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Opioid and Dopamine Genes Interact to Predict Naltrexone Response in a Randomized Alcohol Use Disorder Clinical Trial

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Abstract

Background: While the opiate antagonist, naltrexone, is approved for Alcohol Use Disorder (AUD), not everyone benefits. This study evaluated whether the *OPRM1* SNP rs1799971 interacts with the dopamine transporter gene *DAT1/SLC6A3* VNTR rs28363170 or the catechol-o-methyltransferase (COMT) gene SNP rs4680 in predicting naltrexone response.

Methods: Individuals who met DSM-IV alcohol dependence were randomly assigned to naltrexone (50 mg/day) or placebo based on their *OPRM1* genotype (75 G allele carriers and 77 A allele homozygotes) and also genotyped for DAT1 VNTR (9 vs 10 repeats) or COMT SNP (val/val vs. met carriers). Heavy drinking days (%HDD) were evaluated over 16 weeks and at the end of treatment. Effect sizes (*d*) for naltrexone response were calculated based on genotypes.

Results: Naltrexone, relative to placebo, significantly reduced %HDD among *OPRM1* G carriers who also had *DAT1* 10/10 ($p=0.021$, $d=0.72$) or *COMT* val/val genotypes ($p=0.05$, $d=0.80$), and to a lesser degree in those *OPRM1* A homozygotes who were also *DAT1* 9-repeat carriers ($p=0.09$, $d=0.70$) or COMT met carriers ($p=0.03$, $d=0.63$). All other genotype combinations showed no differential response to naltrexone. Diarrhea/abdominal pain was more prominent in *OPRM1* A homozygotes who were also *DAT1* 9 or *COMT* met carriers.

Conclusions: These results suggest that Individuals with AUD with a more opioid-responsive genotype (*OPRM1* G carriers) respond better to naltrexone if they have genotypes indicating normal/less dopamine tone (*DAT1* 10,10 or *COMT* val, val), while those with a less responsive opioid-responsive genotype (*OPRM1* A homozygotes) respond better to naltrexone if they have genotypes indicating greater dopamine tone (*DAT1* 9-repeat or *COMT* met carriers). These results could lead to more personalized AUD treatments.

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Keywords

Alcohol Treatment; Naltrexone; *OPRM1*; *DAT1*; *COMT*; Pharmacogenetics

Introduction

While alcohol use disorder (AUD) is quite prevalent (Grant et al., 2016, Greenfield et al., 2014) and carries a large burden of social and health consequences (Rehm et al., 2014, Hasin et al., 2007), the majority of Individuals with AUD do not receive treatment (Grant et al., 2016) and an even smaller number receive pharmacotherapy (Mark et al., 2009). One reason for this is the low-moderate efficacy rate for available medications, including naltrexone, one of the more widely used of the FDA-approved medications for AUD (Zindel and Kranzler, 2014, Anton et al., 2006). It has been suggested that variability to medication response, in general, might be due to individual genetic differences, calling for a “personalized or more precise approach” to pharmacotherapy (Stein and Smoller, 2018). One reason for the variable response to naltrexone could lie in a pharmacogenetic interaction (Goldman et al., 2005, Jones et al., 2015). It had been initially suggested in a retrospective analysis that a putatively functional nonsynonymous single nucleotide polymorphism (SNP), rs1799971, at the 118 locus of the mu opioid receptor gene (*OPRM1* A118G), with the minor allele (G) coding a change from asparagine (Asn) to aspartate (Asp) at the 40-position in the receptor protein, might predict naltrexone response (Oslin et al., 2003). While a sub-analysis of the COMBINE Study supported this finding (Anton et al., 2008), other mostly retrospective studies had mixed results (Chamorro et al., 2012). An initial prospective study (Oslin et al., 2015) reported that naltrexone response did not differ from placebo irrespective of *OPRM1* A/G status. More recently, our group (Schacht et al., 2017) reported that, while *OPRM1* A118G status did not predict naltrexone response in an intent-to-treat analysis, in the most adherent subjects who completed the trial, G-allele carriers responded better to naltrexone, and they relapsed more quickly once naltrexone was discontinued. Most recently, a meta-analysis (Kranzler et al., 2019, Hartwell et al., 2020) concluded that although variation in study design, subject-specific factors (e.g., level of drinking, racial heterogeneity, smoking status, medication adherence) might have diluted interpretative results, there was minimal evidence that *OPRM1* A118G allelic differences significantly predicted naltrexone efficacy.

