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Pharmacogenetics of Naltrexone in Asian Americans: A Randomized Placebo-Controlled Laboratory Study

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Recent clinical and laboratory studies have shown that the effects of naltrexone for alcoholism may be moderated by the Asn40Asp single-nucleotide polymorphism (SNP) of the μ-opioid receptor gene (*OPRM1*). Allele frequencies for this polymorphism, however, have been shown to vary substantially as a function of ethnic background, such that individuals of Asian descent are more likely to carry the minor (Asp40) allele. The objective of this study is to test the naltrexone pharmacogenetic effects of the Asn40Asp SNP in a sample of Asian Americans. This study consists of a double-blinded, randomized, placebo-controlled laboratory trial of naltrexone. Participants ($n = 35$, 10 females; 13 Asn40Asn and 22 Asp40 carriers) were non-treatment-seeking heavy drinkers recruited from the community. After taking naltrexone or placebo, participants completed an intravenous alcohol administration session. The primary outcome measures were subjective intoxication and alcohol craving. Results suggested that Asp40 carriers experienced greater alcohol-induced sedation, subjective intoxication, and lower alcohol craving on naltrexone, as compared to placebo, and to Asn40 homozygotes. There results were maintained when controlling for *ALDH2* (rs671) and *ADH1B* (rs1229984) markers and when examining the three levels of *OPRM1* genotype, thereby supporting an *OPRM1* gene dose response. These findings provide a much-needed extension of previous studies of naltrexone pharmacogenetics to individuals of Asian descent, an ethnic group more likely to express the minor allele putatively associated with improved biobehavioral and clinical response to this medication. These findings help further delineate the biobehavioral mechanisms of naltrexone and its pharmacogenetics.

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INTRODUCTION

The opioidergic system has been associated with the pathophysiology of substance-use disorders, including alcoholism (Erickson, 1996; Herz, 1997; Kreek, 1996). Opioid receptors are involved in the rewarding properties of several substances, such as opiates, cocaine, and alcohol. Specifically, alcohol is thought to produce some of its reinforcing effects through the release of endogenous opioids in certain brain areas and through interactions with the dopaminergic system, particularly in the midbrain. Naltrexone is an opioid receptor antagonist, which has been shown to have the highest affinity for μ-opioid receptors (Littleton and Zieglgansberger, 2003). Naltrexone is one of three pharmacotherapies currently approved for the treatment of alcoholism in the United States.

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Results from clinical trials have supported the efficacy, albeit moderate, of naltrexone as a pharmacotherapy for alcohol dependence. Studies have found that naltrexone reduces the occurrence of heavy drinking days (Balldin *et al*, 2003; Monti *et al*, 2001; Rubio *et al*, 2002), increases time to first relapse (Anton *et al*, 1999; Guardia *et al*, 2002; Kiefer *et al*, 2003), yields lower relapse rates (Heinala *et al*, 2001; Latt *et al*, 2002; Volpicelli *et al*, 1992), reduces the number of drinking days (O'Malley *et al*, 1992; Volpicelli *et al*, 1992), the number of drinks per drinking episode (Chick *et al*, 2000; Guardia *et al*, 2002; Morris *et al*, 2001; O'Malley *et al*, 1992), and the latency between first and second drink among social drinkers (Davidson *et al*, 1996). More recently, a large multisite controlled trial has found that naltrexone was an effective treatment for alcohol dependence when delivered in combination with a medically oriented behavioral intervention (Anton *et al*, 2006). A few studies, however, have not found support for the efficacy of naltrexone (Killeen *et al*, 2004; Kranzler *et al*, 2000; Krystal *et al*, 2001).

Human laboratory studies have examined the biobehavioral mechanisms of action of naltrexone (Anton *et al*,

2004; Drobes *et al*, 2004; King *et al*, 1997; McCaul *et al*, 2001; Monterosso *et al*, 2001; Swift *et al*, 1994; Volpicelli *et al*, 1995). Results of such studies revealed that naltrexone dampens feelings of alcohol-induced stimulation (Drobes *et al*, 2004; Swift *et al*, 1994), decreases ratings of liking of the alcohol (McCaul *et al*, 2001), causes an increase in self-reported fatigue, tension, and confusion (King *et al*, 1997), reduces alcohol consumption, and slows down the progression of drinking in a delayed access laboratory paradigm (Anton *et al*, 2004). More recently, pharmacogenetic studies have focused on the gene coding for μ-opioid receptors (ie, *OPRM1* gene), which are the primary targets of naltrexone (Goldman *et al*, 2005; Oslin *et al*, 2003). One of the most widely studied polymorphisms of the *OPRM1* gene is the Asn40Asp single-nucleotide polymorphism (SNP) (rs1799971), as molecular studies have suggested that this substitution affects receptor activity for the endogenous ligand β-endorphin, leading to a gain in function, such that the Asp40 variant was thought to bind β-endorphin three times stronger than the Asn40 allele (Bond *et al*, 1998). Conversely, another study found the Asp40 allele to have deleterious effects on mRNA and protein yield, leading to a loss of function, rather than a gain (Zhang *et al*, 2005).

Several studies have tested the relationship between the Asn40Asp SNP of the *OPRM1* gene and substance-use disorders, particularly alcoholism and opioid dependence with inconsistent results (Arias *et al*, 2006; Bart *et al*, 2005; Bergen *et al*, 1997; Crowley *et al*, 2003; Franke *et al*, 2001; Gelernter *et al*, 1999; Kranzler *et al*, 1998; Loh el *et al*, 2004; Luo *et al*, 2003; Schinka *et al*, 2002; Shi *et al*, 2002; Tan *et al*, 2003; Town *et al*, 1999). Beyond the genetic association studies of diagnostic phenotypes, experimental studies have found that Asp40 carriers display a greater response to the effects of alcohol compared with individuals homozygous for the Asn40 allele, as measured by subjective intoxication, sedation and stimulation, and changes in mood states (Ray and Hutchison, 2004). A recent positron emission tomography study found that male Asp40 carriers had a stronger striatal dopamine response to intravenous alcohol as compared with Asn40 homozygotes (Ramchandani *et al*, 2011). Likewise, a functional neuroimaging study has found greater hemodynamic response in mesocorticolimbic areas both before and after alcohol priming among Asp40 carriers (Filbey *et al*, 2008). Taken together, these studies have shown that this polymorphism may moderate the reinforcing effects of alcohol and have elucidated the neurobiological mechanisms underlying these putative effects.

