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Effects of Opioid Receptor Gene Variation on Targeted Nalmefene Treatment in Heavy Drinkers

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Abstract

Background—Recent studies examining the moderating effects of polymorphic variation in opioid receptor genes have yielded conflicting results. We examined opioid receptor gene polymorphisms as moderators of the therapeutic effects of the opioid antagonist nalmefene.

Methods—Participants (n=272) were subjects who consented to the pharmacogenetic analysis of a multi-site, randomized, placebo-controlled trial of targeted nalmefene for the reduction of heavy drinking. We genotyped two single nucleotide polymorphisms (SNPs) in *OPRM1* (including A118G, a commonly studied SNP that encodes an Asn40Asp amino acid substitution), two SNPs in *OPRD1*, and one SNP in *OPRK1*, which encode the μ -, δ -, and κ -opioid receptors, respectively. Regression analysis served to examine the moderating effects of these SNPs on medication response.

Results—As previously described by Karhuvaara et al. (2007), nalmefene significantly reduced the number of heavy drinking and very heavy drinking days per week, compared with placebo. There were no main or moderating effects of the genotypes examined on these outcomes.

Conclusions—Our finding that the therapeutic effects of targeted nalmefene were not moderated by polymorphic variation in opioid receptor genes is consistent with two recent reports showing that variation in opioid receptor genes does not moderate the response to naltrexone. However, these findings contrast with those from two other studies, in which the Asn40Asp polymorphism in *OPRM1* moderated the naltrexone treatment response. Additional research is needed to clarify the role of variation in opioid receptor genes on the response to opioid antagonist treatment of alcoholism.

Keywords

Alcohol treatment; pharmacogenetics; *OPRM1*; *OPRK1*; *OPRD1*; nalmefene

In 1994, the US Food and Drug Administration (FDA) approved the opioid receptor antagonist naltrexone as an oral formulation to treat alcohol dependence. In 2006, a long-acting, injectable formulation of naltrexone was also approved for this indication. Although naltrexone has been shown to be superior to placebo in the treatment of alcohol dependence,

a substantial number of patients still do not respond to treatment with the medication (Garbutt et al., 2005; Srisurapanont and Jarusuraisin, 2005).

Efforts to identify moderators of naltrexone response have shown a family history of alcohol dependence to be the most consistent clinical predictor (Monterosso et al., 2001; Rubio et al., 2005; Volpicelli et al., 1995). Recently, pharmacogenetic studies have been used in an effort to identify alcohol-dependent individuals who are most likely to benefit from naltrexone treatment. In this regard, the μ -opioid receptor (genetic locus *OPRM1*), which is the primary pharmacologic target for opioid antagonists, has been of greatest interest.

The most widely studied polymorphism in *OPRM1* is A118G, a single nucleotide polymorphism (SNP) that encodes an amino acid substitution (Asn40Asp), which has an impact on receptor binding or expression (Befort et al., 2001; Beyer et al., 2004; Bond et al., 1998; Zhang et al., 2005). Bond et al. (1998) found that the receptor encoded by the Asp40 allele had a three-fold greater binding of the endogenous opioid β -endorphin (but no other endogenous or exogenous opioid ligands, including naloxone) than the receptor encoded by the Asn40 allele. In contrast, Befort et al. (2001) found no difference in the receptor's affinity for various exogenous or endogenous opioids (including β -endorphin), but reduced receptor expression of the variant allele in a mammalian cell line. Beyer et al. (2004) also found reduced expression of the receptor, but no functional change associated with the variant allele. Zhang et al. (2005) found the Asp40 allele to be associated with reduced mRNA and protein levels in both human post-mortem tissue and transfected Chinese hamster ovary cells. In post-mortem brain tissue from humans heterozygous for the Asn40Asp SNP, Zhang and colleagues found the mRNA from the A118 allele to be 1.5–2.5 times more abundant than from the G118 allele. The observed allelic expression imbalance was confirmed in Chinese hamster ovary cells using a paradigm that compared expression of mRNA based on transfection of the *OPRM1* coding sequence with A118, C118, T118, or G118, with a difference from the wild type A118 found only with the G118 allele.