One source of variation is that other genes might be influencing the *OPRM1* effect on naltrexone response in an epistatic manner. A number of animal studies have suggested an interaction between the endogenous opioid, dopamine systems, alcohol reward and consumption (Job et al., 2007, Ramchandani et al., 2011) as well as naltrexone effects (Gonzales and Weiss, 1998). This led us to consider epistatic relationships between *OPRM1* and putatively functional dopamine-related genes (Tunbridge et al., 2019). For instance, it has been well documented that *DAT1/SLC6A3*, the gene encoding the dopamine transporter, which is the primary method of dopamine inactivation in the striatum (Ciliax et al., 1999), has a 40-base pair variable number tandem repeat (VNTR) functional polymorphism (rs28363170) in the 3' untranslated region, with 9 or 10 repeats (9R or 10R) most common. The 9R allele, compared to the 10R allele, has been associated with reduced DAT expression (Fuke et al., 2001) and, among Individuals with AUD, lower striatal DAT availability (Heinz

et al., 2000), potentially leading to relatively increased extra-synaptic DA tone. Furthermore, data from neuroimaging studies suggest 9R carriers show increased brain activation to rewarding stimuli (Aarts et al., 2010, Dreher et al., 2009, Forbes et al., 2009). Using a well-validated alcohol cue reactivity fMRI paradigm in non-treatment-seeking Individuals with AUD, we previously reported that naltrexone, relative to placebo, reduced alcohol cue-induced ventral striatal activation among *OPRM1* G-allele carriers who were also *DAT1* 10R homozygotes, while for *OPRM1* A-allele homozygotes, the reduction was seen among *DAT1* 9R carriers (Schacht et al., 2017). These findings paralleled the pharmacogenetic interaction of naltrexone with *OPRM1* and *DAT1* genotypes on drinking in an alcohol self-administration (bar-lab) paradigm in the same individuals (Anton et al., 2012). *OPRM1* G-allele carriers who were also homozygotes for the *DAT1* 10R allele and *OPRM1* A-allele homozygotes who were also *DAT1* 9R carriers had reduced drinking with naltrexone compared to placebo. These genes also interacted in their effects on alcohol consumption among social drinkers, such that *DAT1* 9R allele carriers had more drinking days than 10R-allele homozygotes, but this effect was attenuated among individuals who were also *OPRM1* G allele carriers (Weerts et al., 2017)

Given the general interaction between the brain opioid and dopamine systems, we also wanted to replicate and extend this concept by evaluating another well-studied functional dopamine system genetic polymorphism, the catechol-O-methyltransferase (*COMT*) rs4680 SNP (G/A also known as val158met), and its interaction with the *OPRM1* A118G SNP. The presence of the *COMT* met allele has been reported as conferring lower enzyme activity compared to the val158val genotype (Lachman et al., 1996, Chen et al., 2004), leading to higher dopaminergic tone. A number of studies have shown variation in both brain imaging and behavioral responses depending on *COMT* val158met genotype, with the majority supporting the hypothesis that met carriers have higher dopamine tone, reward sensitivity and brain activity to rewarding cues, but perhaps altered cognitive capacity (Craddock et al., 2006, Lachman, 2008, Schacht, 2016). Our group has shown that the *COMT* val158met SNP plays a role in blood pressure (Stewart et al., 2009) and in aripiprazole response in Individuals with AUD, in the same direction as *DAT1* VNTR effects (Schacht et al., 2017). Interestingly, there have been some preliminary reports that *COMT* inhibitors might have efficacy in some reward-based disorders dependent on *COMT* val158met genotype both in animals (McCane et al., 2018) and humans (Grant et al., 2013) suggesting, potentially, an important pharmacogenetic interaction.

The current report provides results from an expanded analysis of a single site prospective study in which individuals with DSM-IV alcohol dependence were prospectively genotyped for their *OPRM1* A118G status (G-allele carriers vs. A-allele homozygotes) and then randomized to receive naltrexone or placebo (Schacht et al., 2017). In a pre-planned analysis, these same individuals were also genotyped for the *DAT1/SLC6A3* VNTR and *COMT* val158met SNP to evaluate the epistatic effects of these genotypes with *OPRM1* A118G genotype on naltrexone efficacy. A priori, one might have hypothesized that the *OPRM1* G allele might positively interact with the *DAT1* 9 allele and/or the *COMT* 158met allele to predict higher dopamine/endorphin reward tone, that when blocked by naltrexone, would lead to greater medication response. However, our past work (Anton et al. 2012)

suggested the opposite effect on alcohol consumption and brain activation to alcohol cues (Schacht et. al. 2013), an important reason to explore this further in a clinical trial.

Methods and Materials

Trial Design and Participants

The study as previously described (Schacht et al., 2017) was a 16-week randomized clinical trial ([clinicaltrials.gov #NCT00920829](https://clinicaltrials.gov/ct2/show/study/NCT00920829)) of naltrexone vs. placebo with initial stratification by *OPRM1* A118G genotype (A-allele homozygotes versus G-allele carriers). Although the [clinicaltrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00920829) listing did not prespecify the pharmacogenetic interactions evaluated here, the initial funding proposals did hypothesize these interactions and proposed these analyses a priori. Participants (recruited and treated between July 2009 and June 2015) were between 18–70 years of age, met *DSM-IV* criteria for alcohol dependence by SCID interview (First MB et al., 1997), could not be of African American descent by self-report (due to the low frequency of the A118G G allele among African Americans), consumed, on average, at least 5 drinks per day prior to screening, and were required to be abstinent at least 4 days prior to randomization. Participants did not meet DSM-IV criteria for dependence for any other drug except nicotine. Cocaine or marijuana use prior to screening was allowable, but no psychoactive drug could be evident in the urine at screening. Participants were not taking psychotropic medications other than antidepressants (stable dose for one month required) and did not meet criteria for current major depression, bipolar disorder, psychoses, PTSD, or eating disorders. They had to be medically stable (not having liver enzymes, ALT and AST, more than 3 times normal). Females were required to use a reliable form of contraception or be post-menopausal, and were not pregnant or nursing.