To the extent that this polymorphism moderates the reinforcing effects of alcohol and given naltrexone's blunting of alcohol reinforcement, this SNP represents a highly plausible moderator of naltrexone response. Consistent with this hypothesis, individuals with the Asp40 allele demonstrate enhanced hypothalamic-pituitary-axis (HPA) dynamics in response to an opiate blockade (Wand *et al*, 2002), enhanced cortisol response, and reduced agonist effect of morphine-6-glucuronide after treatment with naloxone (Hernandez-Avila *et al*, 2003), greater naltrexone-induced blunting of alcohol high (Ray and Hutchison, 2007a), and lower relapse rates in clinical trials of naltrexone for alcoholism (Anton *et al*, 2008; Oslin *et al*, 2003). A study of Korean alcohol-dependent patients found that carriers of the Asp40 allele took longer to relapse, but these effects were only observed among treatment adherent patients (Kim *et al*, 2009). Some studies, however, have failed to support this pharmacogenetic effect (Gelernter *et al*, 2007; Tidey *et al*, 2008).

Importantly, studies have highlighted allele frequency imbalance as a function of ethnic background, such that the minor (Asp40) allele frequency is approximately 20% in Caucasians, 5% in individuals of African ancestry, and as high as 50% among individuals of East Asian descent (Arias *et al*, 2006). Therefore, to the extent to which this polymorphism moderates behavioral and clinical responses to naltrexone, ethnicity must be carefully considered to extend the findings from primarily Caucasian samples to ethnic minorities, such as Asian Americans and African Americans. This is critical, as population-specific effects of the Asn40Asp SNP have been reported, such that greater cortisol response to naloxone, an opioid receptor antagonist, among Asp40 carriers was observed among individuals of European Ancestry, but not among individuals of Asian descent (Hernandez-Avila *et al*, 2007).

The objective of this study is to extend the pharmacogenetic literature on naltrexone and the Asn40Asp SNP of the *OPRM1* gene to East Asian Americans, an ethnic group in which the Asp40 allele is notably more frequent. This study examines the moderating role of the Asn40Asp polymorphism on the biobehavioral effects of naltrexone in the human laboratory, namely its attenuation of subjective intoxication and alcohol craving. It is hypothesized that Asp40 carriers will report greater naltrexone-induced blunting of alcohol reward and craving, consistent with findings from primarily Caucasian samples (Ray and Hutchison, 2007a). A secondary objective is to test the pharmacogenetic effects on HPA-axis activation, indexed by cortisol and ACTH levels. In short, this study will help translate the promising findings regarding naltrexone pharmacogenetics for alcoholism to an ethnic minority group that is more likely to express the minor allele associated with a more beneficial clinical response to this medication in previous studies. Hence, these findings have important clinical implications for (1) identifying medication responders and (2) reducing the potential for health disparities associated with pharmacogenetic research (Tate and Goldstein, 2004).

MATERIALS AND METHODS

Participants

This study was approved by the University of California Los Angeles Institutional Review Board and all participants provided written informed consent after receiving a full explanation of the study. Inclusion criteria were as follows: (1) a score of 8 or higher on the Alcohol-Use Disorders Identification Test (AUDIT) (Allen *et al*, 1997), indicating a heavy drinking pattern; (2) self-reported drinking frequency of 3 or more drinks (2 for women) at least twice per week; and (3) East Asian ethnicity (ie, Chinese, Korean, or Japanese). In all, 35 (10 females) non-treatment-seeking heavy drinkers were randomized in this trial. The majority of participants (75.5%) reported being full-time students. The average age was 22.3 (SD = 1.98; range = 21–29), and of

the 35 participants enrolled in this study, 17 (48.6%) were Chinese, 15 (42.9%) were Korean, and 3 (8.5%) were Japanese. All female subjects tested negative for pregnancy and all subjects had a breath alcohol concentration (BrAC) of zero before each session.

Screening and Experimental Procedures

Initial assessment of the eligibility criteria (above) was conducted through a telephone interview. Eligible participants were invited to the laboratory for an additional screening session. Upon arrival at the laboratory, participants read and signed an informed consent form, provided a saliva sample for DNA analyses, and completed a series of individual differences measures. Given that the expected minor allele frequency was approximately 50%, no prospective genotyping was employed, unlike our previous work with primarily Caucasian samples (Ray and Hutchison, 2007a). Before participating in the alcohol challenge, participants attended a physical examination at the UCLA General Clinical Research Center (GCRC) conducted by the study physician (KM). A total of 49 participants (12 women) were screened in the laboratory, 41 completed the physical exam, 3 of whom were ineligible for medical reasons and 4 of whom decided not to participate in the trial, leaving us with 35 participants who enrolled in the study. Of the 35 individuals randomized, 32 completed the entire study and 3 dropped out after completing one alcohol administration session.

Participants completed two experimental sessions, one after taking naltrexone for 4 days and one after taking a matched placebo for 4 days. Active medication and placebo were delivered in a counterbalanced and double-blinded manner. During the experimental sessions, participants were seated in a recliner chair and the i.v. was placed in their non-dominant arm. After completing the baseline assessment, participants received intravenous doses of alcohol and completed identical assessment measures at each of the following points in BrAC: 0.02, 0.04, and 0.06 g/dl. After the infusion procedure was finished, participants were given a meal and asked to stay in the lab until their BrAC was below 0.02 g/dl. Given the importance of the diurnal cycle to the assessment of ACTH and cortisol, participants completed both infusion sessions at the same time of the day (average start time was 1230 hours).

