Published in final edited form as:

Alcohol Clin Exp Res. 2012 November ; 36(11): 2000–2007. doi:10.1111/j.1530-0277.2012.01807.x.

Naltrexone Modification of Drinking Effects in a Sub-Acute Treatment and Bar-Lab Paradigm: Influence of *OPRM1* and Dopamine Transporter (*SCL6A3*) Genes

Raymond F. Anton, M.D.¹, Konstantin K. Voronin, M.D., Ph.D.¹, Patrick K. Randall, Ph.D.¹, Hugh Myrick, M.D.^{1,2}, and Abraham Tiffany, B.S.¹

¹Medical University of South Carolina

²Ralph H. Johnson, Veteran Administration Medical Center

Abstract

Background—Naltrexone is moderately effective for the treatment of alcohol dependence but there is great individual variability. The opioid receptor (*OPRM1*) SNP asn40asp has been shown to alter alcohol and naltrexone response in animals and humans. In addition, the brain opioid and dopamine systems interact and might underlie drinking and craving. This study investigated the effects of the *OPRM1* SNP and dopamine transporter VNTR genetic differences on drinking, alcohol effects, and naltrexone response under controlled conditions in non-treatment seeking alcoholics.

Methods—265 non-treatment seeking individuals with alcohol dependence were genotyped a priori for the *OPRM1* asn40asp SNP and post-hoc for DAT (*SCL6A3*) 9 and 10 VNTR's. Asp40 carriers (n=43) and matched asn40 homozygotes (n= 40) were randomized to naltrexone or placebo for 7 days before receiving a priming drink and limited-access alcohol consumption in a bar-lab setting. Effects of genotypes on natural drinking as well as drinking, alcohol effects, and response to naltrexone in the bar-lab setting were examined by genotype.

Results—There were no significant main effects of naltrexone or *OPRM1* genotype, or any medication by *OPRM1* interaction, on drinking variables. However, in individuals who had at least one DAT 9 VNTR, and who were also *OPRM1* asn40 homozygotes, naltrexone reduced drinks/day consumed under natural conditions (p=0.006) but not in the bar-lab. *OPRM1* asn40 homozygotes (p=0.028) and DAT 9 VNTR carriers (p=0.032) had more stimulation to alcohol after the priming drink.

Conclusions—This study does not support a salient role for the *OPRM1* asp40 alone in predicting drinking or naltrexone effects. However, although exploratory and in need of replication, it introduces the possibility that epistasis between the *OPRM1* gene and DAT gene might need to be taken into account when examining differential genetic response to alcohol or medication treatment, especially in early-stage alcoholics.

Keywords

Alcoholism; Genetics; Medication; Drinking; Opioid Receptors

Corresponding/First Author: Raymond F. Anton, M.D., Medical University of South Carolina, Institute of Psychiatry, 67 President Street, MSC 861, Charleston, SC 29425, (843) 792-1226, antonr@musc.edu.

Presented, in part, at the Research Society on Alcoholism meeting in San Diego CA., June 2010, and at the International Society of Psychiatric Genetics in Athens Greece, October 2010.

BACKGROUND

It is well documented that naltrexone is efficacious in the treatment of alcohol dependence (Anton et al., 2008; Kranzler and Van Kirk, 2001; Streeton and Whelan, 2001) and has been approved by the FDA for this indication since 1994. Nevertheless, the effect is moderate at best and it is recognized that not all individuals with an alcohol use disorder respond to it. Genetic differences have been suggested as one factor that might influence both response to alcohol and the ability of naltrexone to modify this response. There have been a number of animal and human clinical lab studies (Ray et al., 2011) suggesting that a single nucleotide polymorphism (SNP) in the mu opioid gene (A118G)¹ leading to a missense asparagine to aspartate amino acid substitution at position 40 (asn40asp) (rs17799971) can lead to differences in alcohol effects and response to naltrexone. It has been shown that some of this enhanced alcohol response and drinking behavior in rodents engineered to have a homologous SNP is due to increased dopamine release in the nucleus accumbens (Ramchandani et al., 2011) which in general is thought to be a signature of reinforcement and addiction. Of interest, naltrexone has been shown in animals to both reduce nucleus accumbens dopamine release (Gonzales and Weiss, 1998) and to reduce drinking in rodent (Middaugh and Bandy, 2000) and non-human primate drinking models (Kornet et al., 1991). Of great interest, an homologous SNP to that found in humans in the mu opioid receptor gene occurring naturally in non-human primates also appears to confer both sensitivity to alcohol response (Barr et al., 2007) and response to naltrexone in the reduction of alcohol effects and consumption (Barr et al., 2010; Vallender et al., 2010). This finding parallels data in human clinical lab studies (Ray et al., 2011) and clinical trials (Oslin et al., 2003; Anton et al., 2008) where naltrexone appears to exert a stronger effect on those individuals with the asp40 *OPRM1* SNP. However, this finding is not universal as several reports suggest that it may not be as salient (Gelernter et al., 2007; Ooteman et al., 2009). While there is accumulating evidence that this *OPRM1* genetic difference has functional importance in humans, before it can be used clinically, more information is needed especially from prospective and controlled clinical study investigation (O'Brien, 2008).