Although two meta-analyses of case-control studies showed no evidence of an etiologic association of the Asp40Asn polymorphism to substance dependence (Arias et al., 2006), several studies have shown the polymorphism to moderate naloxone-induced activation of the HPA axis in humans (Chong et al., 2006; Hernandez-Avila et al., 2003; Wand et al., 2002). Hernandez-Avila et al. (2007), in a naloxone challenge study conducted in a sample selected to have a high proportion of Asp40 allele carriers, found that an altered cortisol response was associated with the Asp40 allele in subjects of European ancestry, but not those of Asian ancestry.

In a study of intravenous ethanol administration in heavy drinkers, individuals with the Asp40 allele experienced a more intense “high,” and greater subjective intoxication, stimulation, sedation, and happiness from alcohol, and were more likely to report a family history of alcohol use disorders than did Asn40 homozygotes (Ray and Hutchison, 2004). Further, male heavy drinkers with the Asp40 allele reported higher levels of craving when exposed to an alcohol stimulus than did Asn40 homozygotes (van den Wildenberg et al., 2007). However, cue-elicited craving was paradoxically increased in Asp40 allele carriers in a sample of heavy drinkers who were pretreated with naltrexone, while no change in craving was noted for Asn40 homozygotes (McGeary et al., 2006).

Other opioid receptor gene variants have also been examined in relation to the risk for substance dependence. Zhang et al. (2006), in a haplotypic analysis of the risk for alcohol or drug dependence, genotyped 12 intronic SNPs and 1 exonic SNP (Asn40Asp) in *OPRM1* and identified two haplotype blocks, one of which was significantly more common in cases (with alcohol or drug dependence), and another that was significantly more common in

controls. Although the Asn40Asp SNP was in complete linkage disequilibrium with most of the intronic SNPs in one haplotype block, allelic analysis revealed that it was not associated with substance dependence in this sample. Two other studies of *OPRM1* showed an association of substance dependence with haplotypes found mainly in the putative regulatory region of the gene (Hoehe et al., 2000; Luo et al., 2003). An association to substance dependence was also reported for an intronic polymorphism in *OPRM1* (Kranzler et al., 1998).

To date, there have been three studies examining the moderating effect of the Asn40Asp polymorphism on naltrexone treatment response in alcohol dependence and one study in a sample of non-treatment-seeking heavy drinkers. Oslin et al. (2003) studied 130 European American (EA) subjects from three placebo-controlled trials. They found that, among patients treated with naltrexone, those with one or more Asp40 alleles were significantly less likely than Asn40 homozygotes to relapse to heavy drinking. Placebo-treated subjects showed no moderating effect of genotype. Gelernter et al. (2007) examined the moderating effect of polymorphic variation in opioid receptor genes on treatment response in a subset of patients (n=220, 73.6% EAs and 26.4% AAs) from the VA Cooperative Study of Naltrexone Treatment (Krystal et al., 2001). In addition to the Asn40Asp polymorphism, these investigators studied two other *OPRM1* SNPs, three markers in *OPRD1* (which encodes the δ -opioid receptor), and one marker in *OPRK1* (which encodes the κ -opioid receptor). They found no significant interaction between any of the SNPs studied and the response to naltrexone treatment. Anton et al. (2008) examined the moderating effect of the Asn40Asp polymorphism on the response to naltrexone treatment in a sub-sample of subjects from the COMBINE study (Anton et al., 2006). Subjects included in the primary pharmacogenetic analysis (n=297) were EAs who were treated with naltrexone or placebo and medication management. A positive moderating effect on the response to naltrexone was observed for carriers of the Asp40 allele on the percentage of heavy drinking days and on a global measure of treatment outcome. Secondary analyses, which included subjects from all racial groups, as well as those receiving treatment with naltrexone or placebo and an intensive behavioral intervention, showed no moderating effect of the Asn40Asp polymorphism. In a brief cross-over trial of naltrexone versus placebo in a group of non-treatment-seeking heavy drinkers (n=30, 77.8% EA), no difference in treatment response was observed for those with the Asp40 allele (Mitchell et al., 2007).