Recruitment

The study was approved by the IRB at the Medical University of South Carolina and participants signed informed consent before formal assessment and genotyping. Participants, recruited primarily by community advertisement, were not currently receiving other alcohol treatment. After genotyping (blind to subjects and study staff), individuals with at least one G allele and approximately 1/3 of A-allele homozygotes (temporally proximal to G allele carriers) were offered participation. Subsequently, pre-planned genotyping for the *DAT1* VNTR and *COMT* val158met SNP was conducted to form the combined gene groups for this evaluation (see Figure 1, CONSORT Diagram).

Interventions

Medication—After at least 4 days of abstinence, participants were randomized to receive naltrexone (25 mg for 2 days then 50 mg thereafter) or identical placebo (distributed in labeled blister packs) for 16 weeks.

Assessment and Medical Management (MM)—Medical management (MM), a manualized intervention (Pettinati et al., 2005) designed and used previously (Anton et al., 2006), and also utilized in previous naltrexone pharmacogenetic studies (Oslin et al., 2003, Anton et al., 2008) was administered. During each MM session (conducted on weeks 1,2,3,4,6,8,10,12,16) medication adverse effects were collected using the SAFTEE (Johnson

et al., 2005), and adherence was reviewed and encouraged. Participants were also encouraged, but not required, to attend Alcoholics Anonymous meetings.

Assessment

Multiple assessments were administered prior to randomization, including: SCID-IV, Alcohol Dependence Scale (ADS) (Skinner and Allen, 1982), Obsessive Compulsive Drinking Scale (OCDS) (Anton et al., 1996), Form-90 (modified timeline follow-back method for documenting alcohol consumption) (Tonigan et al., 1997), Drinker Inventory of Consequences (DrInC) (Forcehimes et al., 2007), the Clinical Institute Withdrawal Assessment for Alcohol-Revised (Sullivan et al., 1989) and baseline physical complaints. Lab tests included a health screen, liver function tests, pregnancy test (females) and alcohol use markers gamma-glutamyltransferase (GGT) and carbohydrate-deficient transferrin (Helander et al., 2010, Litten et al., 2010, Anton and Youngblood, 2006).

During each study visit, the calendar based timeline follow-back (Sobell and Sobell, 2000) was used to assess daily drinking. Study drop-outs were paid (\$50) to return at week 16 for drinking assessment.

Outcome Measures

The primary a priori defined outcome measure was percent heavy drinking days (5 or more standard drinks per day for men and 4 or more for women) per month, over the course of treatment.

Genetic and Biological Tests/Assays

The Clinical Neurobiology Lab (CNL) at MUSC (RA Director) extracted genomic DNA from peripheral blood mononuclear cells using a commercial DNA extraction kit/procedure (Qiagen Inc., Valencia CA). *OPRM1* A118G (asn40 vs. asp40 alleles) and *COMT* 158 (val vs. met alleles) were determined by the 5' nuclease genotyping assay (TaqMan®) using primers and allele-specific probes (C_8950074-1 Applied Biosystems, Foster City, CA). Samples previously genotyped in the laboratory of Dr. David Goldman (NIAAA Intramural) and in the CNL were used as reference samples (3 each per genotype/assay). The *SCL6A3* (*DAT1*) VNTR was assessed after PCR amplification by specific primers 5'-TGTGGTGTAGGGAACGGCCTGAG-3' and 5'-CTTCCTGGAGGTCACGGCTCAAGG-3' (Thermo Fisher Scientific, Waltham, MA). Amplified samples were electrophoresed on 2.0% agarose gels and visualized with ethidium bromide under ultraviolet light. Previously identified and sequenced controls for each VNTR length (9 or 10 repeats) were amplified in each assay and two raters compared them with subject samples to call genotypes. Four subjects who carried an 11-repeat allele were grouped with the 10 carriers (being greater than 9 carriers) for this analysis. For each polymorphism, genotypes were in Hardy-Weinberg equilibrium and consistent with published frequencies among individuals of European American descent (Kang et al., 1999, Palmatier et al., 1999). RA supervised quality control in a blinded fashion. %CDT was measured with a reference HPLC assay (Helander et al., 2010), other blood chemistries including GGT were measured with an auto-analyzer.

Sample Size Estimates

Sample size estimates for the original study were guided by power analysis based on the magnitude of the *OPRM1* by medication interaction on heavy drinking days in the COMBINE Study (Anton et al., 2008). The sample size for this exploratory epistatic evaluation was limited by that collected for the original *OPRM1* gene evaluation (Schacht et al., 2017).

Randomization

Participating subjects were entered into a pre-constructed randomization program based on *OPRM1* genotype (strata) with the following URN randomization variables: sex, smoking status, alcohol use disorder family history, antidepressant, and recent cocaine use. The program assigned them to a study medication group based on a priori probability assumptions to maintain balance between groups. All subjects, assessment and treatment staff, were blind to both genotype and medication assignment. Since *DAT1* and *COMT* genotyping were done later they did not serve as randomization variables.

