization. Exclusion criteria were: current psychotropic medication use other than antidepressants (for which a stable dose for at least one month was required); current *DSM-IV* Axis I diagnosis or suicidal/

homicidal ideation; history of significant medical illness; liver enzyme (ALT or AST) levels greater than three times the upper limit of normal; and past-month naltrexone, disulfiram, or acamprosate use. Female participants could not be pregnant or nursing. Table 1 lists demographic characteristics for the 145 participants included in the analysis (methylation quality-control measures described below led to the exclusion of one participant's data).

Medication, randomization, and assessment.

Participants were required to maintain abstinence for at least four days before medication randomization, and were then urn randomized (29) to receive naltrexone (25 mg for two days, then 50 mg thereafter) or placebo for 16 weeks. Randomization was stratified by rs1799971 genotype, with sex, smoking status (non-smoker vs. smoker, defined as ≥ 10 cigarettes per day), cocaine use, antidepressant use, and AUD family history balanced across medication groups. Study medications were identically over-encapsulated with 100 mg riboflavin (riboflavin-assessed adherence was high and did not vary between medication groups (27)) and distributed in labeled blister packs. Participants and investigators were blind to genotype and medication assignment. After randomization, participants returned for nine follow-up visits, during which daily drinking since the last visit was assessed with the calendar-based Timeline Follow-back interview (30). Participants who dropped out after randomization were compensated to return at week 16 to provide missing drinking data. Forty participants ultimately dropped out, at similar rates across medication groups, but full drinking data were available for 89% of participants.

DNA collection and genotyping.

Genomic DNA was extracted (Gentra Puragene Blood Kit; Qiagen Inc., Valencia, CA) from peripheral blood mononuclear cells collected at the initial assessment visit, stored at −80° C, and used to genotype rs1799971, rs28363170, and rs4680. Details of these assays were previously reported (7).

Methylation assay.

An Infinium MethylationEPIC BeadChip (Illumina, San Diego, CA), which assays methylation at 866,895 cytosine residues, 99.7% of which are CpG sites, was used to assess genome-wide DNA methylation. For each subject, 500 ng genomic DNA was bisulfite converted, denatured and amplified, fragmented, resuspended, and hybridized to the BeadChip (eight samples per chip). Each chip included four samples each from naltrexone-and placebo-treated participants, with age (median split) and smoking (smoker vs. non-smoker) evenly distributed within each group of four samples. Age and smoking were selected for balancing because both characteristics affect global DNA methylation (31, 32). During hybridization, the amplified and fragmented DNA annealed to fluorophore-linked probes specific to each CpG site (one for methylated and one for unmethylated sites). Processed BeadChips were then scanned on the Illumina iScan System, which excited each probe's fluorophores and recorded their fluorescence. Summaries of the probe interrogations yielded average signals for the proportion of alleles that were methylated, vs. unmethylated, at each CpG site.

Quality control.

RnBeads (33) was used to examine box plots of the quality of the staining, hybridization, extension, target removal, and bisulfite conversion of the genomic DNA. Distributions and medians of negative control box plots were also examined. Based on these metrics, one participant's data were judged to be of low quality and excluded, leaving 145 participants for analysis. Probes were subsequently assessed to ensure they were in CpG positions and did not overlap with SNPs with minor allele frequencies >0.01. Data were normalized in R using the preprocessNoob function in the minfi package (34), which estimates background noise from the out-of-band probes, removes it for each sample separately, and utilizes a subset of the control probes to estimate dye bias.

To further assess data quality and examine convergent validity, participants' age and sex were estimated from the methylation data, and whole-genome methylation differences between smokers (n=57) and non-smokers (n=88) were tested. First, a well-validated algorithm (35) was used to predict participants' ages from the methylation of 30,084 CpG sites; these predictions correlated highly with participants' self-reported ages (r=0.882, p<0.001). Second, the Horvath algorithm (35) and RnBeads also predict sex based on sex chromosome methylation; both predictions exactly matched participants' self-reported sex. Finally, RnBeads was used to identify differentially methylated CpG sites between smokers and non-smokers, and these sites were compared with the sites whose methylation best discriminated current smokers from non-smokers in a large (N=15,907) previous analysis (32). Four of the five most differentially methylated sites in our data (Illumina probe IDs cg05575921, cg21161138, cg21566642, and cg01940273, none of which were among the *OPRM1*, *SLC6A3*, or *COMT* sites analyzed) were among the top five sites in the previous analysis.