Nalmefene, a specific and potent opioid antagonist, has affinity for the three opioid receptor subtypes, all of which have been implicated in the pathophysiology of alcohol dependence (Oswald and Wand, 2004). Nalmefene has a potential therapeutic advantage over naltrexone owing to its lack of hepatic toxicity, longer half-life, and greater bioavailability (Ingman et al., 2005; Mason et al., 1999). Nalmefene's affinity for the μ -, and κ -opioid receptors is similar to that of naltrexone, though its affinity for the δ -opioid receptor is greater than naltrexone's (Emmerson et al., 1994; Michel et al., 1985). A single 50-mg oral dose of nalmefene completely blocked respiratory depression, analgesia, and subjective effects of fentanyl for 48 hours (Gal et al., 1986). In alcohol-dependent individuals, nalmefene treatment reduced drinking during a 5-day natural observation period, as well as after a priming dose of alcohol in a bar-laboratory paradigm (Drobes et al., 2004). Of the three studies of daily nalmefene treatment of alcohol dependence, two showed lower rates of relapse to heavy drinking, more abstinent days/week, and fewer drinks per drinking day in the nalmefene-treated patients compared with those receiving placebo. (Anton et al., 2004; Karhuvaara et al., 2007; Mason et al., 1994; Mason et al., 1999).

In the present study, we examined the moderating effect of opioid receptor gene variants in subjects from the 28-week, multi-center trial by Karhuvaara et al. (2007), the primary aim of which was to test the efficacy and safety of nalmefene when used on an "as needed" basis to

reduce heavy drinking. That study enrolled a total of 403 subjects, who were included based on their self-identification of drinking problems rather than a formal diagnosis of alcohol dependence and no treatment goals were imposed on them. The study showed that targeted nalmefene was significantly better than placebo in reducing heavy drinking days, very heavy drinking days, and drinks per drinking day, and in increasing abstinent days. We hypothesized that one or more SNPs in the genes encoding opioid receptors that had previously been identified as putatively functional would moderate the effects of nalmefene on drinking outcomes. To that end, we examined the interactive effects of five SNPs in the genes encoding the μ -, δ -, and κ -opioid receptors on drinking outcomes following treatment with nalmefene or placebo.

METHODS

The study protocol and consent materials (both of which included a description of the pharmacogenetic substudy) were approved by ethics committees at all of the 15 participating clinical sites in Finland. Institutional review boards at the University of Connecticut Health Center, Yale University, and VA-Connecticut also approved the pharmacogenetic substudy. The methodology for the treatment trial is described in detail in Karhuvaara et al. (2007); a brief summary is provided here.

Recruitment

Subjects were recruited mainly through newspaper advertisements. Following a preliminary telephone interview, eligible subjects were invited to the nearest recruiting site for a screening visit. Inclusion criteria were age ≥ 18 , ≤ 14 consecutive days of abstinence and ≥ 18 heavy drinking days during the preceding 12 weeks, the absence of intoxication and severe withdrawal symptoms at the enrollment visit, a stable address and telephone number, and an identified locator person. Exclusion criteria included the presence of any severe medical, psychiatric or social problem that required resolution or that would interfere with the conduct of the study or impair treatment compliance; drug dependence or illicit drug use; previous participation in studies of nalmefene or recent participation in other drug studies; recent treatment with disulfiram or naltrexone; and current pregnancy or nursing.

Assessments

Following medical and psychiatric screening, including clinical laboratory testing, subjects' drinking during the 90 days prior to enrollment was quantified using the Timeline Follow-Back method (TLFB; Sobell and Sobell, 1992). Subjects also completed the Alcohol Dependence Scale (ADS; Skinner and Allen, 1982), Drinker Inventory of Consequences (DrInC; Miller et al., 1995), Beck Depression Inventory (BDI; Beck et al., 1996), and the Beck Anxiety Inventory (BAI; Beck and Steer, 1993). The Structured Clinical Interview for DSM-IV (SCID-I; First et al., 2001) was used to characterize the subjects' alcohol problems at the outset of the study.

Medication Treatment

Eligible subjects were randomly assigned (in a 3:2 ratio) to nalmefene 20 mg or matching placebo groups using random permuted blocks. Subjects were instructed to take one tablet of the study medication 1–2 hours before any intake of alcohol, when drinking seemed imminent. Only one dose of study drug was allowed per day. After two weeks of treatment, the dose could be doubled (i.e., to 40 mg once daily) if the treatment response was considered by the investigator to be inadequate, or it could be halved due to adverse effects. During the 28-week treatment period, subjects returned to the study site nine times (initially weekly, then biweekly, and later monthly) for research assessments and medication

dispensing. At each visit, alcohol use and study medication intake since the previous visit were recorded using the TLFB and a report of adverse events was elicited.