Statistical Analysis

Chi-Square or ANOVA were used to evaluate differences in baseline demographic and alcohol use data between the 2 combinations of 8 treatment groups: medication (naltrexone vs. placebo) x *OPRM1* genotype (A-allele homozygotes vs. G-allele carriers) x either *DAT1* genotype [10R homozygotes vs. 9R carriers] or *COMT* genotype (val-allele homozygotes vs. met-allele carriers) (Table 1). Two baseline predictors of percent heavy drinking days during the trial, “current employment” and “time since last drink prior to randomization”, were used as covariates in the intent-to-treat (ITT) analysis (all subjects with at least 1 week of drinking data) using a linear mixed model (SPSS ver.22, - Linear Mixed, IBM, Armonk NY) with an unstructured variance/covariance matrix focusing on 4 bins of monthly percent heavy drinking data with medication group, time, *OPRM1* genotype, and either *DAT1* or *COMT* genotypes as factors. Based on previous data (Anton et al., 2008, Schacht et al., 2017) the initial protocol stipulated that the last month of treatment would be analyzed independently as contrasts within the mixed model. Simple (lower order) gene by medication interactions (Table 2 and Table 3) were analyzed and significant p values were confirmed using a false discovery rate (Benjamini et al., 2006) calculated using the R statistical package cp4p (Gianetto et al., 2019). Effect sizes of naltrexone compared to placebo were calculated for each gene combination for comparative/illustrative purposes. Since nicotine use was found to affect naltrexone response (Schacht et al., 2017, Anton et al., 2018) a sensitivity analysis was performed to evaluate the effect of smoking as a factor in the main ITT analysis.

Results

Randomization and Baseline Characteristics

Of those initially screened, 358 individuals consented to participate, and 152 were randomized with equal distribution between *OPRM1* gene groups (Figure 1). Since 6 did not have valid outcome data, there were 146 evaluable individuals. Of those, 40 terminated the

study early (similar across all gene/medication groups in numbers and reasons), but full 16-week drinking data was available on 126 (range 84–91% across gene/medication groups). Determined by pill count, 82% of subjects took at least 80% of study medication, again similar across all groups.

A 2 *OPRM1* gene x 2 allele (independently for *DAT1* and *COMT* alleles) x 2 medication group ANOVA showed there were no statistical differences between genotype/medication groups across a range of demographic and drinking variables, including sex, age, nicotine use, antidepressant and cocaine use, baseline drinking, biomarker, and severity measures (Table 1).

Drinking Outcome Measures

The main ITT analysis on percent heavy drinking days (HDD%) included the evaluable participants in each group for the combination of *OPRM1* x *DAT1* (Figure 2) or *OPRM1* x *COMT* (Figure 3). As Figure 2 shows, there was a significant interaction between study medication and *OPRM1/DAT1* genotypes over time ($F= 3.63$ DF, 3, 123.2, $p=0.015$) such that, over the course of 16 weeks, naltrexone was more effective than placebo in reducing %HDD in those with the *OPRM1* A/A genotype who also carried at least one *DAT1* 9 VNTR, and in those with an *OPRM1* G allele who also had the *DAT1* 10/10 VNTR genotype compared to the other genotype and study medication combinations.

Figure 3 shows while there was no interaction of study medication and genotype over time ($F= 1.89$, DF, 3, 123.9, $p=0.14$), there was a significant interaction between study medication, *OPRM1*, and *COMT* genotypes ($F= 4.25$, DF, 1, 131.7, $p=0.041$) such that, averaged across all study weeks, naltrexone was more effective than placebo in reducing %HDD in those with the *OPRM1* A/A genotype who also carried at least one *COMT* met allele, and in those with an *OPRM1* G allele who also had the *COMT* val/val genotype compared to the other genotype and study medication combinations.

In order to compare genotype by medication differences across the spectrum of genotypes, and to illustrate the clinical significance of the pharmacogenetic interactions, the univariate analyses in each genotype group and the effect size of naltrexone over placebo, for the full 16 weeks of treatment, as well as during the last 4 weeks of treatment, is given in Table 2.

Planned simple effects analyses showed that at the 16-week (end of study) time point, differences between the groups were generally in the same direction as the full study interval (Table 2). However, for the *OPRM1* x *DAT1* interaction, only those *OPRM1* G-allele carriers, who also had the *DAT1* 10/10 genotype, were significantly more responsive to naltrexone than placebo ($F= 8.17$, 1, 119, $p=0.005$). For the *OPRM1* x *COMT* interaction, only those *OPRM1* G-allele carriers, who also had *COMT* val/val genotypes, were significantly more responsive to naltrexone than placebo ($F=7.3$, 1, 123, $p=0.008$). As seen, while effect sizes remained large for the gene/medication groups discussed above, *OPRM1* G-allele carriers who were also either *DAT1* 10R homozygotes or *COMT* val/val homozygotes benefitted the most from naltrexone, both during the course of the study and at the end of treatment.