Regions analyzed.

Supplemental Figures 2–4 show the CpG sites on the BeadChip located in each region analyzed and mean methylation at each site. Not every CpG site in each region was represented on the BeadChip, but all available sites within each region were included. For *OPRM1*, the promoter was defined, consistent with a recent study of *OPRM1* methylation effects on naltrexone response (24), as the 130 nucleotides upstream and 600 nucleotides downstream of the transcription start site (TSS). For *SLC6A3*, the promoter was defined, consistent with prior studies, as the 1500 nucleotides upstream and 300 nucleotides downstream of the TSS. *COMT* has isoforms that encode both soluble and membrane-bound COMT, each of which has its own promoter (P1 and P2, respectively) (36). The four CpG sites on the BeadChip in the P1 promoter and eight in the P2 promoter were included. Finally, CpG sites in the *SLC6A3* VNTR region were also analyzed. The BeadChip includes three sites in this region (cg15600751, cg1632193, and cg10838500), but one (cg10838500) occurs only in the 10R allele (37). Thus, methylation at cg15600751 and cg1632193 was averaged for this analysis.

Statistical analysis.

Interactions between methylation (averaged across the proportion of methylated alleles at each CpG site in each region) and medication were tested with linear mixed models (SPSS

v. 25 MIXED) in which methylation and medication group (naltrexone vs. placebo) were between-subjects factors and time in study (month 1 to 4) was a repeated within-subjects factor. Methylation averages in each region of interest were normally distributed and met assumptions for linear modeling. The dependent variable, as in previous analyses (7, 27), was percent heavy drinking days (PHDD; i.e., the proportion of study days on which women/men drank 4/5 or more standard drinks). Significant methylation by medication interactions indicated that naltrexone effects on PHDD across all study months differed as a function of methylation; significant methylation by medication by time interactions indicated that these effects differed by both methylation and time in study. For each model, the highest-level significant interaction was interpreted by median-splitting methylation levels for each gene and testing, *post hoc*, the simple effect of medication within each combination of methylation level (e.g., high vs. low *OPRM1*, *SLC6A3*, and *COMT* methylation). Effect sizes (Cohen's *d*) were calculated for groups in which this simple effect was significant.

Three primary models were tested (Table 2): one included *SLC6A3* promoter methylation, *OPRM1* promoter methylation, medication, time, and all interactions of these factors; and the second and third substituted either *COMT* promoter methylation or *SLC6A3* VNTR methylation for *SLC6A3* promoter methylation. Alpha for these models was Bonferroni-corrected to p=0.0167. Alpha for *post hoc* tests, which were conducted only to interpret higher-level interactions, was left at p=0.05. Each model used a first-order autoregressive variance-covariance matrix and covaried age. On an exploratory basis, the interaction of every combination of individual CpG sites in each region of interest was also tested to determine whether specific combinations of individual sites interacted with medication group to predict PHDD (Supplemental Tables 1–3). To test whether methylation effects could be accounted for by the previously reported epistatic interactions (7), additional models were tested in which *OPRM1* rs1799971 and *SLC6A3* rs28363170 genotypes (dichotomized as G-allele carriers vs. A-allele homozygotes and 9R-allele carriers vs. 10R-allele homozygotes) and their interactions with medication and time were added to the first and third models, and rs1799971 and *COMT* rs4680 (met-allele carriers vs. val-allele homozygotes) genotypes and their interactions were added to the second model.

Results

SLC6A3 promoter and *OPRM1* promoter.