At each visit, a brief psychosocial intervention based on the BRENDA model (Volpicelli et al., 2001) was provided to all subjects, the main emphasis of which was on the correct use of the medication. The intervention included biopsychosocial assessment, feedback to the subject on the assessment, simple advice to reduce alcohol drinking, and the monitoring of progress in treatment. No specific treatment goals were set.

Subjects

Complete data were available for 272 subjects (i.e., 67.3% of the subjects from the clinical trial, including 106 subjects from the placebo group and 166 subjects from the nalmefene group). Enrollment and blood samples for DNA began after the medication trial was underway, and subjects who had already dropped out by that time could generally not be reached or persuaded to give a blood sample, which is reflected in the significantly greater completion rate for subjects who participated in the pharmacogenetic sub-study (85%), compared with the 131 subjects who did not provide a sample for genetic analysis (21%) [$\chi^2(1)=152.8$, $p < .001$]. Table 1 shows the demographic and clinical characteristics of the participants in the pharmacogenetic sub-study, by medication group. All subjects were Caucasian and of Finnish ancestry and 80% were male.

Genes and Genotyping

DNA was extracted from whole blood using standard methods. Some of the SNPs (Table 2) examined were possible functional variants (*OPRM1*: rs1799971, rs648893, *OPRK1*: rs963549); the other SNPs (*OPRD1*: rs678849, rs2234918) were selected because they were genotyped in previous studies examining their association to substance dependence ((Gelernter, 2000; Luo et al., 2003; Zhang et al., 2006); described in more detail in (Gelernter et al., 2007)).

Four of the SNPs (rs1799971, rs648893, rs678849, and rs2234918) were genotyped using the TaqMan fluorogenic 5' nuclease assay (Livak et al., 1995) and the ABI PRISM 7900 Sequence Detection System (ABI, Foster City, CA, USA). All Taqman reactions were run in duplicate using 2ng of DNA and with 100% concordance. *OPRK1* rs963549 was genotyped as an RFLP using 10ng of DNA, 0.5M Betaine, PC and KlenTaq; cycling parameters were 95° C for 5 min followed by 35 cycles of 95°-53°-72°- 30s/30s/30s. PCR product was digested overnight with 10U of BstNI and run out on a 3% Metaphor agarose gel. At least 8% of genotypes were repeated for quality control, with complete concordance.

Statistical Analysis

The number of heavy drinking days (HDD) per week (during the 28-week treatment period), defined as a day on which men consumed ≥ 5 standard drinks and women consumed ≥ 4 drinks, was the primary efficacy variable in the treatment trial. A standard drink contained approximately 12 grams of ethanol. Secondary efficacy variables included the weekly number of days of abstinence (ABS) and of very heavy drinking days (VHDD: men: ≥ 10 drinks; women: ≥ 8 drinks).

Hierarchical multiple regression was used to test the interactive effects of genotype and treatment. To ensure an adequate number of subjects in all subgroups, for each of the SNPs the minor allele homozygote group was combined with the heterozygote group to yield a binary genotype variable. However, for SNPs rs2234918 and rs678849, where the genotype subgroups had adequate numbers, we also used two dummy codes to contrast the more common homozygote to both the less common homozygote and the heterozygote group.

Covariates (i.e., gender, family history of alcoholism, number of previous alcohol treatments, the number of DSM-IV criteria met, ADS score, BDI total score, and BAI total score) were included in the regression models if they demonstrated a significant bivariate association with the dependent measures. We also covaried the respective pretreatment drinking measure (i.e., number of heavy drinking days, very heavy drinking days, and abstinent days per week in the 90 days prior to randomization) in all analyses. Thus, significant effects can be interpreted as accounting for variance in residual change in the outcomes from baseline to the end of treatment.

We entered the predictors in three blocks: first we entered the control variables and the treatment condition dummy code (0 = control, 1 = treatment). Second, we entered all five genotype dummy codes. We chose this strategy (a) because the genotype dummy codes were virtually uncorrelated (of 10 pair wise associations, only three genotypes were significantly related; the strongest association was between rs648893 and rs1799971, $\phi = .318$) and (b) to control the Type I error rate (i.e., we only interpreted unique effects if the omnibus test for the change in r^2 was significant). In the third block, we entered the five treatment $\times$ genotype product terms to test for the interactive effects. Again, to protect against inflating the Type I error rate, unique effects were only examined in the context of a significant change in r^2 . Regarding effect size, we report squared semi-partial correlations (i.e., Δr^2 due to entry of the individual predictor) and 95% confidence intervals for the unstandardized partial regression coefficients.