Linear Effects of DAT1 and COMT Allele Load

To further explore these epistatic interactions, the linear effects of allele load at each polymorphism was evaluated. Although the low frequency of the *OPRM1* G allele, even in this enriched sample, precluded evaluation of the linear effect of G-allele load, we tested the linear effect of the number of *DAT1* 9R and *COMT* val alleles as they interacted with dichotomized *OPRM1* genotype. Cell sizes for these analyses were somewhat unbalanced for *DAT1*, owing to the lower overall frequency of the 9R allele, but were more evenly distributed for *COMT* (see Supplementary Table 1). Results indicated that the Medication x *OPRM1* x *DAT1* (number of 9R alleles) x Time interaction trended towards significance ($p=0.076$) such that more 9R alleles in the *OPRM1* AA genotype indicated greater naltrexone efficacy but in *OPRM1* G carriers, less efficacy. For the *COMT* genotypes, a Medication X *OPRM1* X *COMT* (number of val alleles) linear interaction ($p=0.038$) was observed, such that a larger number of val alleles was associated with improved naltrexone response among *OPRM1* G carriers, but with less naltrexone response in AA homozygotes (Supplementary Figure 1). See the supplementary table 1. for the univariate comparisons and naltrexone efficacy effect sizes for each genotype.

Sensitivity and Moderator Analyses

Given our previous findings that nicotine use/smoking status might influence naltrexone response (Schacht et al., 2017, Anton et al., 2018) and despite similar nicotine use between all gene and medication groups (Table 1), a sensitivity analysis, whereby nicotine-use was entered as a covariate into the ITT analyses, found that the pharmacogenetic results were not materially changed.

Other potential modifiers of these reported pharmacogenetic effects might have been antidepressant use or other drug use during the trial. While there was no difference in the rate of antidepressant use between the various medication and genetic groups at the time of randomization (Table 1) we performed a sensitivity analysis where antidepressant use was evaluated in the ITT mixed models previously described, either as a covariate or as a predictor variable. Antidepressant use did not materially affect the significance of the primary analyses or their interpretation. Similarly, when within-study cocaine or marijuana use (determined by drug screens) were entered into the ITT mixed models, there was no material impact on the significance of the results or their interpretation.

Although subjects recruited for this study reported their race as non-African American (primarily European American), an additional step was taken to evaluate population stratification accounting for the observed effects. In order to evaluate racial ancestry in the participants and its distribution across study groups, data available on an Illumina Infinium MethylationEPIC BeadChip assay (collected for other purposes) was used. Along with the ~850,000 epigenetic markers, this assay contains 56 common SNPs for sample identification purposes. Comparison of population allele frequencies and MethylationEPIC results allows for the utilization of many of these SNPs for a basic ancestry analysis (<https://github.com/ttriche/infiniumSnps>). Ancestry analysis was performed with ADMIXTURE v1.3 (Alexander et al., 2009) on data from the present study along with 2,504 subjects from the 1000 Genomes Project (1KGP) phase 3 data (Genomes Project et al., 2015), classifying the

subjects into five ancestry clusters (Steinley, 2004) and providing a probability of membership to each. Each cluster was labeled according to the race available on 2,254 of the 1KGP subjects. Agreement between reported race and each subject's maximum cluster probability was high (Cohen's $\kappa = 0.77$), suggesting that the 56 SNPs correctly identified race for the 1KGP 2,254 subjects. Per Census estimates, residents of the Charleston-North Charleston-Summerville Metropolitan Statistical Area, from which subjects for this study were recruited, are 69% European American and 29% African American; thus, the principal goal of the ancestry analysis was to identify possible misclassification of individuals with self-reported European ancestry who might have had predominantly African ancestry. The clustering algorithm described above correctly assigned 99.4% of 1KGP subjects who self-reported African American ancestry to the African ancestry cluster, and assigned only one subject who self-reported European ancestry (and who also identified as Hispanic) to that cluster. Because of this, and the other assignment accuracy as detailed above, we evaluated the genetic ancestry estimation of our subjects using the five cluster-assignments identified. The ancestry cluster probabilities were evaluated for their distributional balance across study groups. Using one-way ANOVAs, the mean probabilities for each of the five ancestry clusters were compared across medication and primary gene (*OPRM1*, *DAT*, *COMT*) groups. There were no significant differences in these ancestry indicators across treatment comparison groups, indicating that racial variation was ostensibly balanced across the subgroups included in the medication by genotype analyses.