The highest-level significant interaction was between *SLC6A3* promoter methylation, *OPRM1* promoter methylation, medication group, and time (Table 2). When *SLC6A3* and *OPRM1* methylation were median-split (Figure 1; medians were *SLC6A3*=0.150, *OPRM1*=0.170), naltrexone, relative to placebo, significantly reduced PHDD at month 2 (F(1, 217.46)=4.87, mean difference between naltrexone and placebo=16.2% HDD (95% CI=1.7–30.7%), d=0.61, p=0.028) and month 3 (F(1, 232.53)=4.79, mean difference between naltrexone and placebo=16.5% HDD (95% CI=1.7–31.4%), d=0.62, p=0.030) among individuals with lower methylation of both promoters. The simple effect of medication was not significant at any time point among individuals with any other combination of *SLC6A3* and *OPRM1* methylation except those with high *SLC6A3* and

low *OPRM1* methylation, among whom this effect was significant at month 3 (F(1, 241.14)=5.35, mean difference between naltrexone and placebo=25.7% HDD (95% CI=3.8–47.7%), d=0.98, p=0.022), as a function of increased PHDD in the placebo group at that point. The *SLC6A3* by *OPRM1* by medication by time interaction remained significant even when rs28363170 genotype, rs1799971 genotype, and their interactions with each other and with medication and time were included in the model, suggesting that the methylation by medication interaction was independent of these other effects.

COMT promoter and *OPRM1* promoter.

The highest-level significant interaction was between *COMT* promoter methylation, *OPRM1* promoter methylation, and medication group (Table 2). Across all study months, naltrexone, relative to placebo, reduced PHDD more among individuals with lower *COMT* and *OPRM1* methylation. When *COMT* and *OPRM1* methylation were median-split (Figure 2; medians were *COMT*=0.341, *OPRM1*=0.170), naltrexone, relative to placebo, significantly reduced PHDD across all study months only among individuals with lower methylation of both promoters (F(1, 154.12)=5.41, mean difference between naltrexone and placebo=19.7% HDD (95% CI=3.0–36.5%), d=0.85, p=0.021). This interaction remained significant even when rs4680 genotype, rs1799971 genotype, and their interactions with each other and with medication and time were included in the model, suggesting that the methylation by medication interaction was independent of these other effects.

SLC6A3 VNTR and *OPRM1* promoter.

The highest-level significant interaction was between *SLC6A3* VNTR methylation, *OPRM1* promoter methylation, and medication group (Table 2). Across all study months, naltrexone, relative to placebo, reduced PHDD more among individuals with lower *SLC6A3* VNTR and *OPRM1* methylation. When *SLC6A3* VNTR and *OPRM1* promoter methylation were median-split (Figure 3; medians were *SLC6A3*=0.618, *OPRM1*=0.170), naltrexone, relative to placebo, significantly reduced PHDD across all study months only among individuals with lower methylation of both regions (F(1, 146.43)=9.93, mean difference between naltrexone and placebo=28.5% HDD (95% CI=10.6–46.3%), d=1.25, p=0.002). This interaction remained significant even when rs28363170 genotype, rs1799971 genotype, and their interactions with each other and with medication and time were included in the model, again suggesting that the methylation by medication interaction was independent of these other effects.

Discussion

Taken together, these data suggest that methylation differences in genes underlying opioid signaling and dopamine reuptake and inactivation interact to predict naltrexone treatment effects on heavy drinking among AUD outpatients. Specifically, methylation of the *SLC6A3* and *COMT* promoters interacted with *OPRM1* promoter methylation to influence naltrexone efficacy, as did, in an exploratory analysis, methylation of the *SLC6A3* 3’ UTR VNTR region. Effect sizes for naltrexone, relative to placebo, on heavy drinking in the methylation-defined subgroups in which it was most effective were in the medium to large range (d=0.61–1.25), greater than the overall small effect of naltrexone on heavy drinking across

all AUD individuals (38). Although preliminary, these findings suggest potentially novel epigenetic predictors of naltrexone response.