RESULTS

A breakdown of pretreatment variables is shown in Table 1. There were significant baseline differences in abstinent days and a trend toward a significant difference in the number of heavy drinking days between the treatment groups. This possible bias was handled by the baseline correlation analysis.

Results from the multiple regression analysis for heavy drinking days are shown in Table 3. The analyses did not differ substantially for the other outcome measures, particularly when the potential for Type 1 error was accounted for. Consequently, the data are not shown for the analysis of VHDD or ABS. Block one accounted for a significant amount of variance for all three outcomes. The pretreatment drinking covariate was a significant predictor in all models. Treatment condition was a significant predictor in the HDD and VHDD models, but not in the ABS model. Given the coding of treatment condition, the coefficients shown in Table 3 represent the covariate-adjusted mean differences in HDD per week during treatment.

Entry of blocks two (the genotype dummy codes) and three (the treatment $\times$ genotype product terms) into the models did not result in significant changes in r^2 . As a check, we re-estimated the models examining each genotype separately along with its interaction with medication condition. Specifically, these models included only the genotype of interest, medication condition, the treatment $\times$ genotype product term, and the corresponding pretreatment levels of drinking (the only significant covariate). The substantive findings were generally the same for this and the other outcome measures. Thus, the null findings for the treatment $\times$ genotype interactions were not due to redundancy among the predictors or multicollinearity.

We also modeled rs2234918 and rs678849 as 3-level nominal variables (using two dummy codes and two genotype $\times$ treatment interaction predictors); the findings did not change substantively. Finally, examination of the residuals from all three models showed no departure from normality. Some evidence of heteroscedastic residuals was found in the

VHDD model; however, results using a log-transformed variable were identical to those reported above.

DISCUSSION

We found no evidence that allelic variation in any of the three genes studied moderated the response to nalmefene treatment. Specifically, there was no evidence to support a treatment interaction with the Asn40Asp polymorphism (rs1799971), or the other polymorphisms that were examined (including rs2234918 and rs678849 in *OPRD1*, and rs963549 in *OPRK1*). The lack of a moderating effect of the Asn40Asp polymorphism differs from two of the four studies that have examined the moderating effect of this SNP on naltrexone treatment effects (Anton et al., 2008; Mitchell et al., 2007; Oslin et al., 2003).

There are a number of possible explanations for the lack of a moderating effect of the Asn40Asp polymorphism on the response to nalmefene treatment. The underlying hypothesis is based on an unclear mechanism of interaction between medication and genotype. Although there is consistent evidence to support an altered physiologic response to naloxone based on the presence of the Asn40Asp polymorphism (Chong et al., 2006; Hernandez-Avila et al., 2007; Hernandez-Avila et al., 2003; Wand et al., 2002), a similar effect for naltrexone or nalmefene has yet to be demonstrated. Further, although the structure of nalmefene is similar to that of naltrexone, and the general mechanism of action in alcohol dependence is thought to be the same (Mason et al., 1999), the genetic moderators of the effects of these medications may differ as a result of their subtle but potentially significant pharmacokinetic and pharmacodynamic differences. It is also possible that the schedule of administration (i.e., targeted in the nalmefene study, daily in the studies of naltrexone) could have influenced the moderating effect of opioid receptor genotype on treatment outcome. The medication may need to be present and pharmacologically active on a daily basis in order to provide a pharmacogenetic effect on drinking outcomes. It has also been proposed that the duration of treatment (i.e., the initial “acute” phase of several weeks vs. longer term treatment), and the expectation that pharmacogenetic effects will be most prominent early in treatment, with the effect not being evident over the course of a treatment trial, may explain the lack of findings of genetic moderators of treatment with opioid antagonists (Gelernter et al., 2007). Specifically, neuroadaptive changes in opioid receptor regulation due to chronic exposure may result in tolerance to some aspects of the response to opioid antagonists, which could include pharmacogenetic effects (Lesscher et al., 2003).

