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Variation in *OPRM1* Moderates the Effect of Desire to Drink on Subsequent Drinking and its Attenuation by Naltrexone Treatment

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Abstract

To evaluate the role of the functional Asn40Asp polymorphism in the mu-opioid receptor gene on drinking behavior and naltrexone's ability to attenuate drinking, we used a daily diary method in a 12-week, randomized clinical trial of naltrexone to reduce drinking. Participants (N=158 problem drinkers) were assigned to receive either daily or targeted naltrexone 50 mg (N = 81) or matching placebo (N = 77). Patients reported by telephone each evening their current desire to drink and their drinking during the previous night and during the reporting day. We examined genotype, medication, and desire to drink and their interactions as predictors of nighttime drinks consumed, controlling for drinking earlier in the day. Asp40 carriers showed a stronger positive association between evening desire (deviations from their mean levels) and later night drinking levels than Asn40 homozygotes ($p = 0.019$). The desire $\times$ genotype $\times$ medication condition interaction was also significant ($p = 0.009$), with a significant desire $\times$ genotype interaction for the placebo group ($p = 0.001$), but not the naltrexone group ($p = 0.74$). In summary, when the evening level of desire to drink was relatively high, Asp40 allele carriers were at greater risk than Asn40 homozygotes to drink more, which was attenuated by naltrexone. Although average measures across the study were not informative, daily reports helped to demonstrate the moderating effects of genetic variation on the relation between desire to drink and alcohol consumption, and the effects of naltrexone on that phenotype.

Keywords

Desire to Drink; Heavy Drinking; Naltrexone; Pharmacogenetics; Treatment Response

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Author Contributions

HRK, SA, and HT were responsible for the study concept and design. HRK, JC, and HT acquired the clinical data. JC performed the genetic analysis. SA conducted the data analysis and HRK and HT assisted in the interpretation of findings. HRK drafted the manuscript. All authors provided critical revision of the manuscript for important intellectual content. All authors critically reviewed content and approved final version for publication.

Introduction

There is growing interest in personalized approaches to the treatment of alcohol dependence (AD), including the genetic moderation of pharmacological effects. The most widely studied genetic moderator of alcohol treatment response is a variant in *OPRM1*, the gene encoding the μ -opioid receptor (MOR). The MOR is a target for the opioid antagonist naltrexone, which is efficacious in reducing heavy drinking in individuals with AD (Rösner et al. 2010). A common A→G single nucleotide polymorphism (SNP) in exon 1 of *OPRM1* (Bergen et al. 1997) encodes the substitution of an aspartic acid residue for an asparagine residue (Asn40Asp) in the N-terminal extracellular domain of the receptor. Although there is evidence that this SNP is functional, its effects at the molecular, cellular and behavioral levels and on naltrexone's ability to attenuate drinking have varied with different study designs and populations (reviewed in Kranzler and Edenberg 2010, Ray et al. 2011).

Recently, Ramchandani et al. (2011), in a placebo- and pharmacokinetically-controlled alcohol challenge in social drinkers, measured striatal dopamine release by [11C]-raclopride displacement using positron emission tomography. In this study, dopamine release was evident only in carriers of the Asp40 allele. Further, using brain microdialysis in two humanized mouse lines carrying the human sequence variant for the SNP, these investigators saw a peak response to an alcohol challenge in animals homozygous for the Asp40 allele that was four times that of Asn40 homozygotes. Together, these studies provide evidence of a neurochemical effect of the Asn40Asp SNP following alcohol administration.