Epigenetic changes in genes that may underlie naltrexone's effects on dopamine signaling interacted with *OPRM1* methylation to predict its effects on drinking. Alcohol acutely elicits striatal dopamine release (39) and naltrexone blocks this phenomenon (3, 4). DAT and COMT are the primary methods of dopamine inactivation in the striatum (13) and PFC (14), respectively. *SLC6A3* rs28363170 and *OPRM1* rs1799971 variation have previously been reported to interact in their effects on acute response to alcohol, such that individuals carrying the gain-of-function alleles of each polymorphism (i.e., 10R and G) displayed lower hedonic response (40). Since lower *OPRM1, SLC6A3,* and *COMT* promoter methylation have been associated with relatively increased expression of these genes (17, 18, 26), naltrexone might more effectively reduce heavy drinking among individuals with lower methylation of these regions because this pattern of methylation predisposes greater MOR availability and more effective synaptic dopamine clearance after alcohol-induced dopamine release. With respect to methylation of the *SLC6A3* 3' UTR VNTR, CpG methylation outside of gene promoters can also regulate gene expression (41), and 3' UTRs contain regulatory regions that can influence a variety of posttranscriptional modifications that affect gene expression (42). The *SLC6A3* VNTR modulates *SLC6A3* expression (43), potentially via transcription factor binding in this region (44). Thus, greater *SLC6A3* VNTR methylation could modulate transcription or posttranscriptional functions that affect *SLC6A3* expression. We previously reported epistatic genetic effects on naltrexone efficacy in this sample, but the significance of the methylation effects persisted when these genetic effects were included in the models, suggesting that, even after accounting for variance attributable to epistatic effects, interactions between *OPRM1* methylation and *SLC6A3* and *COMT* methylation independently predicted naltrexone effects.

Consistent with a secondary analysis of another AUD naltrexone RCT (24) and a meta-analysis of *OPRM1* rs1799971 pharmacogenetic effects (45), *OPRM1* methylation did not independently moderate naltrexone response (i.e., *OPRM1* by medication interactions were significant only in the context of higher-level interactions with *SLC6A3* or *COMT* methylation). The rs1799971 meta-analysis included the primary analysis of the data used in the current study (27), which also did not support this pharmacogenetic effect. Collectively, these findings suggest that, despite the fact that naltrexone directly antagonizes the MOR, the effects of genetic or epigenetic alteration of *OPRM1* alone are not sufficiently large to consistently affect naltrexone response.

Several factors limit interpretation of these findings. First, brain-based interpretations of these data rely upon extrapolation of methylation levels in peripheral leukocytes, from which DNA was extracted, to neural tissue; methylation levels vary between cell types (46), and this extrapolation may not be valid. However, peripheral *SLC6A3* and *COMT* promoter methylation has been reported to correlate highly with methylation of these regions in a variety of brain regions, including the substantia nigra and PFC (18, 25). Second, the use of a whole-genome methylation chip afforded the ability to analyze multiple regions of interest without pyrosequencing each region individually, at the cost of including every CpG site within each region. Illumina chose the CpG sites on the BeadChip to maximize

coverage of promoter and enhancer regions, and CpG sites near each other generally share a large amount of variance in their methylation levels, so the sites included in these analyses are a reasonable proxy for the methylation of the larger regions in which they are located. Nevertheless, inclusion of CpG sites that affect the binding of specific transcription factors could have altered results; future studies should further explore this possibility. Although this paper employed a hypothesis-driven, candidate gene approach, a genome-wide pharmacoepigenetic analysis would also be valuable, as the methylation status of genes involved in other AUD-related pathways (e.g., alcohol metabolism) or naltrexone pharmacokinetics/pharmacodynamics might also influence naltrexone efficacy. Third, blood samples were collected only at baseline; the lack of a post-treatment sample precluded testing whether naltrexone or drinking status *altered* methylation of the genetic regions of interest. Finally, although the study was powered to detect an interaction between *OPRM1* rs1799971 genotype and medication group, sample size limits interpretation of gene-by-gene methylation interactions.