The use of “tag” SNPs representing haplotype blocks in *OPRM1* that have been associated with risk of alcohol and drug dependence and the inclusion of SNPs in both *OPRD1* and *OPRK1* provide good representation of the opioid gene variation that could moderate the response to nalmefene treatment. However, in this analysis only opioid receptor genes were examined, so that other genetic effects were not detectable. The Finnish population examined in this study is an isolated one that is ancestrally homogeneous, even compared to other European populations (Finnish Genome Center, www.genome.helsinki.fi). As much of the pharmacogenetic research with Asn40Asp and other opioid gene variants has been in more heterogeneous European American populations, it is possible that there are trans-acting genetic effects specific to the Finnish population that could obscure a pharmacogenetic effect.

A factor that could potentially explain the discrepancy in the findings reported here and studies of the moderating effects of the Asn40Asp polymorphism on the response to naltrexone treatment of alcohol dependence is that the targeted nalmefene study did not require a goal of abstinence. This is similar to the study by Mitchell et al. (2007), but in contrast to the studies by Oslin et al. (2003) and Anton et al. (2008), which had total

abstinence as their treatment goal. This interpretation is not supported by the findings of Gelernter et al. (2007), whose subjects were drawn from the VA Cooperative Study of Naltrexone, the goal of which was abstinence. In view of recent evidence that variation in GABA-A subunit genes may influence the response to the psychotherapeutic treatment of alcoholism (Bauer et al., 2008), it is possible that the different psychosocial treatments in the studies examining the moderating effects of opioid gene variation on medication response could have confounded the pharmacogenetic effects.

The strengths of the present study are the homogeneous sample, the positive finding of a medication treatment effect (which provides a clearer basis for a pharmacogenetic effect), and the examination of polymorphisms in all three opioid receptor genes. Despite these strengths, detection of pharmacogenetic effects may require greater statistical power than was afforded by a comparatively small sample size. Using data from Oslin et al. (2003), which compared the risk of relapse to heavy drinking within the group treated with naltrexone, we estimated the effect size to be .695 (Chinn, 2000), which is a medium effect size (Cohen, 1988). Using a similar approach, data from Anton et al. (2008) on the likelihood of a good clinical outcome showed an effect size of .966 for the naltrexone in the Asp40 group. The analysis we conducted for heavy drinking days/week had power of $>.8$ to detect an effect size of $>.6$, with $\alpha = .05$ and as many as 12 regressors, thus arguing against type II error. Reducing the number of covariates (e.g., using only medication, genotype, and pre-treatment heavy drinking days) yields higher power to detect an effect size of this magnitude. However, given the possibility that the true pharmacogenetic effect for nalmefene could be smaller than that for naltrexone, we acknowledge that type II error could explain the lack of a pharmacogenetic effect in this analysis.

In addition to reducing the size of the sample, the potential exists for bias to have resulted from the fact that only two-thirds of the participants in the clinical trial by Karhuvaara et al. (2007) provided blood for genetic analysis. The difference in study completion rates between subjects in the pharmacogenetic analysis and those who did not offer blood samples was considerable (85% vs. 21%), and suggests that the subset of subjects included in the pharmacogenetic analysis were more adherent to treatment. Since greater adherence to and/or motivation to participate in treatment may lead to better outcomes in treatment trials, even when patients receive a placebo, it is possible that this may have obscured a pharmacogenetic effect (Oslin et al., 2002). However, the fact that the pharmacogenetic analysis subset showed a robust medication treatment effect versus placebo argues against such confounding. The greater exposure to medication in the subset of subjects participating in the pharmacogenetic analysis also supports the validity of our findings, as these subjects would have likely received enough exposure to the medication to demonstrate a gene by medication treatment interaction. The frequency of the Asp40 allele in the pharmacogenetic sample was higher than the range of frequencies seen in other European populations (Arias et al., 2006) and could reflect differential attrition in the sample participating in the pharmacogenetic substudy. The presence of a clear pharmacological effect in the subsample for which we have genetic information argues against a relevant bias. Although the targeted medication approach used in the nalmefene study could make detection of a pharmacogenetic effect more difficult, it is unclear why this would not apply equally to the detection of a pharmacological effect.