Alcohol Administration and Medication Procedures

Given the importance of effectively controlling blood alcohol levels to reduce experimental variability in alcohol challenge studies (Li *et al*, 2001; O'Connor *et al*, 1998; Ramchandani *et al*, 1999), in this study alcohol was administered intravenously, using procedures developed in our previous work (Ray and Hutchison, 2004; Ray *et al*, 2007b). The infusion was performed using a 5% ethanol i.v. solution and a nomogram was developed, taking into account participant's gender and weight. Infusion rates were: 0.166 ml/min × weight (in kg) for males and 0.126 ml/min × weight for females. Target BrACs were as follows: 0.02, 0.04, and 0.06 g/dl. Upon reaching each of the target levels of intoxication, participants' infusion rates were reduced to half, to maintain stable BrAC during testing. The ethanol infusion yielded highly controlled BrACs, such that the observed mean (SD) BrACs were as follows: 0.020 (0.001), 0.041 (0.001), and 0.060 (0.002) g/dl across medication conditions. Time to each target BrAC was, on average, 20.1, 57.7, and 96.2 min, respectively.

Participants completed one infusion session after taking naltrexone for 4 days (25 mg for days 1 and 2 and 50 mg for days 3 and 4) and one session after taking a matched placebo for 4 days (7-day wash-out period between conditions). Participants were required to take the study medication (naltrexone or placebo) once a day for 3 days before the first experimental session and on the morning of their appointment. Participants reported any side effects to the study physician. There were no dropouts as a result of medication side effects. Medication compliance was examined by packing the medication and placebo into capsules with 50 mg of riboflavin. Urine samples were collected before each ethanol infusion session and were analyzed for riboflavin content under an ultraviolet light, a procedure that makes the riboflavin detectable (Del Boca *et al*, 1996). All samples tested positive for riboflavin content.

Behavioral Assessments

During the laboratory screening session, participants completed a battery of individual difference measures that included demographics and drinking behavior. During the ethanol infusion, measures of subjective responses to alcohol and alcohol craving were administered at baseline and at each target BrAC. As a check-on-blind, participants reported which medication (naltrexone *vs* placebo) they believed to have received before each infusion session.

The following measures were used: (1) *Time Line Follow Back (TLFB)*: a 30-day TLFB was administered in face-to-face interview format to assess alcohol-use frequency and quantity over the past month (Sobell *et al*, 1986). (2) *Side-Effect Checklist*: The short form of the Systematic Assessment for Treatment Emergent Events (SAFTEE) was administered before each infusion session. The SAFTEE consists of 24 common drug side effects and has been recommended for use in clinical trials (Jacobson *et al*, 1986; Levine and Schooler, 1986). (3) *Alcohol Urge Questionnaire (AUQ)*: The AUQ consists of eight items related to urge to drink alcohol, each rated on a seven-point Likert scale, anchored by 'Strongly Disagree' and 'Strongly Agree'. The AUQ has demonstrated high internal consistency in alcohol studies (Bohn *et al*, 1995; MacKillop, 2006). (4) *Subjective High Assessment Scale (SHAS)*: The SHAS was used to assess subjective feelings of alcohol intoxication (Schuckit, 1984). (5) The *Biphasic Alcohol Effects Scale (BAES)* assesses feelings of alcohol stimulation and sedation, each consisting of seven items rated on a 0–10 scale. The BAES has been shown to be reliable and valid in investigations of the subjective effects of alcohol (Erblich and Earleywine, 1995; Martin *et al*, 1993).

Genotyping

Saliva samples were collected under researcher observation for DNA analyses using Oragene saliva collection kits. Genotyping was performed at the UCLA Genotyping and

Sequencing (GenoSeq) Core. Polymerase chain reaction (PCR) primers were labeled with fluorescent dye (6-FAM, VIC, or NED), and PCR was performed on Applied Biosystems dual block PCR thermal cyclers. SNP sequencing was run on an AB 7900HT Fast Real-Time PCR System and analyzed using the Sequence Detection Systems software version 2.3. Each run included two positive control samples (individual 2 in CEPH family 1347; Coriell Institute). Genotypes were automatically scored by the allele calling software and verified by visual inspection. In process validation checks, the UCLA GenoSeq Core has average call, reproducibility, and concordance rates of 96%, 99.7%, and 99.8%, respectively. In addition to the *OPRM1* SNP of interest, markers in the alcohol dehydrogenase gene (*ADH1B*, rs1229984) and aldehyde dehydrogenase gene (*ALDH2*, rs671) were assayed to serve as control variables.

Cortisol and ACTH Assays

Blood samples for cortisol and ACTH analyses were collected at baseline (BrAC = 0.00 g/dl) and again at the final target BrAC (0.060 g/dl). ACTH samples were collected in 1–5 ml EDTA tube, whereas cortisol samples were collected in 1–5 ml BD tube. Within 15 min of collection, blood was centrifuged at 4 °C and the serum transferred to cryovial tubes stored at −70 °C. The UCLA GCRC Core Laboratory tested ACTH and Cortisol on Siemens IMMULITE1000 Immunoassay System and protocol instructions were followed exactly as directed. The sensitivity of these assays is 9 pg/ml and the inter-assay precision is 4.9%.