In conclusion, this study found that AUD individuals with less methylation of the *OPRM1* and *SLC6A3* or *COMT* promoters, or the *SLC6A3* 3'UTR VNTR, were more likely to benefit from naltrexone, relative to placebo, than individuals with other combinations of methylation in these regions. These data should be tested for replication in other naltrexone RCT datasets, and, if replicated, could support the utility of a novel pharmacoepigenetic approach to naltrexone treatment in AUD.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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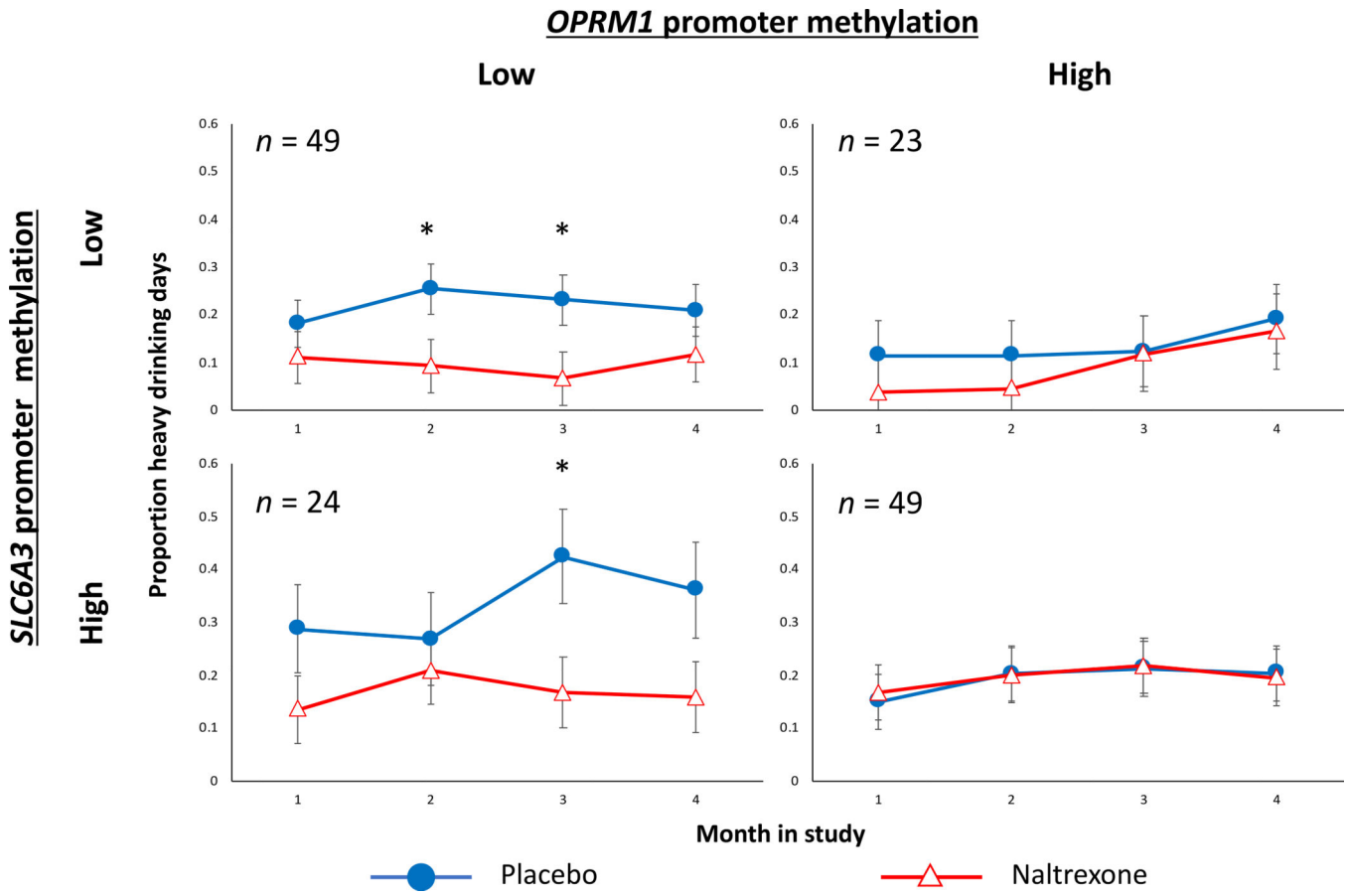

Figure 1.

Effect of naltrexone (open symbols), relative to placebo (closed symbols), on percent heavy drinking days (PHDD) during the 16-week trial as a function of *SLC6A3* promoter and *OPRM1* promoter methylation. *SLC6A3* and *OPRM1* methylation are median split into low and high groups for display purposes. Naltrexone, relative to placebo, reduced PHDD more in study months 2 and 3 among participants with lower *SLC6A3* promoter and *OPRM1* promoter methylation, and in study month 3 among participants with higher *SLC6A3* promoter and lower *OPRM1* promoter methylation. Asterisks indicate time points at which the effect of naltrexone vs. placebo was significant. Figures are estimated marginal means (± standard errors) from the linear mixed model.