Pharmacogenetic analysis of treatment trials in alcohol dependence has focused on the moderating effects of opioid receptor gene variation. It appears from the variable findings obtained in these studies that additional studies of multiple opioid receptor gene variants are required to clarify the genetic basis for treatment response to naltrexone and nalmefene. In addition, the study of other genes that may influence the response to pharmacological treatment of alcoholism is a fertile area of investigation. There is growing evidence that

variation in *GABRA2*, which encodes the alpha-2 subunit of the GABA-A receptor, is associated with alcohol dependence (Covault et al., 2004; Edenberg et al., 2004; Enoch et al., 2006; Fehr et al., 2006; Lappalainen et al., 2005; Soyka et al., 2007). Variation in that gene has also been shown to moderate the subjective response to acute alcohol administration (Pierucci-Lagha et al., 2005) and to predict drinking behavior as both a main effect and in combination with specific psychotherapies in Project MATCH (Bauer et al., 2008). To date, however, there are no published studies of the role of this gene in predicting the response to pharmacotherapy. Other genes that could moderate the response to alcoholism treatment include dopaminergic, serotonergic, glutamatergic, cholinergic, endocannabinoidergic, and neuroendocrine-related genes (Edenberg and Kranzler, 2005; Gelernter and Kranzler, in press). Studies of the molecular significance of variation in these genes, as well as their functional evaluation in human laboratory studies, will help to determine their potential as moderators of the response to alcohol pharmacotherapy.

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Table 1Demographic and clinical characteristics by treatment condition.¹

	Nalmefene (n=166)	Placebo (n=106)	Statistic	P-value
Age [mean yr (SD)]	49.8 (9.1)	49.3 (8.8)	F(1,270)=.22	P=.64
Women (%)	32 (19.3%)	20 (18.9%)	$\chi^2 = .01$, df=1	P=.93
Family history of alcoholism (%)	92 (55.4%)	63 (59.4%)	$\chi^2 = .43$, df=1	P=.52
Previous alcohol treatments (#)	1.3 (2.9)	1.5 (3.7)	F(1,270)=.28	P=.60
Number of DSM-IV alcohol dependence symptoms)	6.6 (1.6)	6.8 (1.4)	F(1,270)=.53	P=.47
Alcohol Dependence Scale score	17.5 (6.9)	18.3 (7.2)	F(1,270)=.83	P=.36
Heavy drinking days ¹ per week	3.8 (1.7)	4.2 (1.7)	F(1,270)=3.81	P=.052
Very heavy drinking days ² per week	2.0 (1.8)	2.1 (1.7)	F(1,270)=.08	P=.77
Abstinent days per week	2.5 (1.7)	2.1 (1.7)	F(1,270)=3.94	P=.048
Beck Depression Inventory score	13.8 (7.5)	14.2 (8.6)	F(1,270)=.14	P=.71
Beck Anxiety Inventory score	9.2 (7.5)	11.0 (10.0)	F(1,270)=2.78	P=.097

* Values are mean (SD) or number (%)

¹ Days on which men consumed ≥ 5 and women consumed ≥ 4 standard drinks

² Days on which men consumed ≥ 10 and women consumed ≥ 8 standard drinks

Table 2

Genotype and Allele Frequencies as a Function of Medication Group

	<u>Placebo</u> <u>(n=106)</u>	<u>Nalmefene</u> <u>(n=166)</u>	<u>Statistic</u>	<u>P-value</u>
<u><i>OPRM1</i> rs648893</u>				
AA (%)	66 (62.3%)	103 (62.1%)	$\chi^2 = .008$, df=2	P=.996
AG (%)	37 (34.9%)	58 (34.9%)		
GG (%)	3 (2.8%)	5 (3.0%)		
f(A)	.80	.80	$\chi^2 = .003$, df=1	P=.995
f(G)	.20	.20		
<u><i>OPRD1</i> rs2234918 (T921C)</u>				
AA (%)	42 (39.6%)	59 (35.5%)	$\chi^2 = .1.29$, df=2	P=.526
AG (%)	45 (42.5%)	82 (49.4%)		
GG (%)	19 (17.9%)	25 (15.1%)		
f(A)	.61	.60	$\chi^2 = .02$, df=1	P=.888
f(G)	.39	.40		
<u><i>OPRM1</i> rs1799971 (Asn40Asp)</u>				
AA (%)	59 (55.7%)	108 (58.1%)	$\chi^2 = 2.412$, df=1	P=.120
AG (%)	43 (40.6%)	53 (28.5%)		
GG (%)	4 (3.7%)	25 (13.4%)		
f(A)	.76	.72	$\chi^2 = .917$, df=1	P=.338
f(G)	.24	.28		
<u><i>OPRD1</i> rs678849</u>				
TT (%)	28 (26.4%)	32 (19.3%)	$\chi^2 = 2.012$, df=2	P=.366
TC (%)	54 (50.9%)	90 (54.2%)		
CC (%)	24 (22.6%)	44 (26.5%)		
f(T)	.52	.46	$\chi^2 = 1.57$, df=1	P=.211
f(C)	.48	.54		
<u><i>OPRK1</i> rs963549</u>				
TT (%)	74 (69.8%)	131 (78.9%)	$\chi^2 = 3.815$, df=2	P=.149
TC (%)	31 (29.2%)	32 (19.3%)		
CC (%)	1 (1.0%)	3 (1.8%)		
f(T)	.84	.89	$\chi^2 = 1.94$, df=1	P=.164
f(C)	.16	.11		