Studies of the effects of the Asn40Asp polymorphism on the desire to drink and drinking behavior as measured in the human laboratory and naturalistically have yielded mixed findings (Ray et al. 2011). In an initial human laboratory study, following alcohol administration, Ray and Hutchison (2004) found that healthy subjects with the Asp40 allele reported greater feelings of intoxication, stimulation, sedation, and happiness than Asn40 homozygotes. In a subsequent laboratory study by these investigators, non-treatment-seeking heavy drinkers with the Asp40 allele also reported greater alcohol-induced high, but less alcohol craving, than Asn40 homozygotes (Ray and Hutchison 2007). This contrasts with findings reported by van den Wildenberg et al. (2007) in which Dutch male heavy drinkers with the Asp40 allele reported higher levels of craving following alcohol cue exposure than those homozygous for the Asn40 allele. Ray (2011) found that non-treatment-seeking heavy drinkers with the Asp40 allele reported greater alcohol-cue-induced craving than Asn40 homozygotes (Ray 2011). Finally, a naturalistic study of non-treatment-seeking heavy drinkers using ecological momentary assessment (EMA) showed that, although Asp40 carriers consumed more alcohol per drinking episode than Asn40 homozygotes, Asp-40 carriers showed a weaker relationship between urge to drink and subsequent drinking than the Asn40 homozygote group (Ray et al. 2010).

There are also mixed findings on the moderating effect of the Asn40Asp SNP on naltrexone's attenuation of drinking behavior. In the laboratory study by Ray and Hutchison (2007), naltrexone attenuated the alcohol-induced high more among Asp40 carriers than Asn40 homozygotes (Ray and Hutchison 2007). Setiawan et al. (2011) replicated these findings in a sample of Canadian social drinkers, where there was greater attenuation by naltrexone of alcohol's subjective effects in individuals with the Asp40 allele. In this study, however, the moderating effect of genotype appeared to be greater in women than men and the decreased subjective effects did not translate into decreased alcohol self-administration (Setiawan et al. 2011). Ray et al. (2012) conducted a double-blinded placebo-controlled study of naltrexone in a sample of community heavy social drinkers of East Asian ancestry who were administered alcohol intravenously in a laboratory setting. In this study, Asp40 carriers reported greater alcohol-induced sedation and subjective intoxication and less

alcohol craving when treated with naltrexone than Asn40 homozygotes. An exploratory comparison of Asp40 homozygotes and heterozygotes was consistent with a dose effect, with trend-level or significantly greater effects of alcohol and naltrexone when two copies of the variant allele were present.

Contradictory findings were obtained in a placebo-controlled study of non-treatment seeking heavy drinkers, in which Asp40 carriers treated with naltrexone reported greater cue-induced craving for alcohol than Asn40 homozygotes (McGeary et al. 2006). Analysis of a larger sample [including subjects from McGeary et al. (2006)] in a naturalistic follow-up failed to demonstrate moderation by the Asn40Asp SNP on the observed naltrexone treatment effects (Tidey et al. 2008). Similarly, a placebo-controlled study of naltrexone's effects in non-treatment seeking heavy drinkers failed to show a moderating effect of the Asn40Asp SNP (Mitchell et al. 2007). A cue-exposure study in Dutch alcohol-dependent individuals yielded no evidence of moderation by the Asn40Asp SNP on the response to 21 days of treatment with naltrexone (Ooteman et al. 2009).

Treatment trials have also yielded variable findings on the moderating effect of the Asn40Asp SNP on naltrexone treatment response. Oslin et al. (2003) first reported a moderating effect of the polymorphism using data from three placebo-controlled trials of naltrexone. These investigators found that naltrexone-treated patients with one or two Asp40 alleles were less likely than Asn40 homozygotes to relapse to heavy drinking. Placebo-treated subjects showed no moderating effect of genotype. One subsequent multi-center trial of naltrexone for AD showed no evidence that the SNP moderated the effect of the medication (Gelernter et al. 2007), but a second trial showed robust moderation, at least among individuals who received medication management rather than more intensive psychotherapy (Anton et al. 2008). In a 12-week, open-label trial in 63 Koreans with alcohol dependence, time to relapse for medication adherent individuals (N=32) with the Asp40 allele was significantly longer than for Asn40 homozygotes (Kim et al. 2009). Although not statistically significant, the Asp40 group had a relapse rate that was much lower than that of the Asn40 homozygote group. In contrast, a recent open-label study in which 100 alcohol-dependent Australians received daily naltrexone (Coller et al. 2011) showed reduced weekly alcohol consumption, mean corpuscular volume, gamma-glutamyltranspeptidase concentration and alcohol craving during the 12-week treatment period. However, the Asn40Asp SNP did not moderate any of these effects.