Data Analytic Plan

Analyses were conducted using a multilevel model framework (Singer, 1998) using PROC MIXED in SAS to test genotype group differences on medication response. In all analyses, we modeled individual intercepts and linear slopes across rising BrAC levels. Specifically, in the multilevel models, Medication and Genotype were Level 1 variables (nested within subjects), whereas subject and BrAC were Level 2 variables. The analyses examined the effects of *Medication*, a two-level within-subjects factor (naltrexone *vs* placebo, coded 0 and 1), *Genotype*, a two-level between-subjects factor (Asn40 hyomozygotes *vs* Asp40 carriers, coded 0 and 1), *BrAC*, a four-level within-subjects factor (BrAC = 0.00, 0.02, 0.04, and 0.06 g/dl, coded 0–3), and their *interactions*. The dependent variables were measures of subjective responses to alcohol (SHAS and BAES), alcohol craving (AUQ), and hormonal indices (cortisol and ACTH). Follow-up analyses controlled for the *ALDH2* (rs671) and *ADH1B* (rs1229984) markers to validate the main results.

RESULTS

Baseline Comparisons

A series of pre-test comparisons were conducted to determine whether the *OPRM1* groups differed on drinking and demographic variables. Results revealed no genotype group differences on demographic or drinking variables (Table 1). All urine samples tested positive for riboflavin, suggesting that individuals were compliant with the medication instructions immediately before each appointment. Regarding the integrity of the medication blind, 55% of the participants guessed correctly while in the placebo condition and 63% of the participants guessed correctly while in the naltrexone condition, which was higher than what would be expected by chance (ie, 50%). However, there was no significant difference in correct guesses as a function of medication, $\chi^2(1) = 1.37$, $p = 0.24$. A series of Fisher's exact tests, a non-parametric test appropriate for small cell sizes (Fisher, 1922), were conducted comparing the medication *vs* placebo on each of the 24 items from the side effects checklist (SAFTEE). Results revealed a significant medication effect on difficulty sleeping, which occurred in 20% of patients taking naltrexone, as compared to 13% of patients on placebo (Fisher's exact test, $p < 0.05$). There was no significant medication effect on any of the remaining 23 side effects measured by the SAFTEE (Fisher's exact test, $p > 0.05$) and there were no significant differences in side effects as a function of genotype (Fisher's exact test, $p > 0.05$).

Table 1 Pre-test Differences between the Genotype Groups

Variable[a]	Asn40Asn ($n = 13$)	Asn40Asp/Asp40Asp ($n = 22$)	Test for difference
Gender (% female)	30.8	27.3	$\chi^2(1) = 0.05$, $p = 0.83$
Ethnicity (% Chinese, Korean, Japanese)	61, 31, 8	41, 50, 9	$\chi^2(2) = 1.44$, $p = 0.49$
Age	22 (1.9)	22.4 (2.1)	$t(33) = -0.59$, $p = 0.56$
Alcohol-Use Disorders Identification Test[b]	13.4 (4.0)	13.1 (4.1)	$t(33) = 0.23$, $p = 0.82$
Rutgers Alcohol Problem Index (RAPI)[c]	23.85 (16.5)	21.82 (12.9)	$t(33) = 0.41$, $p = 0.69$
Drinking frequency in past year[d]	6.6 (1.8)	7.3 (1.9)	$t(33) = -1.10$, $p = 0.28$
Drinks per drinking episode in past year	5.4 (1.5)	5.8 (2.1)	$t(33) = -0.54$, $p = 0.59$
Number of drinking days in past 30 days[e]	11.4 (4.4)	8.0 (5.6)	$t(33) = 0.49$, $p = 0.63$
Drinks per drinking day in past 30 days[e]	5.1 (1.8)	5.3 (2.5)	$t(33) = -0.26$, $p = 0.80$

[a]Standard deviations appear within parentheses below the means of continuous variables.
[b]AUDIT score $\geqslant 8$ indicates a hazardous drinking pattern; possible range of scale: 0–40.
[c]RAPI score of 21–25 were noted in clinical samples (White and Labouvie, 1989); possible range of scale: 0–69.
[d]A score of 6 corresponds to once per week; 7 corresponds to twice per week.
[e]Assessed by the Timeline Follow Back (TLFB) interview.

Allele frequencies for the *OPRM1*, *ALDH2*, and *ADH1B* genes for the screening and experimental samples are shown in Table 2. The allele frequencies observed in the screening sample were in conformity with Hardy–Weinberg equilibrium expectations for the *OPRM1* ($\chi^2(2) = 0.08$; $p = 0.78$), *ALDH2* ($\chi^2(2) = 0.63$; $p = 0.43$), and *ADH1B* ($\chi^2(2) = 0.49$; $p = 0.48$) SNPs. In addition, there was no allele frequency imbalance between *OPRM1* and *ADH1B* ($\chi^2(4) = 6.28$; $p = 0.18$) or between *OPRM1* and *ALDH2* ($\chi^2(2) = 1.63$; $p = 0.44$). Likewise, there was no differential dropout as a function of *OPRM1* (Fisher's exact, $p = 0.73$), *ADH1B* (Fisher's exact, $p = 0.21$), or *ALDH2* (Fisher's exact, $p = 0.84$) genotype.

Pharmacogenetic Effects: Subjective Intoxication

Analyses of alcohol-induced stimulation revealed no effect of medication ($\beta = -0.18$, SE = 0.24, $t = -0.76$, $p = 0.45$), genotype ($\beta = -0.51$, SE = 0.46, $t = -1.12$, $p = 0.26$), or medication × genotype interaction ($\beta = 0.24$, SE = 0.23, $t = 1.04$, $p = 0.30$). As expected, there was a main effect of BrAC ($\beta = 0.50$, SE = 0.18, $t = 2.70$, $p < 0.05$), such that participants reported higher levels of stimulation across rising levels of BrAC. Regarding alcohol-induced sedation, there was no effect of medication ($\beta = 0.24$, SE = 0.21, $t = 1.15$, $p = 0.25$); however, there was a main effect of genotype ($\beta = 1.43$, SE = 0.40, $t = 3.62$, $p < 0.001$) and a significant medication × genotype interaction ($\beta = -0.90$, SE = 0.21, $t = -4.30$, $p < 0.001$), such that Asp40 carriers reported higher sedation across rising levels of BrAC and greater sedation on naltrexone relative to placebo, and as compared to Asn40 homozygotes. There was a significant effect of BrAC ($\beta = 0.66$, SE = 0.15, $t = 4.48$, $p < 0.0001$), such that participants reported higher sedation across rising BrAC levels (Figure 1).