Adverse Events – Pharmacogenetic Effects

One important aspect of any pharmacotherapy is medication tolerance as reflected by adverse effects (AEs). Naltrexone has a number of reported AEs, many of which are gastrointestinal complaints. Using the SAFETEE interview, differences in AEs between the genotype combinations depicted in Table 2 were tested to evaluate whether epistatic interactions were associated with specific AEs and, secondarily, to evaluate whether those AEs impacted the pharmacogenetic effect on naltrexone efficacy in reducing drinking reported in Table 2. Diarrhea and/or abdominal pain, reported by 26% of placebo participants and 58% of naltrexone participants ($p < 0.0001$), were the most frequent AEs, consistent with previous reports (Anton et al., 2006). The number of unique individuals reporting diarrhea/abdominal complaints, as well as the proportion of times (out of a maximum 9 independent assessments) these AEs were reported across individuals, broken down by genotype, is presented in Table 3. The greatest difference between naltrexone and placebo in diarrhea/abdominal pain reports was seen in the *OPRM1* AA/*DAT1* 9-Carrier and the *OPRM1* AA/*COMT* Met-Carrier groups as expressed both in the number of individuals reporting this complaint at least once ($p = 0.001$ and $p = 0.003$ respectively) or multiple times ($p = 0.001$ and $p = 0.003$ respectively) with all significant values meeting a 5% false discovery rate criterion after correction for multiple comparisons. Since naltrexone was also superior to placebo in reducing drinking in these two genotype groups, especially over the 4 months of the trial, we reanalyzed the drinking data accounting for the presence of diarrhea/abdominal pain complaints. The significance of the naltrexone vs. placebo effect on heavy drinking days was not materially affected, indicating that the diarrhea/abdominal complaints were independent of the pharmacogenetic efficacy effects.

Discussion

This study had high internal validity, being conducted at one site with the same staff providing MM throughout the study and using a priori *OPRM1* genotyping and selection. Data collection was performed blind to all genotypes and medication group assignment. Individuals of clinical interest (some taking antidepressants and some with cocaine use) were included, and equally distributed across study groups. The analyses of *DAT1* and *COMT* genotypes were hypothesis driven, and pre-planned, with the genotyping done prior to any *OPRM1* outcome analyses.

In our initial report (Schacht et al., 2017), while there was a significant main effect of naltrexone over placebo overall, *OPRM1* A118G status was not predictive of response in the ITT analysis (but did have some predictive value in the most adherent individuals). In the current expanded analysis of the epistatic interaction of the *OPRM1* 118 genotypes with several dopamine genes, significant medication by genotype interactions emerged. As the results show, those *OPRM1* G allele carriers who were also *DAT1* 10,10 or *COMT* val/val homozygotes responded remarkably well to naltrexone compared to placebo (effect sizes of 0.7 and 0.8). In addition, those *OPRM1* A/A homozygotes who were either *DAT1* 9R-carriers or *COMT* met-carriers also responded better to naltrexone than placebo (effect sizes 0.70 and 0.63 respectively). These effect sizes are much greater than the small to moderate effect sizes of naltrexone efficacy in unselected Individuals with AUD across many trials (Srisurapanont and Jarusuraisin, 2005, Maisel et al., 2013), and may explain the inconsistent results reported in past naltrexone/*OPRM1* A118G pharmacogenetic studies. In this study, individuals with *OPRM1* G allele who were *DAT1* 9R-carriers or *COMT* met-carriers or who were *OPRM1* AA homozygotes who were *DAT1* 10,10 or *COMT* val/val homozygotes were, on average, naltrexone non-responders. Although exploratory, due to smaller sample sizes, a linear analysis of allele load suggested that the presence of a greater number of indicator alleles (homozygous versus heterozygous) predicted more naltrexone efficacy, further supporting these observations.

As to the biological basis of this finding, while the basic science literature is not completely consistent regarding *OPRM1* A118G effects on endogenous opiate binding and receptor production (Bond et al., 1998, Zhang et al., 2005), there has been consistent suggestions that the *OPRM1* A118G SNP affects the mu opiate receptor in the human brain. Several studies have shown that AUD or smoking individuals who are G carriers have less mu opiate receptor opioid binding (Weerts et al., 2013, Ray et al., 2011). This could be caused by decreased mu opiate receptor availability or adaptation to chronic increased endogenous opioid release (Weerts et al., 2013, Ray et al., 2011). Nevertheless, a number of studies have shown that AUD *OPRM1* G carriers respond more positively to alcohol cues (Ray et al., 2013), alcohol stimulation, positive mood (Ray and Hutchison, 2004), and intoxication (Ray et al., 2012). Our reported pattern of epistatic interactions might suggest that Individuals with AUD who are *OPRM1* G carriers might need to also have low/normal dopamine-tone, since genotypes normally reflective of heightened brain dopamine (*DAT1* 9R and *COMT* met) were not associated with naltrexone response in these individuals, while those genotypes that reflect low-normal dopamine tone (*DAT1* 10R and *COMT* val/val) generally were most responsive. Of course, there is no way of knowing exactly if this pharmacogenetic

argument is specifically germane to the ventral striatum-affiliated systems underlying reward saliency, since opiate and dopamine systems co-exist, and likely interact, in cortical areas underlying reward salience and cognitive control. It should be noted that work of others (Filbey et al., 2008) and ourselves (Schacht et al., 2013) have identified pharmacogenetic interactions between brain dopamine and opioid systems in Individuals with AUD. Future pharmacogenetic examination of interaction of these areas using neuroimaging paradigms coupled with neurocognitive examination should be undertaken.