Conflicting findings on the Asn40Asp SNP's moderation of the effects of alcohol and the efficacy of naltrexone reflect a modest impact of the SNP and limited statistical power in most of the available studies (due to both small sample size and a low prevalence of the Asp40 allele in non-Asian populations; Arias, Feinn, & Kranzler, 2006). To examine the moderating effects of the Asn40Asp SNP on drinking and on its attenuation by naltrexone, we chose a phenotype (i.e., the daily association between desire to drink and subsequent drinking) that is more ecologically valid than that used in human laboratory studies because it reflects free choice behavior in the individual's usual environment. Further, in contrast to outcomes typically used in treatment trials, we measured drinking behavior on a daily, rather than a weekly, monthly or quarterly basis. Our micro-longitudinal design allowed us to (a) measure desire to drink and drinking behavior relatively free of recall error and bias and (b) to focus on the within-person associations between desire to drink and alcohol consumption, thus reducing error variance and enhancing statistical power. Specifically, we examined daily data from problem drinkers participating in a 12-week, placebo-controlled trial of naltrexone to reduce drinking (Kranzler et al. 2009). Using multiple measurements of the relations between desire to drink and drinking behavior, we were able to examine the moderating effects of the Asn40Asp SNP on the desire to drink-drinking relationship and its attenuation by naltrexone.

Materials and Methods

Overview

We used daily reports in the context of a randomized, placebo-controlled trial to examine whether 1) variation in *OPRM1* genotype moderated the effect of desire to drink on subsequent drinking and 2) this effect was attenuated by naltrexone. As described in detail in Kranzler et al. (2009), problem drinkers from a comparison of daily vs. targeted naltrexone reported by telephone daily (using interactive voice response, IVR) their current desire to drink and their drinking from the previous night (after they completed the prior day's survey) and current day (up to reporting time). This design allowed us to examine whether desire to drink reported on day t predicted subsequent drinking that night (reported on day $t + 1$) controlling for pre-survey drinking on day t . Further, we examined two variables, Asn40Asp genotype and treatment with naltrexone or placebo, as potential moderator variables, on the hypothesis that Asp40-allele carriers would show greater drinking following reports of high desire to drink and that naltrexone treatment would attenuate that response.

Subjects were recruited through advertisements and referrals by area clinicians. Following a telephone screening interview, eligible participants were given a complete description of the study procedures and potential risks. They gave written, informed consent to participate in the study, which was approved by the institutional review board of the University of Connecticut Health Center. They were paid to complete daily reports and research assessments conducted at the end of treatment. The study was registered as NCT00369408 on www.clinicaltrials.gov.

Briefly, subjects were 18–65 years old and able to read English at an eighth grade or higher level. They reported an average weekly alcohol consumption of 24 standard drinks (men) and 18 standard drinks (women) and were judged to be able to give informed consent and to complete study assessments accurately. Women of childbearing potential were non-lactating and practicing a reliable method of birth control and had a negative serum pregnancy test prior to the initiation of treatment. Subjects were excluded if they had a clinically significant physical or psychiatric illness requiring medical treatment, a current DSM-IV diagnosis of drug (other than nicotine) dependence, a lifetime diagnosis of opioid dependence, regular use of opioids or other psychoactive medications in the preceding month, a current diagnosis of alcohol dependence that was clinically severe, a recent unsuccessful attempt to reduce drinking or a history or present evidence of significant alcohol withdrawal symptoms.

A total of 163 subjects were randomized to treatment condition (see Kranzler et al. 2009). There were no genetic data available for four individuals and one did not have daily data, resulting in a final sample of 158 subjects for analysis, including 153 European-Americans, 3 African Americans, and 2 individuals who identified themselves as other and not Hispanic. Most of the subjects were male (57.6%) and included 36 Asp40-allele carriers (22.8%). Subjects' mean age was 49.1 years (SD = 9.4), with a mean of 15.4 years (SD = 2.4) of education.