A similar pattern of results was found for subjective intoxication, measured by the SHAS. There was no effect of medication ($\beta = 0.50$, SE = 2.16, $t = 0.23$, $p = 0.82$); however, there was a main effect of genotype ($\beta = 7.65$, SE = 3.84, $t = 1.99$, $p < 0.05$) and a significant medication × genotype interaction ($\beta = -4.83$, SE = 2.12, $t = -2.27$, $p < 0.05$), such that Asp40 carriers had reported higher subjective intoxication across rising BrAC levels and greater feelings of subjective intoxication on naltrexone relative to placebo, and as compared to Asn40 homozygotes (see Figure 1). There was also a significant effect of BrAC ($\beta = 9.63$, SE = 1.52, $t = 6.35$, $p < 0.0001$) and a significant medication × BrAC interaction ($\beta = -1.75$, SE = 0.89, $t = -1.96$, $p = 0.05$), such that participants reported higher subjective intoxication across rising levels of BrAC and naltrexone increased subjective intoxication across BrACs, as compared to placebo (Figure 2).

Follow-up analyses controlling for *ALDH2* and *ADH1B* markers did not change any of the results reported above. In addition, there was no significant main effect of *ALDH2* or *ADH1B* markers on stimulation ($p = 0.60$ and 0.46, respectively), sedation ($p = 0.31$ and 0.81, respectively), or subjective intoxication ($p = 0.97$ and 0.76, respectively)

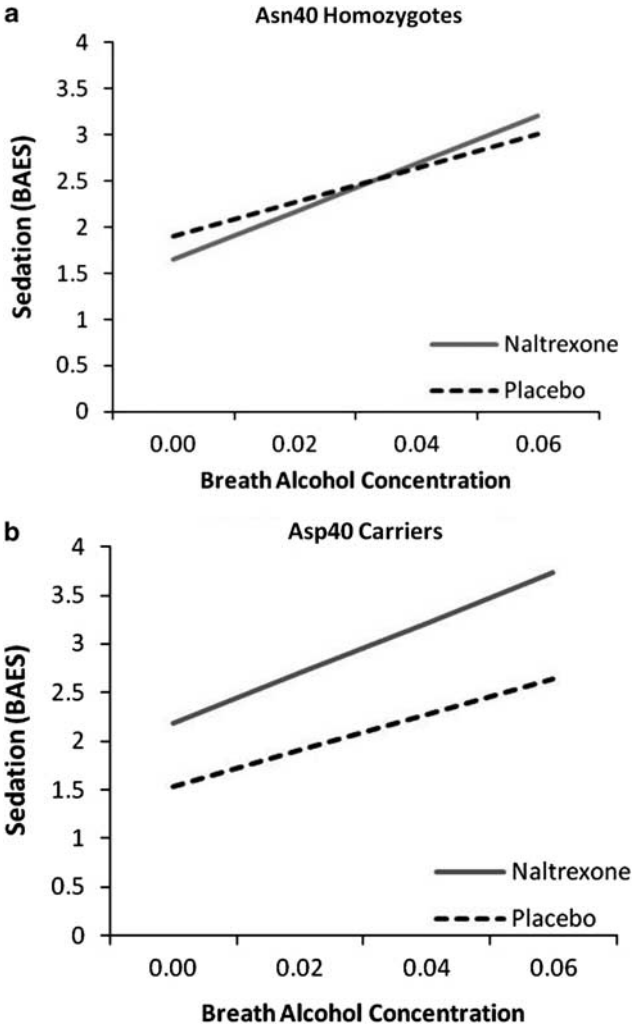

Figure 1 Predicted values for sedation as a function of breath alcohol concentration (BrAC) for on naltrexone (NTX) and placebo (PLAC) conditions for Asn40 homozygotes (a) and Asp40 carriers (b).

Table 2 Allele Frequencies for the Single-Nucleotide Polymorphisms (SNPs) of Interest

Initial sample ($n = 49$)								Experimental sample ($n = 35$)							
OPRM1			**ALDH2**[a]		**ADH1B**			**OPRM1**			**ALDH2**		**ADH1B**		
Asn40Asn	**Asn40Asp**	**Asp40Asp**	***1/*1**	***1/*2**	***1/*1**	***1/*2**	***2/*2**	**Asn40Asn**	**Asn40Asp**	**Asp40Asp**	***1/*1**	***1/*2**	***1/*1**	***1/*2**	***2/*2**
21	22	6	39	10	5	18	26	13	17	5	26	9	4	12	19

[a]There were no *ALDH2* *2/*2 observations in this sample.