It is possible that this interaction between endogenous opioid and dopamine systems in AUD (as extrapolated from functional genotyping) might provide valuable insights into the brain pathophysiology of AUD and suggest a way to unravel the complex effects of alcohol on brain transmitter systems. The fact that response to a targeted (and pharmacologically specific) medication like naltrexone could be differentially impacted by these interactions is both novel and noteworthy. It also suggests that multiple genes might need to be examined to provide the precision that personalized treatment of AUD might require, not a surprise given the biological and pharmacological response heterogeneity of the disorder.

Of note, the findings reported here were guided by previous work done by our group in non-treatment seeking Individuals with AUD using fMRI where we found that naltrexone reduced alcohol-cue brain activation in the ventral striatum more strongly in G carriers who were also *DAT1* 10,10 homozygotes (Schacht et al., 2013). This finding directly paralleled drinking response to naltrexone in a bar lab setting in the same study such that both *OPRM1* G carriers who were *DAT1* 10,10 homozygotes and AA homozygotes who were *DAT1* 9R carriers both reduced drinking to placebo (Anton et al., 2012). The confluence of results from two independent studies on Individuals with AUD (one a clinical lab study, as above, and one a clinical trial, presented here) lends both biological and clinical validity to the *OPRM1/DAT1* findings. The fact that the *OPRM1/COMT* findings are consistent with opioid-dopamine directions, as suggested by the *OPRM1/DAT1* findings, further buttress the overall validity of the hypothetical construct and pharmacogenetic efficacy findings.

Since we previously reported that nicotine-use/smoking was associated with better naltrexone response in the current trial (Schacht et al., 2017, Anton et al., 2018), we analyzed its impact on the pharmacogenetic findings and found that nicotine-use/smoking did not materially impact the results presented here. However, future larger studies might further explore more subtle interactions of nicotine-use and genotype in AUD treatments.

Of additional interest is our finding that the *OPRM1* gene seems to interact with dopamine-related genes (*COMT* and *DAT1*) in predicting gastrointestinal effects of naltrexone. Opiate receptors in the bowel interact with other neurotransmitters and, when agonized, cause constipation and, when blocked by naltrexone-like compounds, constipation is reversed and/or diarrhea may occur (Pannemans and Corsetti, 2018). To our knowledge, this is the first time that an opiate antagonist medication's effect on the GI tract seemed to be differentially affected by pharmacogenetic epistasis. It is especially interesting that several of the genotype combinations that predicted naltrexone response (to various degrees) also were associated with GI complaints, perhaps suggesting a similar biological interaction in the GI tract and the brain. This needs further exploration.

Finally, a comment should be made about past reports of the interaction of *OPRM1* A118G and particularly *DAT1* VNTR's. While beyond the scope of this report, it should be mentioned that there has been a lack of consistency in findings on how these genes interact on drinking measures and alcohol response. While most studies do report interactions between these functional brain genetic polymorphisms, the lack of consistency should make researchers reconsider how these brain neurochemical systems might interact, as well as how these systems might interact differently in social drinkers (Weerts et al., 2017) heavy drinkers (Ray et al., 2014) , non-treatment seeking heavier drinking Individuals with AUD (Anton et. al. 2012) and even heavier drinking AUD treatment-seekers (the current study). This could have implications for both the clinical models themselves, and for treatment predictions extrapolated from these models.

This study has a few limitations. Given the original hypothesis and sample size available, individuals could not be genotyped and subsequently randomized based on two/three genes, so we cannot rule out some systematic selection and treatment assignment bias. Also, sample size limitation did not allow valid analysis of 4-way interactions between all three genotypes and medication response. Finally, while African Americans and Asians were not studied, and thereby reducing the confounding effects of unrecognized race-based epistasis, the findings cannot be automatically extended to these, or other, racial groups. This needs future evaluation. It is further recognized that focusing on one or several "target polymorphisms" could lead to type 1 errors due to population stratification (although our ancestry analysis minimizes this possibility), sampling errors, or chance. However, for pharmacogenetic studies, a targeted approach might be more meaningful if it is based on an a priori understanding of the underlying biology/pharmacological interactions that informs hypothesis driven (non-exploratory) investigation, as was the case in this study.

Despite the above limitations, these findings are underpinned by previous scientific findings, a priori hypotheses formulation, and a well-executed clinical trial. Nevertheless, replication in other data sets would be useful. Data of this nature could inform a more precise application of pharmacotherapy for AUD that might be more accepted and appreciated by patients and their treatment providers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1: Consolidated Standards of Reporting Clinical Trials Diagram

Those with valid data had more than one week of drinking data reported post-randomization.

Figure 2: Percent Heavy drinking days/month in those treated with naltrexone or placebo by *OPRM1* (AA or G carrier) and *DAT1* (9R carrier or 10/10) genotypes.

ITT main effect of naltrexone, genotype, by time ($F=3.63$ DF, 3, 123.2, $p=0.015$). See text.

Figure 3. Percent Heavy drinking days/month in those treated with naltrexone or placebo by *OPRM1* (AA or G carrier) and *COMT* (met carrier or val/val) genotypes.

ITT main effect of naltrexone by genotype ($F= 4.25$, $DF, 1,131.7$, $p=0.041$). See text.

Table 1.

Demographic, alcohol use and severity measures.