Study Medication

Participants were randomly assigned to receive treatment with naltrexone 50 mg/day (N=81) or an indistinguishable placebo tablet (N=77) and a daily or targeted schedule of medication administration. Medication was dispensed in a quantity sufficient for a two-week period. Subjects in the daily conditions were encouraged to take one tablet daily. Subjects in the targeted groups received were encouraged to use 3–5 tablets per week by taking them in anticipation of high-risk drinking situations, with a maximum of one tablet every 24 hours.

Brief Skills Training

In counseling sessions held every two weeks, subjects received brief coping skills therapy focusing on the identification and management of high-risk drinking situations to enable them to reduce their drinking to non-hazardous levels. Details on the goals and content of the sessions and on the recommended drinking levels can be found in Kranzler et al. (2009).

Assessments

The *Time-Line Follow-Back Assessment Method* (TLFB; Sobell and Sobell 1992) was used to estimate alcohol consumption during the 90-day pre-treatment period. Patients were given a blank calendar and were asked to reconstruct their drinking behavior over the preceding three-month period.

Interactive Voice Response Technology (IVR) is a telephone-based method for the administration of survey questions. Its use in alcohol treatment trials has been described previously (Kranzler et al. 2004, 2009, 2011). Briefly, at the initiation of treatment, subjects met with a research assistant for a brief IVR training session and were given a toll-free number to use to contact the IVR system. They were asked to call the system daily from a touch-tone phone between 5:00 and 9:00 PM to report on the day's experience. Subjects who did not call the IVR system by 8:00 PM were called automatically at a preferred telephone number to remind them to complete the interview.

Each day, patients reported their desire to drink and their drinking during the previous night (i.e., after the last IVR call) and for that day by using the telephone keypad, with responses entered automatically in a database. They reported the number of standard drinks in each of three categories of beverage: beer, wine, and liquor. Using IVR, patients also reported whether they consumed a study tablet since the previous call.

Genotyping

DNA was prepared from whole blood samples using a commercial kit (Gentra Puregene, Qiagen, Valencia, CA). The Asn40Asp *OPRM1* polymorphism (rs1799971) was genotyped using a TaqMan™ 5' nuclease assay. 10 µl reactions were prepared containing 25 ng of genomic DNA, 1 ng of BSA, 1× TaqMan master mix (Applied Biosystems, Inc. Foster City, CA), 6 pM of each primer (CCCAGCCCCGGTTCCT and TGATGGCCGTGATCATGGA), 0.3 pM of a Vic-MGB probe for the Asp40 allele (Vic-AGATGGCGACCTGTCC-MGB) and 0.6 pM of a Fam-MGB probe for the Asn40 allele (Fam-AGATGGCAACCTGTCC-MGB). Samples were PCR amplified using 35 thermocycles with annealing-extension at 58.5°C for 60 sec. Post-PCR Vic and Fam fluorescence were measured using an ABI 7700 instrument (Applied Biosystems, Inc. Foster City, CA) and scatter plot of Vic vs. Fam fluorescence used to assign genotypes. Reference samples with known genotypes were included in each assay plate as positive controls. DNA samples were genotyped twice. DNA from 4 subjects did not provide reliable results and were not included in the analysis. The results for the remaining samples were 100% concordant on repeat genotyping.

Data configuration and analysis

We first tested the main and interaction effects of genotype and medication condition as predictors of mean daily drinking levels using standard linear regression. Specifically, we entered genotype (coded Asp40-allele carriers = 0 vs. Asn40-allele homozygotes = 1), medication group (coded placebo = 0 vs. naltrexone = 1) in a first step, followed by the genotype × medication condition product term in step 2.