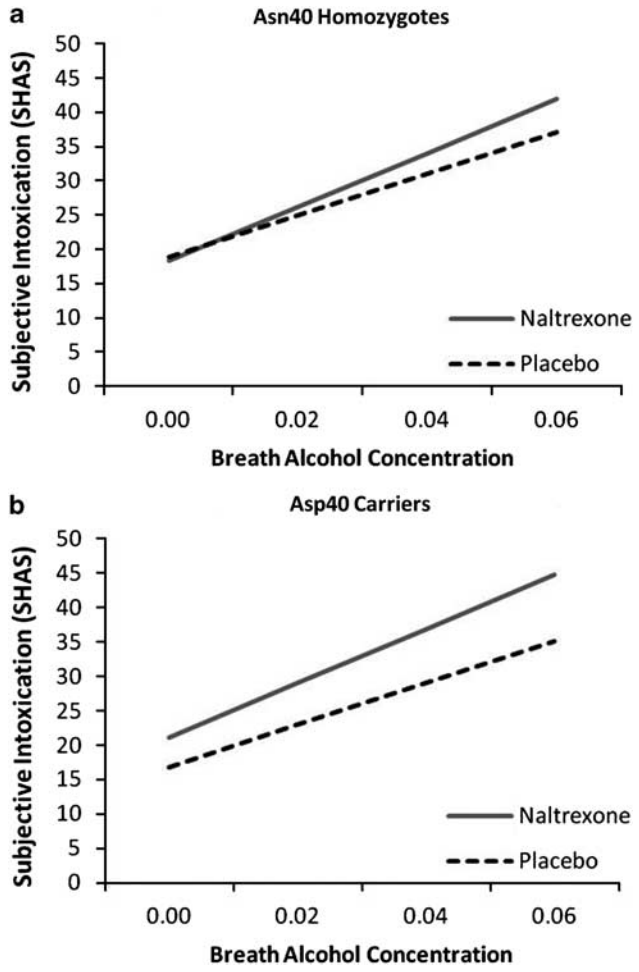

Figure 2 Predicted values for subjective intoxication as a function of breath alcohol concentration (BrAC) for on naltrexone (NTX) and placebo (PLAC) conditions for Asn40 homozygotes (a) and Asp40 carriers (b).

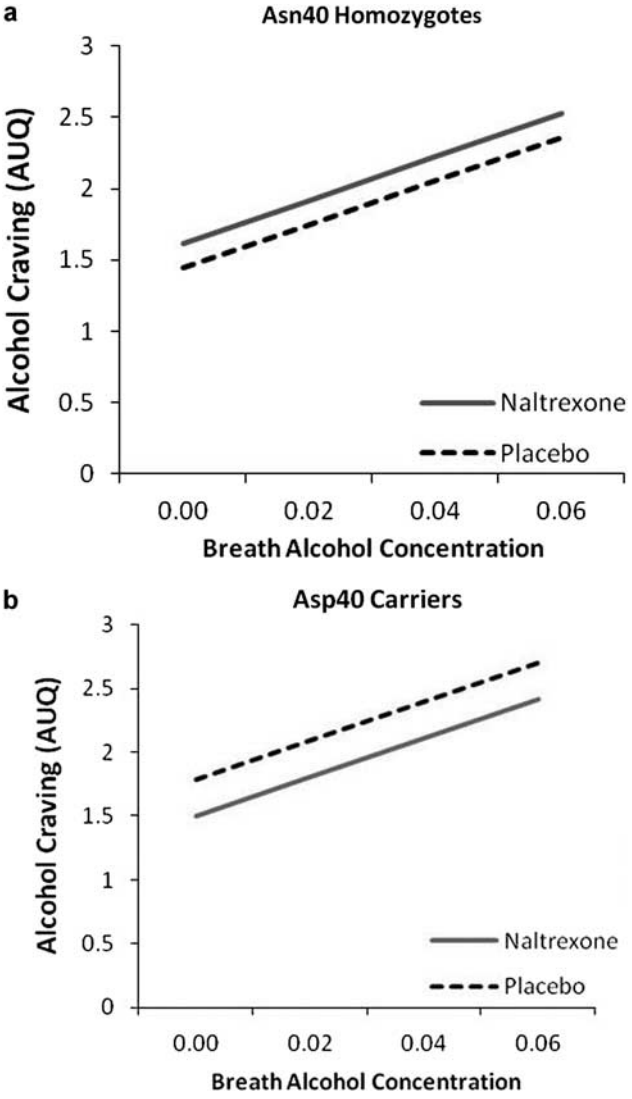

Figure 3 Predicted values for alcohol craving as a function of breath alcohol concentration (BrAC) for naltrexone (NTX) and placebo (PLAC) conditions for Asn40 homozygotes (a) and Asp40 carriers (b).

when these markers were added to the models testing the effects of medication, BrAC, *OPRM1*, and their interaction.

Pharmacogenetic Effects: Alcohol Craving

Analyses revealed no significant main effect of medication ($\beta = -0.17$, SE = 0.16, $t = -1.08$, $p = 0.28$) or *OPRM1* genotype ($\beta = -0.57$, SE = 0.40, $t = -1.42$, $p = 0.16$). However, there was a significant medication × genotype interaction ($\beta = 0.46$, SE = 0.20, $t = 2.33$, $p < 0.05$), such that Asp40 carriers reported greater naltrexone-induced blunting of alcohol craving, as compared to Asn40 homozygotes. There was a significant effect of BrAC ($\beta = 0.30$, SE = 0.07, $t = 4.58$, $p < 0.0001$), such that participants reported higher craving across rising levels of BrAC (Figure 3). These results remained significant after controlling for *ALDH2* ($p = 0.88$) and *ADH1B* ($p = 0.81$) in the models.

Pharmacogenetic Effects: Cortisol and ACTH

Analyses of cortisol levels revealed a significant effect of BrAC ($\beta = -2.50$, SE = 0.66, $t = -3.79$, $p < 0.001$), such that alcohol administration dampened cortisol levels relative to baseline. However, there was no significant effect of medication ($\beta = -0.22$, SE = 1.12, $t = -0.20$, $p = 0.84$), genotype ($\beta = 2.03$, SE = 2.44, $t = 0.83$, $p = 0.41$), or medication × genotype interaction ($\beta = -0.91$, SE = 1.38, $t = -0.66$, $p = 0.51$). A similar pattern emerged for analyses of ACTH levels, such that there was a significant effect of BrAC ($\beta = -4.82$, SE = 2.37, $t = -2.04$, $p = 0.05$), indicating that alcohol administration lowered ACTH levels as compared to baseline. There was no significant effect of medication ($\beta = 0.38$, SE = 3.67, $t = 0.10$, $p = 0.92$), genotype ($\beta = 8.60$, SE = 7.29, $t = 1.18$, $p = 0.24$), or medication × genotype interaction ($\beta = -3.03$, SE = 4.51, $t = -0.67$, $p = 0.51$). Adding *ALDH2* and *ADH1B* markers did not alter the results reported above and neither of the two markers was associated with cortisol ($p = 0.14$ and 0.28, respectively) or ACTH ($p = 0.24$ and 0.37, respectively) levels.