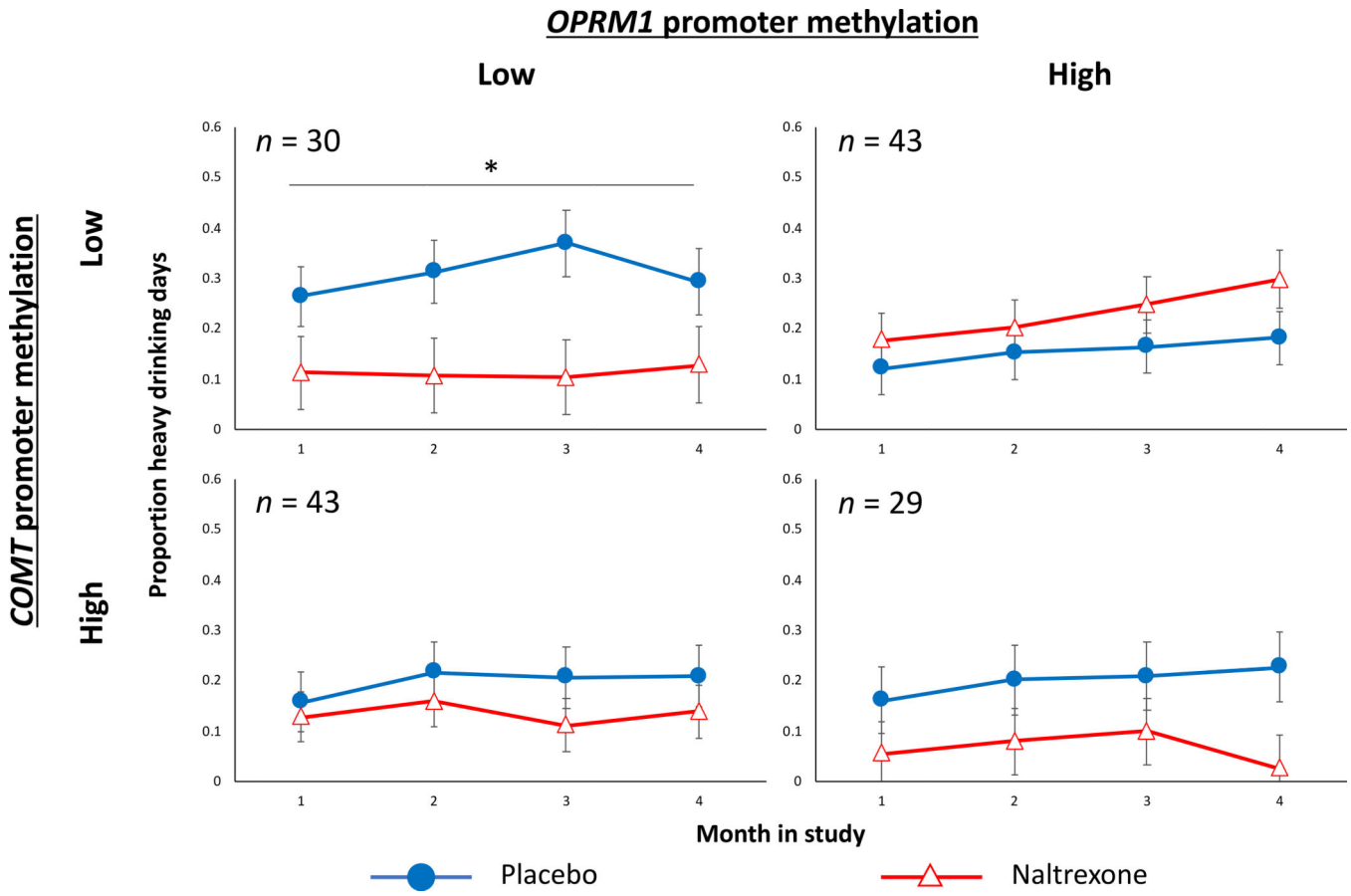

Figure 2.