Next, we used Generalized Estimating Equations (GEE) to test whether the association between evening desire to drink and subsequent nighttime drinking varied as a function of *OPRM1* genotype and medication condition, controlling for daytime drinking levels. Specifically, we predicted nighttime drinking (reported on day $t+1$ for the previous night) from desire to drink and daytime drinking levels (reported on day t). Because nighttime drinking was reported one day subsequent to evening reports of desire to drink and daytime alcohol consumption, two consecutive days of daily reporting were required for a completed full day of reporting. Thus, one missing daily report resulted in two missing data points for analysis. We also included six day-of-the-week dummy codes (using Saturday as the reference day) to control for weekly drinking cycles. Given the count nature of the outcome (and the large number of zeros and positive skew at the daily level of analysis), we estimated a negative binomial regression with log link (Coxe, West, & Aiken, 2009). To reduce the influence of extremely high reports of daytime or nighttime drinking (which occurred on 0.4% of the total person days), we recoded any value greater than 20 to 20. We person-mean-centered daily reports of desire to drink (i.e., by subtracting each person's overall mean from their daily reports) to examine how daily deviations from individuals' mean desire levels were related to nighttime drinking. Person-mean centering yields an unbiased estimate of the overall within-person association (see Stone and Shiffman, 1994) between desire to drink and drinking and allowed us to address the question of how individuals' nighttime drinking levels changed on days characterized by relatively higher or lower levels of desire to drink. Each person's overall mean desire to drink was also included in the models. Sex was initially included as a control variable, but because it was not significant, it was removed from the model. Although at the person level, the cell sizes became very small when schedule of administration (i.e., daily vs. targeted) was included in the analysis, because the study design included that factor, we explored whether the four-way interaction of desire to drink, genotype, medication group, and schedule of administration predicted daily drinking.

Results

Treatment and Research Adherence

We received a total of 10,929 daily reports. Of these, 9,789 were complete reports (i.e., both day t and $t+1$ values) for analysis. This represents 74.6% of the possible 13,114 person-days [i.e., 158 patients $\times$ 83 days (because drinking data for day 84 were incomplete as there was no report of nighttime drinking for the last treatment day)].

The number of Asp40 carriers vs. Asn40 homozygotes did not differ by medication group ($\chi^2[1] = 1.81, p = 0.18$). Table 1 shows the demographic and pretreatment variables by genotype and medication condition, where it can be seen that there were significant differences across the four genotype and medication condition groups on the number of reporting days, education level, and pretreatment drinking days. Follow-up focused tests indicated that, in the naltrexone condition, Asp40 carriers provided more reports than Asn40 homozygotes. Further, Asp40 carriers in the placebo condition were younger than Asn40 homozygotes in the naltrexone condition. Finally, Asp40 carriers in the placebo condition reported fewer pretreatment drinking days than each of the other three groups.

Subjects' Drinking Behavior During Treatment

Subjects reported drinking on 79.2% of days, consuming 4.6 drinks ($SD = 2.27$) per drinking day. Daytime drinking (i.e., prior to the daily calls) was reported on 37.5% of the days and subjects reported drinking a mean of 3.1 drinks ($SD = 2.4$) per daytime drinking period. Nighttime drinking was reported on 69.9% of the days and subjects reported drinking 3.6 drinks ($SD = 2.7$) per nighttime drinking period.

Average Drinking as a Function of Genotype and Medication Condition

We included sex, years of education and pretreatment proportion of drinking days as control variables in the first step of the multiple regression model. Results indicated that neither genotype ($b = -0.23$, $SE = 0.45$, 95% CI: -1.12 to 0.65 , $p = 0.60$) nor medication condition ($b = 0.07$, $SE = 0.37$, 95% CI: -0.67 to 0.80 , $p = 0.86$) significantly predicted mean daily drinking levels. The genotype $\times$ medication condition interaction, entered in step 2, also was not a significant predictor ($b = 0.85$, $SE = 0.90$, 95% CI: -0.94 to 2.63 , $p = 0.35$).