Follow-Up: *OPRM1* Genotype Dose Response

Given the higher frequency of the Asp40 allele in this sample, follow-up analyses were performed across the three

levels of *OPRM1* genotype in the experimental sample: Asn40Asn ($n=13$), Asn40Asp ($n=17$), and Asp40Asp ($n=5$). Although exploratory, these linear models in effect test the *OPRM1* gene dose response, which has not been possible to date given the very low frequency of the Asp40Asp genotype in primarily Caucasian samples. Results of analyses using the three levels of *OPRM1* genotype supported the results reported above, such that there was a significant main effect of *OPRM1* genotype ($\beta=1.13$, SE $=0.28$, $t=4.08$, $p<0.001$) and a significant medication $\times$ genotype interaction ($\beta=-0.67$, SE $=0.15$, $t=-4.63$, $p<0.0001$) on sedation, in the same direction as reported above. Likewise, the main effect of *OPRM1* genotype ($\beta=7.01$, SE $=2.69$, $t=2.60$, $p<0.01$) and the medication $\times$ genotype interaction ($\beta=-4.05$, SE $=1.47$, $t=-2.74$, $p<0.01$) were maintained for subjective intoxication. The same was true for the medication $\times$ genotype interaction ($\beta=0.36$, SE $=0.14$, $t=2.66$, $p<0.01$) regarding alcohol craving. The null findings for stimulation, cortisol, and ACTH also remained unchanged when examining the three levels of *OPRM1* genotype. To further probe for the gene dose effect, follow-up analyses compared Asn40Asp ($n=17$) to Asp40Asp ($n=5$). Results revealed trend-level differences on medication $\times$ genotype interactions regarding sedation ($\beta=-0.51$, SE $=0.30$, $t=-1.71$, $p=0.09$), subjective intoxication ($\beta=-4.50$, SE $=3.02$, $t=-1.49$, $p=0.13$), and a significant difference regarding stimulation ($\beta=-0.94$, SE $=0.31$, $t=-3.01$, $p<0.01$). Taken together, these results suggest that the pharmacogenetic effects are maintained using the three levels of *OPRM1* genotype and that there is some preliminary evidence to support a gene dose response with regard to its pharmacogenetic effects on subjective intoxication.

DISCUSSION

This study sought to examine the pharmacogenetic effects of the *OPRM1* Asn40Asp SNP and naltrexone in a sample of heavy-drinking East Asian Americans. This is an important empirical and clinical question for two reasons. First, the minor Asp40 allele of the *OPRM1* gene found to predict a more positive response to naltrexone in the human laboratory (Ray and Hutchison, 2007a) and in clinical trials (Anton *et al*, 2008; Kim *et al*, 2009; Oslin *et al*, 2003) is markedly more prevalent among individuals of Asian descent (Arias *et al*, 2006). Second, the vast majority of the pharmacogenetic studies completed to date have been in Caucasian samples. Only two studies have examined the moderating role of the *OPRM1* Asn40Asp SNP in response to opioid blockade in individuals of Asian descent. One was a clinical trial of naltrexone in Korean alcohol-dependent patients who found a positive pharmacogenetic effect when restricting the analyses to individuals who were medication compliant (Kim *et al*, 2009). The other study compared HPA-axis activation in response to a naloxone challenge among Caucasians and individuals of Asian descent and found that the pharmacogenetic effect was population-specific and was only observed in Caucasians (Hernandez-Avila *et al*, 2007). Taken together, these findings suggest that careful evaluation of population-specific pharmacogenetic effects are warranted to (a) further understand mechanisms of medication response, and (b) prevent health disparities in personalized medicine (Tate and Goldstein, 2004).

This study consisted of a randomized, double-blinded, cross-over, placebo-controlled study of naltrexone. Its rigorous experimental design allowed us to interrogate pharmacogenetic effects on important mechanisms of naltrexone efficacy for alcoholism, namely reductions in alcohol craving and subjective intoxication (Anton *et al*, 2004; King *et al*, 1997; Volpicelli *et al*, 1995). Results revealed a significant pharmacogenetic effect with regard to alcohol craving, such that naltrexone blunted alcohol craving during alcohol exposure more strongly among Asp40 carriers relative to Asn40 homozygotes, and as compared to placebo. These novel findings suggest a mechanisms by which naltrexone may be differentially effective among Asp40 carriers of East Asian descent. In addition, there is preliminary evidence to support an *OPRM1* gene dose effect on naltrexone pharmacogenetics.

Analyses of subjective intoxication indicated a significant pharmacogenetic effect such that naltrexone potentiated alcohol-induced sedation and subjective intoxication among Asp40 carriers, as compared to Asn40 homozygotes and to placebo. These findings are of great interest as dimensions of alcohol sedation and subjective intoxication are thought to capture the negative and aversive effects of alcohol (Ray *et al*, 2009b). And importantly, a recent prospective study has shown that higher sedation during alcohol administration is a protective factor against binge drinking and the subsequent development of alcohol-use disorders in heavy drinkers (King *et al*, 2011). Although previous studies of primarily Caucasian samples have suggested greater alcohol 'high' among Asp40 carriers (Ray and Hutchison, 2004; Ray *et al*, 2010b; Setiawan *et al*, 2011) and greater naltrexone blunting of alcohol 'high' (Ray and Hutchison, 2007a), it appears that the pharmacogenetics of naltrexone in Asian Americans may be associated with an increase in the aversive effects of alcohol as opposed to dampening of the reinforcing effects of alcohol. Of note, there was a significant effect of alcohol on stimulation, such that participants reported higher stimulation across rising levels of BrAC. However, there was no pharmacogenetic effect on this neurobehavioral marker.