In addition to the *OPRM1* gene, there have been reports that functional genetic differences in several dopamine system genes alter reward based brain mechanisms. The dopamine transporter (DAT1) is responsible for clearing dopamine from the synapse and, as such, it is important in DA synaptic control and neurotransmission. The DAT1 gene (SCL6A3) has a 40-bp variable number of tandem repeat (VNTR) sequences located in the 3' untranslated region, the most common being nine and ten VNTR's. The ten VNTR has been reported to show higher DAT expression than the nine or lesser VNTR (Fuke et al., 2001). Therefore, hypothetically, nine VNTR individuals (either homozygote or heterozygote) are likely to have less DAT and higher synaptic dopamine. It has been reported that 9/9 or 9/10 carriers compared to 10/10 carriers have a greater brain ventral striatal response to both anticipation and receipt of reward during fMRI paradigms (Dreher et al., 2009; Forbes et al., 2009), suggesting that greater synaptic DA availability might underlie reward salience. While nine VNTR carriers also were reported to have greater ventral striatal responses to smoking cues (Franklin et al., 2009; Franklin et al., 2011), to our knowledge this has not been explored with alcohol consumption or cues and/or how it might predict medication response.

Since it has been speculated that dopamine tone/release might underlie the stimulant response to alcohol observed in heavy drinkers (King et al., 2002) and early stage alcoholics

¹Note that although this SNP is referred to in the literature, as well as this manuscript, as the asn40asp (or the A118G SNP), this designation has been recently updated in the public bioinformatics databases (ABI, NCBI, HapMap) as it has been determined that the mu-opioid receptor may contain an additional 62 amino acids. The new designation of this SNP on the NCBI Human Genome Assembly 36 is asn102asp (or A355G) (<http://www.ncbi.nlm.nih.gov/SNP>).

(Thomas et al., 2004) and since naltrexone reduces ventral striatal dopamine output in rodents (Gonzales and Weiss, 1998) and also reduces alcohol-induced stimulation in man (Anton et al., 2004; Ray and Hutchison, 2007) it is conceivable that there might be a salient interaction between the opiate and dopamine systems that might be genetically based and modified by naltrexone. For instance, DAT 9-repeat carriers might be more likely to have elevated dopamine levels in nucleus accumbens after alcohol consumption or cue-presentation and therefore be more likely to respond to naltrexone - secondary to its ability to decrease alcohol or cue-induced dopamine release.

The primary aim of this study was to examine whether naltrexone would alter alcohol consumption and effects based on *OPRM1* genotype (with asp40 naltrexone-treated individuals drinking the least) using a previously validated (O'Malley et al., 2002; Drobos et al., 2003; Anton et al., 2004) acute dosing and bar-lab paradigm in non-treatment seeking alcoholics. A secondary aim was to explore the role of DAT genetic differences on this effect (that DAT 9 VNTR carriers would respond the best to naltrexone and potentially enhance the *OPM1* genetic effect).

MATERIALS AND METHODS

Participants

Two hundred and sixty five non-treatment seeking alcoholics between the ages of 21 and 65 were screened and genotyped for this study and 83 were selected for participation in the double blind protocol. Only Caucasian subjects (by verbal report) who met DSM-IV criteria for alcohol dependence (APA, 1994) were included secondary to low asp40 allele frequency in African Americans.

Exclusion criteria were as follows: current DSM-IV criteria for drug dependence (excluding nicotine) by verbal report and urine drug screens, other major DSM-IV Axis I disorders, psychoactive medication or substance use (except marijuana) in the past 30 days or a positive urine drug screen, current suicidal or homicidal ideation, past history of alcohol-related medical illness, liver enzymes 3 times above normal, or significant health problems.