Nighttime Drinking as a Function of Evening Desire to Drink, Genotype, and Medication Condition

We first estimated the GEE negative binomial regression without any of the multiplicative terms (see Table 2, step 1). Consistent with findings from published daily studies of drinking (Armeli et al. 2000, Todd et al. 2003), there were day-of-the-week effects on drinking, with subjects drinking more on Saturday nights compared to all other nights except Fridays. Earlier day drinking and the mean level of desire to drink were positively associated with nighttime drinking. Of central interest, evening desire to drink (i.e., deviations from mean levels) was significantly related to nighttime drinking: specifically, on evenings when individuals reported a greater desire to drink than their mean level, they reported greater drinking later that night. Exponentiation of this slope reveals the rate increase in drinking for a unit change in desire [i.e., $\exp(0.248) = 1.28$]; it showed that for every one unit that daily desire exceeded mean desire, drinking increased by 28%.

In step 2, we entered the 2-way product terms between genotype, medication condition, and daily desire to drink. Only the genotype $\times$ daily desire to drink interaction was significant, with Asp40 carriers ($b = 0.39$, $SE = 0.07$, 95% CI: 0.24 to 0.53 , $p < 0.001$) showing a stronger positive association between changes in desire to drink and subsequent drinking level than Asn40 homozygotes ($b = 0.21$, $SE = 0.028$, 95% CI: 0.15 to 0.26 , $p < 0.001$). We next entered the 3-way product term for the genotype $\times$ medication condition $\times$ daily desire to drink interaction in step 3, which was significant; the form of this interaction is shown in Figure 1. Probing of the lower-order 2-way interactions indicated that the genotype $\times$ desire to drink interaction effect on drinking was significant for individuals who were treated with placebo ($b = -0.40$, $SE = 0.12$, 95% CI: -0.65 to -0.16 , $p = 0.001$), but not for individuals taking naltrexone ($b = -0.03$, $SE = 0.07$, 95% CI: -0.17 to 0.12 , $p = 0.74$).

Next, in an exploratory fashion we included schedule of administration (coded $-1 =$ daily, $1 =$ targeted) in the model to test the four-way interaction of genotype, medication group, schedule of administration and desire to drink on daily drinking. Specifically, we included all the necessary 2-way and 3-way product terms along with the 4-way product term. The 4-way effect was not statistically significant ($b = -0.009$, $SE = 0.14$, 95% CI: -0.29 to 0.28 , $p = 0.95$), indicating that the effect of the three-way interaction was comparable for the targeted and daily medication administration groups (for targeted group $b = .35$; for daily group $b = .38$). Moreover, the genotype $\times$ medication $\times$ daily desire to drink interaction remained significant ($b = 0.37$, $SE = 0.14$, 95% CI: 0.08 to 0.65 , $p = 0.01$) in the presence of the additional product terms.

Because there were significant medication condition and genotype differences for reporting days, education, and pretreatment drinking days, we also estimated several supplemental models to rule out possible confounding effects. Specifically, we re-estimated the model tested above including each of these variables as an additional predictor (in separate models) and modeled its interactive effect with daily desire to drink. Results indicated that neither the daily desire to drink $\times$ education interaction ($b = -0.01$, $SE = 0.01$, 95% CI: -0.03 to 0.01 , $p = 0.31$) nor the daily desire to drink $\times$ completed reporting days interaction ($b = -0.002$,

SE = 0.002, 95% CI: -0.005 to 0.001, $p = 0.31$) was significant. Moreover, inclusion of these terms did not affect the significance of the genotype $\times$ daily desire to drink interaction.

In contrast, the daily desire to drink $\times$ pretreatment drinking days interaction was significant ($b = -0.009$, SE = 0.002, 95% CI: -0.012 to -0.005, $p < 0.001$), indicating that the association between daily changes in desire to drink and nighttime drinking was less positive for individuals who drank more often during pretreatment. Inclusion of this interaction rendered the genotype $\times$ daily desire to drink interaction marginally significant ($p = 0.06$). However, the 3-way interaction of pretreatment drinking days, medication, and daily desire to drink, which was the primary analysis of interest, was not significant ($b = 0.004$, SE = 0.003, 95% CI: -0.001 to 0.01, $p = 0.13$) and the genotype $\times$ medication condition $\times$ daily desire to drink interaction remained significant in this model ($b = 0.22$, SE = 0.11, 95% CI: 0.01 to 0.438, $p = 0.04$), indicating that these effects were not confounded by the baseline difference in the desire to drink $\times$ pretreatment drinking days interaction.