The concept of subjective intoxication to alcohol is rather unique in Asian Americans as studies have shown that genetic differences affecting the metabolism of alcohol produce a 'flushing response' (Eng *et al*, 2007). Studies have shown that 'flushers' also experience significant alcohol stimulation, and that even though their response to alcohol is not predominantly negative, the flushing response remains protective against heavy drinking (Wall *et al*, 1992). Taken together, it is plausible to hypothesize that the aversive effects of alcohol may be more potent determinant of future drinking among Asian Americans. To that end, the observed pharmacogenetic effects of naltrexone in increasing alcohol sedation and subjective intoxication among Asian Americans may be highly clinically significant. Importantly, while genetic markers of alcohol metabolism were genotyped in this sample, they did not contribute to the phenotypes of interest, likely because the sample selection required significant alcohol exposure and drinking at a hazardous level. Such criteria likely excluded

individuals with significant flushing responses to alcohol from our sample. Follow-up analyses controlling for ALDH2 and ADH1B markers supported the OPRM1 effects and bolstered confidence in these results.

The secondary aim of this study was to examine biomarkers of HPA-axis activation (ACTH and cortisol) as outcome measures of naltrexone and *OPRM1* pharmacogenetics. Previous studies of HPA-axis challenges with naloxone (Hernandez-Avila *et al*, 2003, 2007; Wand *et al*, 2002), and more recently with metyrapone (Ducat *et al*, 2011), have suggested an intriguing pharmacogenetic effect. Consistent with our previous study (Ray *et al*, 2009a), results revealed a significant main effect of alcohol, such that it decreased both cortisol and ACTH relative to baseline. However, in contrast to our previous work in a Caucasian sample, there was no medication effect on either ACTH or cortisol and no medication by genotype interaction. Previous studies have shown a negative association between cortisol levels and alcohol craving, suggesting that naltrexone's ability to raise cortisol levels may help account for its clinical effects (O'Malley *et al*, 2002). A similar pattern has been recently observed for GABAergic neuroactive steroids and naltrexone pharmacogenetics (Ray *et al*, 2010a). Taken together, these findings are consistent with a previous study of naloxone pharmacogenetics, suggesting that the HPA-axis reactivity to opioid blockade may be specific to Caucasians and not present among Asian Americans (Hernandez-Avila *et al*, 2007). Although this study did not include a Caucasian control sample, it enrolled a larger number of Asian American individuals, such that it increased statistical power to detect both a medication main effect and pharmacogenetic interaction. On balance, these results support the null hypothesis proposed by Hernandez-Avila and co-workers (2007), and suggest that additional polymorphisms are critically implicated in HPA-axis responsivity to opioid blockers, beyond the Asn40Asp SNP.

These results, if supported and extended in clinical trials, may be especially useful in targeting the use of naltrexone in Asian populations in the United States and worldwide. Although there is convincing evidence of genetic protective factors against alcoholism in Asian populations (Eng *et al*, 2007), recent epidemiological studies have suggested that alcoholism represents a sizeable public health problem in East Asian countries (Hao *et al*, 2005; Higuchi *et al*, 2007). Specifically, studies have estimated that the prevalence of hazardous drinking (AUDIT $\geqslant 8$) is 23.6% among Japanese men (Higuchi *et al*, 2007), whereas approximately 3.4% of Chinese (Hao *et al*, 2005) and 16.2% of Koreans (Cho *et al*, 2011) are affected by an alcohol-use disorder in their lifetime. Although cultural and sociodemographic factors are likely to play a role in the risk for developing an alcohol-use disorder in Asian countries (Guo *et al*, 2009; Hao *et al*, 2005; Higuchi *et al*, 2007), patients stand to benefit from the optimization of naltrexone for alcohol dependence on the basis of *OPRM1* genotype in light of the higher prevalence rates of the minor (Asp40 allele) among individuals of East Asian descent. Should the preliminary *OPRM1* gene dose response pharmacogenetic effect observed in this study be supported in future trials, even greater optimization may be achieved in the pharmacogenetic prescription of naltrexone among individuals of Asian descent.

This study must be interpreted in the context of its strengths and limitations. Study strengths include the randomized, double-blinded, placebo-controlled design. The cross-over design is also a strength of the study as it allowed individuals to serve as their own controls, hence increasing statistical power to detect medication effects and pharmacogenetic interactions. In addition, the high retention and medication compliance in this study suggest that the medication was well tolerated in its proposed acute titration schedule. The theory-driven approach and the combination of biological and behavioral markers of response to alcohol represent strengths of the study. Study limitations include the subclinical nature of the sample and the absence of prospective genotyping for the candidate gene of interest. The lack of a control condition for alcohol (ie, saline infusion) and ethnicity (ie, Caucasian comparison sample) also represent study limitations.

On balance, this study addresses an important gap in the emerging literature on naltrexone pharmacogenetics. It does so by examining whether the functional Asn40Asp mutation of the *OPRM1* gene moderates responses to alcohol among Asian Americans. Results suggest that while pharmacogenetic effects were found to support the potential clinical utility of this Asp40 allele as a predictor of medication response, the mechanisms underlying these effects may be population-specific. As such, it is plausible to hypothesize that naltrexone may be differentially effective among Asp40 carriers by potentiating the aversive effects of alcohol in Asian American heavy drinkers, as compared to a primary mechanism of dampened reinforcement and alcohol 'high' observed in Caucasian samples. These findings help further delineate the biobehavioral mechanisms of naltrexone and its pharmacogenetics.

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DISCLOSURE

The authors declare no conflict of interest.

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