Characteristic	Total Sample (n=146)		OPRM1x DAT1	OPRM1x COMT
			Statistic ^a	Statistic ^a
Demographics			P value	P value
Age, y (mean (SD))	49.3	(10.1)	0.54	0.10
Sex, M, No. (%)	101	(69.2)	0.88	0.97
Married, No. (%)	94	(64.4)	0.06	0.12
Employed, No. (%)	114	(78.1)	0.92	0.74
Education 12 years, No. (%)	24	(16.4)	0.54	0.17
Current Nicotine Use, No. (%)	58	(39.7)	0.88	0.89
Cocaine Use, No. (%)	19	(13.0)	0.76	0.49
Antidepressant use, No. (%)	48	(32.9)	0.73	0.83
Alcohol use and severity indicators, mean (SD)				
Drinking days (%) ^b	85.3	(19.3)	0.75	0.22
Heavy drinking days (%) ^b	79.7	(22.3)	0.34	0.28
Drinks per drinking day ^b	11.2	(4.8)	0.84	0.44
Drinks per day ^b	9.6	(5.0)	0.88	0.39
Days from last drink to randomization	6.9	(4.4)	0.22	0.44
ADS score ^c	15.4	(6.4)	0.96	0.25
OCDS score ^d	25.6	(8.1)	0.89	0.58
Drinking consequences ^e	41.4	(18.6)	0.54	0.23
No. (%)				
GGT>63 IU/L	46	(31.5)	0.79	0.37
dCDT 1.7% No. (%) ^f	80	(56)	0.25	0.59

^aTest of differences across medication groups and two genes. ANOVA testing linear variables and Chi Square testing categorical variables.^bDrinking calculated during the 90 days prior to screening.^cAlcohol Dependence Scale^dObsessive Compulsive Drinking Scale^eDrinker Inventory of Consequences^fDisialo-Carbohydrate Deficient Transferrin – biomarker of heavy drinking (n=143)

Table 2.

Significance and effect sizes of the interaction of various *OPRM1* and DAT or COMT genes and naltrexone response on percent heavy drinking days over the course of the trial (16 weeks) and at the end of the study (last 4 weeks).

Genotypes		Treatment Effects				
		All 4 months			Last Month	
OPRM1 ^a	DAT ^b					
		N	p value *	Effect Sizes **	p value *	Effect Size **
A,A	9 Carriers	26	0.085	0.70	0.25	0.46
A,A	10,10	47	0.4	0.25	0.82	0.07
G Carriers	9 Carriers	33	0.62	-0.17	0.5	-0.24
G Carriers	10,10	40	0.021	0.72	0.005	0.89
OPRM1	COMT ^c					
A,A	Met Carriers	51	0.03	0.63	0.27	0.32
A,A	Val,Val	22	0.78	-0.12	0.94	-0.03
G Carriers	Met Carriers	53	0.69	0.11	0.92	0.03
G Carriers	Val,Val	20	0.05	0.80	0.008	1.09

* Univariate analysis testing in the interaction of medication group (naltrexone x placebo) in each genetic combination. Bolded p values remain significant after testing a false discovery rate of 10%.

** Effect size for naltrexone over placebo in reduction of percent heavy drinking days

^a A or G allele at the 118 position in the *OPRM1* gene, coding for asn40 or asp40, respectively

^b Number of repeats (i.e., 9 or 10) of the 40-base-pair variable number tandem repeat polymorphism in the *DAT1* gene

^c Val or Met allele at the 158 position in the *COMT* gene

Table 3.

Number of individuals, and number of total reports across individuals, who reported diarrhea/abdominal pain in each medication by genotype group.

		Percent Individuals Positive			Percent (SD) Positive Reports (out of 9 possible)		
		Placebo	Naltrexone	p value *	Placebo	Naltrexone	p value **
<i>OPRM1</i> ^a	<i>DAT1</i> ^b						
AA	9 Carriers	7%	67%	0.001	1 (3) %	25 (24) %	0.001
AA	10,10	29%	57%	0.06	5 (10) %	15 (19) %	0.032
G Carriers	9 Carriers	19%	44%	0.13	7 (16) %	21 (32) %	0.11
G Carriers	10,10	42%	67%	0.12	13 (24) %	17 (18) %	0.55
<i>OPRM1</i> ^a	<i>COMT</i> ^c						
AA	Met Carriers	19%	60%	0.003	4 (9) %	19 (23) %	0.003
AA	Val, Val	25%	60%	0.10	3 (6) %	16 (16) %	0.014
G Carriers	Met Carriers	33%	52%	0.17	10 (20) %	19 (27) %	0.14
G Carriers	Val, Val	25%	67%	0.07	13 (23) %	18 (20) %	0.58

* chi square (bolded p values remain significant after testing a false discovery rate of 5%)

** Univariate ANOVA (bolded p values remain significant after testing a false discovery rate of 5%)

^a A or G allele at the 118 position in the *OPRM1* gene, coding for asn40 or asp40, respectively

^b Number of repeats (i.e., 9 or 10) of the 40-base-pair variable number tandem repeat polymorphism in the *DAT1* gene

^c Val or Met allele at the 158 position in the *COMT* gene