Effect of naltrexone (open symbols), relative to placebo (closed symbols), on percent heavy drinking days (PHDD) during the 16-week trial as a function of *COMT* promoter and *OPRM1* promoter methylation. *COMT* and *OPRM1* methylation are median split into low and high groups for display purposes. Naltrexone, relative to placebo, reduced PHDD more among participants with lower *COMT* promoter and *OPRM1* promoter methylation. The asterisk indicates the effect of naltrexone vs. placebo was significant across all study months. Figures are estimated marginal means (± standard errors) from the linear mixed model.

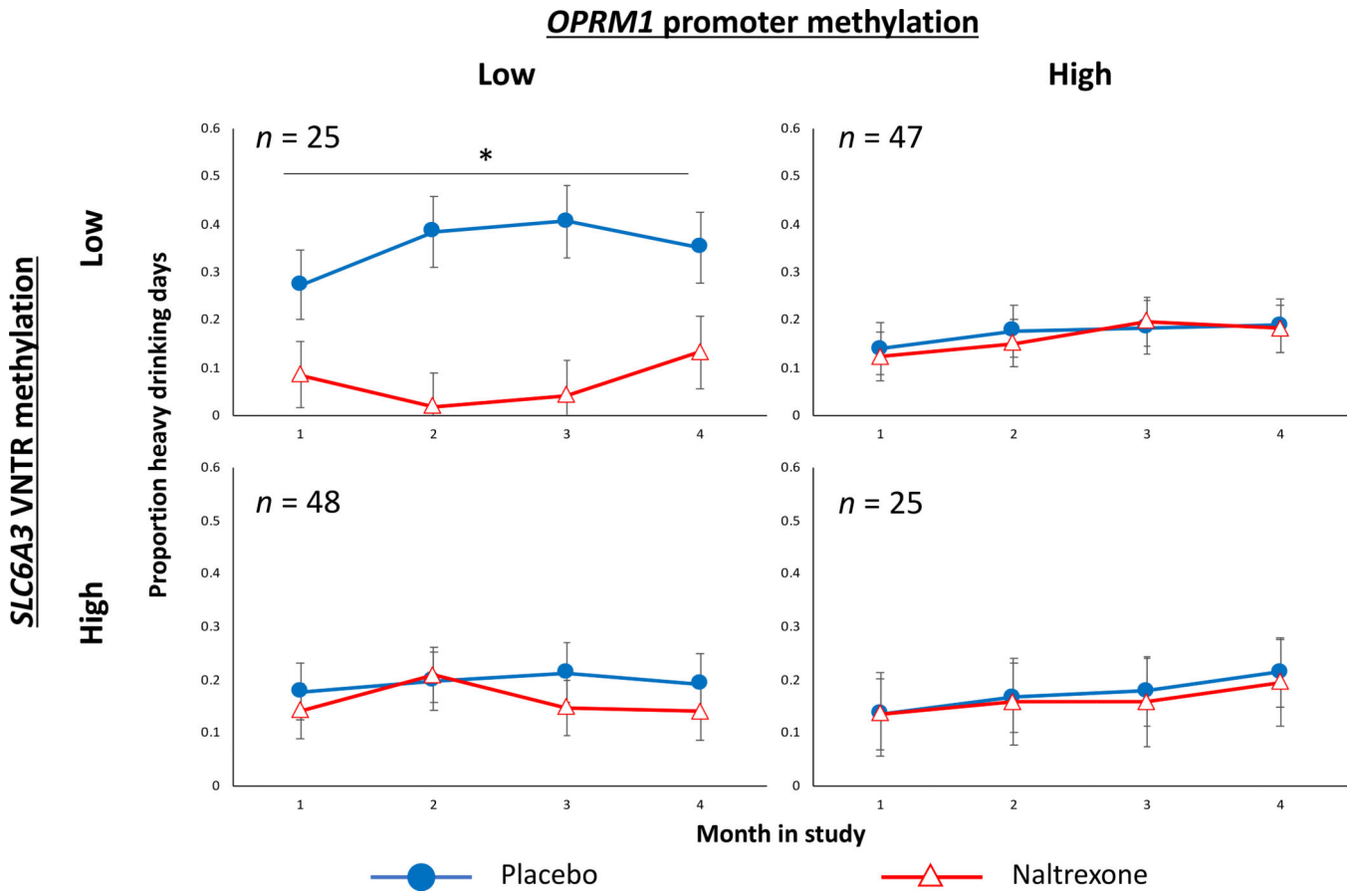

Figure 3.