Discussion

The findings reported here provide evidence that the Asp40 allele of *OPRM1* moderates the relation between desire to drink in the evening and subsequent drinking behavior that night. These findings are consistent with a number of studies showing greater reactivity to alcohol-related cues and alcohol administration in carriers of the Asp40 allele. However, the findings contrast with those from the only other study that we are aware of examining the moderating effect of the Asn40Asp SNP on the relation between desire to drink and subsequent drinking (Ray et al. 2010). Specifically, Ray et al. (2010) found that urge to drink (measured using EMA after the first two drinks of a drinking episode) was positively associated with subsequent drinking the same day and that Asp40 allele carriers consumed more alcohol than Asn40 homozygotes. However, they found that urge to drink within the drinking episode was less strongly associated with the number of drinks consumed in Asp40 carriers than Asn40 homozygotes. The difference in the findings between the two studies may be due to the fact that Ray et al.'s subjects had all recently consumed one or two drinks, in contrast to the subjects in the present report for whom drinking prior to the evening report occurred on about 38% of days, though on those days they consumed a mean of approximately 3 drinks. Further, the subjects in our study who drank prior to reporting their desire to drink may have done so hours before, further limiting the comparison of findings between the two studies.

There are other methodological differences that may also help to explain the different findings in these studies. First, subjects in our study were treatment-seeking individuals, more than 90% of whom met DSM-IV criteria for alcohol dependence and approximately 30% of whom had previously received alcohol treatment. These individuals drank on more than 85% of days during the pretreatment period, drinking heavily on more than 60% of days. In contrast, although of the heavy drinkers studied by Ray et al. (2010), 61% met criteria for alcohol dependence, subjects were recruited to exclude those seeking alcohol treatment or who had ever received such treatment. During the pretreatment period, they drank on less than 65% of days and drank heavily on less than 45% of days. Thus, overall, the subjects we studied were more severely affected individuals, in whom the moderating effect of genotype on the relation between desire to drink and drinking behavior could differ from the less severely affected individuals in the study by Ray et al. (2010).

Our findings with respect to the impact of naltrexone on the relation between desire to drink and subsequent drinking, as moderated by the Asn40Asp SNP, adds to a unique dimension to the growing, but controversial, literature on the pharmacogenetics of alcohol drinking and dependence treatment (Oslin et al. 2003, Anton et al. 2008, McGeary et al. 2006, Gelernter

et al. 2007, Mitchell et al. 2007, Ray and Hutchison 2007, Tidey et al. 2008, Kim et al. 2009, Ooteman et al. 2009, Setiawan et al. 2011, Collier et al. 2011). Interestingly, we found no overall evidence of moderation by the SNP on drinking or on the attenuating effects of naltrexone on drinking in the clinical trial. Nor did we find effects of naltrexone or genotype on the mean relation between desire to drink and drinking (data not presented). These null findings may reflect the modest size of the overall effect of the SNP on drinking and its attenuation by naltrexone and the limited sample size and attendant statistical power, which is often a limiting factor in pharmacogenetic studies. However, the moderating effects of the SNP are evident when more proximal relations (i.e., between evening desire and nighttime drinking) were examined. This suggests that a within-person approach to phenotyping provides insights that are not evident in traditional measures used in clinical trials, which are the platform on which pharmacogenetic studies are generally conducted.

The biological basis for the findings reported here are consistent with some, but not all, of the molecular functional effects that have been seen with the Asn40Asp SNP (Kranzler and Edenberg 2010, Ray et al. 2011). Our findings are most consistent with a gain-of-function of the MOR encoded by the variant allele. That is, in the context of a greater desire to drink, the effects of alcohol are more rewarding because of greater reward mediated by the opioidergic system. Presumably, Asp40 allele carriers have learned of this effect through prior experience, resulting in the observed greater alcohol consumption during periods of greater alcohol urges. It is under such circumstances that naltrexone blockade of the MOR would be most efficacious, as reflected in the drug's effects relative to those of an inactive placebo in the Asp40-allele carriers.