Table 3

Multiple regression results for heavy drinking days per week

Block	F	Δ^2	p-value	Predictor	B	SE	t	p-value	Lower	Upper	Δ^2
1	19.752 ^a	0.271	<0.001	Pre-treatment HDD	0.440	0.050	8.877	<0.001	0.342	0.537	0.216
				ADS total score	-0.010	0.018	-0.540	0.590	-0.044	0.025	0.001
				BAI total score	0.010	0.013	0.749	0.455	-0.016	0.037	0.002
				AD criteria	0.069	0.065	1.071	0.285	-0.058	0.197	0.003
				Medication condition	-0.509	0.177	-2.874	0.004	-0.859	-0.160	0.023
2	0.663 ^b	0.009	0.652	rs648893 (0 = aa, 1 = ag+gg)	0.198	0.187	1.062	0.289	-0.169	0.566	0.003
				rs2234918 (0 = aa, 1 = ag+gg)	-0.014	0.184	-0.075	0.940	-0.377	0.349	0.000
				rs1799971 (0 = aa, 1 = ag+gg)	0.191	0.187	1.023	0.307	-0.177	0.560	0.003
				rs678849 (0 = tt, 1 = ct+cc)	-0.121	0.203	-0.597	0.551	-0.520	0.278	0.001
				rs963549 (0 = cc, 1 = ct+tt)	-0.227	0.204	-1.113	0.267	-0.628	0.174	0.003
3	0.704 ^c	0.010	0.621	Medication × rs648893	0.443	0.387	1.145	0.253	-0.319	1.206	0.004
				Medication × rs2234918	0.395	0.379	1.041	0.299	-0.352	1.142	0.003
				Medication × rs1799971	0.260	0.384	0.677	0.499	-0.497	1.017	0.001
				Medication × rs678849	0.029	0.427	0.068	0.946	-0.812	0.869	0.000
				Medication × rs963549	-0.284	0.412	-0.689	0.491	-1.095	0.527	0.001

Note. Values represent the effects of the block of interest. Df numerator for all F-ratios = 5; df denominator =

^a₂₆₆,

^b₂₆₁,

^c₂₅₆.

HDD=Heavy Drinking Days Per Week; ADS=Alcohol Dependence Scale; BAI=Beck Anxiety Inventory; AD criteria=current DSM-IV Alcohol Dependence criteria; Medication condition (0 = placebo, 1 = nalmefene).

Table 4

Interaction results from individual genotype models

<i>Dependent Variable</i>	<i>B</i>	<i>SE</i>	<i>t</i>	<i>p-value</i>	<i>95% CI for B</i>		<i>Δr²</i>
					<i>Lower</i>	<i>Upper</i>	
Medication × rs648893	0.363	0.361	1.007	0.315	-0.347	1.074	0.003
Medication × rs2234918	0.388	0.362	1.070	0.286	-0.326	1.101	0.003
Medication × rs1799971	0.106	0.362	0.292	0.771	-0.607	0.818	0.000
Medication × rs678849	-0.059	0.412	-0.142	0.887	-0.871	0.753	0.000
Medication × rs963549	-0.309	0.400	-0.772	0.441	-1.098	0.479	0.002

Note. Results for medication, genotype and pre-treatment drinking not shown. Df for all tests = 267.