Effect of naltrexone (open symbols), relative to placebo (closed symbols), on percent heavy drinking days (PHDD) during the 16-week trial as a function of *SLC6A3* VNTR and *OPRM1* promoter methylation. *SLC6A3* and *OPRM1* methylation are median split into low and high groups for display purposes. Naltrexone, relative to placebo, reduced PHDD more among participants with lower *SLC6A3* VNTR and *OPRM1* promoter methylation. The asterisk indicates the effect of naltrexone vs. placebo was significant across all study months. Figures are estimated marginal means (± standard errors) from the linear mixed model.

Table 1.

Demographic characteristics and baseline alcohol use.

	Naltrexone (*n* = 73)		Placebo (*n* = 72)		
	No.	**%**	**No.**	**%**	**Test for difference**
Demographics					
Sex, M	51	69.9	49	68.1	$p = 0.81$
Employed	56	76.7	57	79.2	$p = 0.72$
Education ≤ 12 years	12	16.4	11	15.3	$p = 0.85$
Current nicotine user	32	43.8	26	36.1	$p = 0.34$
Recent cocaine use	8	11.0	11	15.3	$p = 0.44$
Current antidepressant use	21	28.8	27	37.5	$p = 0.26$
	Mean	**SD**	**Mean**	**SD**	
Demographics					
Age, years	50.7	9.3	48.1	10.8	$p = 0.13$
Alcohol use					
Drinks per drinking day	11.9	5.2	10.5	4.3	$p = 0.11$
Drinks per day	10.3	5.3	9.0	4.5	$p = 0.08$
Heavy drinking days (%)	79.7	21.5	80.2	22.9	$p = 0.89$
Days from last drink to randomization	6.6	4.1	7.3	4.7	$p = 0.33$

p values indicate significance of χ^2 and *t* tests for differences between naltrexone and placebo groups.

Table 2.

Fixed effects tests from models testing interactions between *OPRM1*, *SLC6A3*, and *COMT* methylation, medication group, and time in study on percent heavy drinking days.

Model term	df	*F*	*p*
***Model 1: SLC6A3* promoter and *OPRM1* promoter methylation**			
SLC6A3 promoter X group	1, 150.74	0.35	0.56
OPRM1 promoter X group	1, 148.75	0.35	0.55
SLC6A3 promoter X group X time	3, 371.40	4.48	0.004
OPRM1 promoter X group X time	3, 370.65	4.14	0.007
SLC6A3 promoter X *OPRM1* promoter X group	1, 149.22	0.33	0.57
***SLC6A3* promoter X *OPRM1* promoter X group X time**	**3, 370.82**	**4.17**	**0.006**
***Model2: COMT* promoter and *OPRM1* promoter methylation**			
COMT promoter X group	1, 148.10	7.18	0.008
OPRM1 promoter X group	1, 147.75	8.07	0.005
COMT promoter X group X time	3, 371.63	2.45	0.063
OPRM1 promoter X group X time	3, 371.43	2.38	0.070
***COMT* promoter X *OPRM1* promoter X group**	**1, 147.85**	**8.13**	**0.005**
COMT promoter X *OPRM1* promoter X group X time	3, 371.50	2.20	0.088
***Model 3: SLC6A3* VNTR and *OPRM1* promoter methylation**			
SLC6A3 VNTR X group	1, 156.63	9.52	0.002
OPRM1 promoter X group	1, 153.86	8.87	0.003
SLC6A3 VNTR X group X time	3, 376.12	2.17	0.092
OPRM1 promoter X group X time	3, 374.91	2.07	0.10
***SLC6A3* VNTR X *OPRM1* promoter X group**	**1, 154.33**	**8.38**	**0.004**
SLC6A3 VNTR X *OPRM1* promoter X group X time	3, 375.14	1.97	0.12

Figures are degrees of freedom (df), *F* statistics, and corresponding *p* values from linear mixed models that included all main effects and lower-level interactions and covaried age. For each model, the highest-level interaction that was significant at the Bonferroni-corrected threshold ($p = 0.0167$) is bolded.