Limitations of the present study include the fact that it uses reports of nighttime drinking that were obtained the next day. However, such recall bias is likely to be modest. Despite balanced randomization, there were group differences in age and pretreatment drinking frequency (and in the number of daily reports provided by the groups). In the absence of a consistent pattern of such differences, however, it is not possible to ascertain whether they may have biased the observed effects. The sample provided only limited statistical power to analyze schedule of administration as a factor in the four-way interaction. Larger studies that use this approach are needed to ensure adequate power for all study variables in the analysis. Although there were no effects of genotype or medication when either desire to drink or drinking behavior were considered as mean values across subjects, such effects are small and large sample sizes are required to detect such effects. The comparatively small sample sizes in most pharmacogenetic studies of naltrexone may help to explain some of the variability in results that have been reported. Based on the findings reported here, we believe that the use of a daily design, which by including many repeated measures across days of the study, afforded us greater statistical power to test interesting theoretical questions regarding medication effects in subgroups of patients. Such an approach may have important implications for the design of pharmacogenetic studies, which aim to personalize the treatment of a variety of psychiatric and medical disorders.

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Figure 1.

Three-way interaction of daily desire to drink, Asn40Asp genotype and medication condition in predicting nighttime drinking. High and low daily desire ratings correspond to ± 2 standard deviations from the mean.

Table 1
Demographics, Pretreatment Drinking, and Frequency of Complete Reports During Treatment

	Naltrexone		Placebo		<i>p</i> -value
	Asp40 carriers	Asn40/Asn40	Asp40 carriers	Asn40/Asn40	
N	22	59	14	63	.18
Male (%)	55	54	79	59	.41
European American (%)	100	97	100	95	.64
Age (yr)	49.5 (8.2)	50.0 (9.0)	47.0 (12.3)	48.7 (9.6)	.71
Education (yr)	15.2 (2.6)	16.1 (2.3)	14.0 (1.6)	15.2 (2.4)	.02
Pretreatment DDs (%)	88.7 (15.9)	87.9 (14.6)	77.8 (21.2)	91.4 (13.0)	.02
Pretreatment HDDs (%)	66.2 (25.9)	57.4 (28.7)	53.4 (28.5)	64.8 (25.1)	.24
Complete days of reporting	68.6 (15.1)	56.6 (23.8)	67.0 (11.2)	63.6 (18.8)	.05

DDs, Drinking Days; HDDs, Heavy Drinking Days

Note. *p*-values are for omnibus tests across the four subgroups. Numbers in parentheses are SDs. Complete days of reporting are daily observations for which daytime desire to drink and drinking are paired with the next day's report of the previous night's drinking (maximum=83 days).

Table 2

Results for the Full Model Predicting Nighttime Drinking

Step	Parameter	B	SE	95% CI		p-value
				Lower	Upper	
1	Sunday	-.223	.044	-.309	-.137	<.001
	Monday	-.250	.052	-.353	-.148	<.001
	Tuesday	-.217	.051	-.316	-.117	<.001
	Wednesday	-.134	.053	-.238	-.030	.011
	Thursday	-.130	.047	-.223	-.038	.006
	Friday	-.035	.037	-.107	.037	.338
	Mean Desire to Drink	.408	.072	.267	.548	<.001
	Daytime Drinks	.036	.014	.008	.063	.010
	Daily Desire to drink	.248	.030	.189	.306	<.001
	<i>OPRM1</i> Asn40Asp Genotype	-.022	.110	-.237	.193	.839
	Medication	.026	.102	-.174	.226	.801
2	Daily Desire × Genotype	-.174	.080	-.330	-.017	.030
	Daily Desire × Medication	.024	.056	-.087	.134	.675
	Genotype × Medication	.164	.214	-.256	.585	.444
3	Daily Desire × Genotype × Medication	.377	.145	.093	.660	.009

Note: Coefficients are from the block of entry.