Baseline Assessment Procedures

The Investigational Review Board approved this study and subjects signed informed consent. Genotyping was done within 5 days of blood draw. All subjects with the 118 AG (asn40asp) or 118 GG (asp40asp) genotype, and those matched/yoked to them (see below) who had the 118 AA (asn40asn) genotype were asked to return in 2-3 days for further evaluation with a number of standard interview, questionnaire, and medical diagnostic procedures as previously reported by our group (Anton et al., 2004; Voronin et al., 2008). Interview procedures included a demographic form, the SCID (First MB et al., 1997) and a timeline follow-back interview (Sobell et al., 1988) to quantify drinking during the preceding 90 days. The Obsessive-Compulsive Drinking Scale (OCDS) (Anton et al., 1996) and the Alcohol Dependence Scale (ADS) (Skinner and Allen, 1982) were administered. Finally, a urine drug screen, blood tests for health screening, and a physical exam were conducted.

In addition, several scales were employed during the bar-lab setting to identify effects of naltrexone based on *OPRM1* and DAT genes on acute alcohol effects and craving: the Biphasic Alcohol Effects Scale (BAES) (Martin et al., 1993), Subjective High Assessment Scale (SHAS); (Schuckit, 1984), and Alcohol Urge Questionnaire (AUQ) (Bohn et al., 1995).

Randomization and Medication Dosing

Participants who passed all screening and eligibility criteria were urn-randomized (based on sex and smoking status) to receive 25 mg (2 days) and 50 mg (5 days) of naltrexone $n=38$ (19 asp40; 19 asn40) or identical placebo capsules $n=45$ (24 asp40; 21 asn40) for seven days in a blinded fashion. While subjects remained blind to their genotype, investigators attempted to select/match the more frequent asn40asn genotype subjects to the more rare asp40 subjects based on gender, smoking, alcohol problem severity (e.g. ADS scores) and alcoholism family history to minimize salient differences between the genetic sub-groups. All study medications were blister packed and administered in identical standard gel caps with 25 mg riboflavin added.

Experimental Procedures and Outcome Assessments

Participants were given no explicit instructions regarding their drinking behavior for Days 1 – 5 but were required to abstain completely on Day 6 and the morning of day 7. On Day 6, subjects were assessed for alcohol withdrawal using the Clinical Institute Withdrawal Assessment for Alcohol – Revised (Sullivan et al., 1989) (highest score was 3) and urine sample was collected to ascertain riboflavin levels for medication compliance. A 6-Day version of the timeline follow-back interview (which recorded alcohol consumption during the medication period) was done. On that evening, they underwent an fMRI brain scan paradigm (Myrick et al., 2008) that will be reported separately.

On Day 7 participants were observed to ingest the last dose of study medication at 11:30am and 30 minutes later were provided a standard caloric lunch (weight and gender adjusted). At 2:00 pm, the curtain in the bar lab was opened to reveal bar-like cues and they consumed a standard dose of spirits (vodka, gin, rum or bourbon) calculated to achieve a breath alcohol concentration (BAC) level of about 20-30mg% (0.3 gm/L) (Watson, 1989). The drink was 1 part spirits diluted in 3 parts fruit juice (both of the subject's choosing) and consumed over 5 minutes. Serial BAC measurements were done at 10, 20, and 30 minutes after drink consumption. The Biphasic Alcohol Effects Scale (BAES), Subjective High Assessment Scale (SHAS), and Alcohol Urge Questionnaire (AUQ) were done prior to each BAC measurements. At 40 minutes after the initial drink participants were brought the first tray of 4 "mini-drinks" (each consisting of 1/2 the alcohol consumed in the initial drink) and were told that they could consume as many as they desired over the next hour period. Another 4 "mini-drinks" were made available for consumption over the second hour. A BAC assessment was done at the end of each hour of free access.

As per our previous paradigms and consistent with that of O'Malley (O'Malley et al., 2002), we chose to assess alcohol consumption in the context of an alternative reinforcer, so subjects was given a "bar-credit" of \$16 to buy up to 8 mini-drinks (\$2 each) or choose to receive any money not spent. Participants had access to one bag of potato chips (28.3 gram/ bag) per tray to simulate a normal bar environment and water was also provided.

After the procedure, participants remained until 10:00 pm and were given dinner and could read, listen to music, or watch videos - but were also provided educational materials regarding alcohol effects to motivate a change in drinking behavior. A breathalyzer reading below 20 mg% was required at departure and they were driven home by a friend or taxi. The following day, a counseling session was given to educate participants about alcohol harm and to increase motivation to reduce drinking or seek treatment. Participants received \$400 one week later.

Genotyping

OPRM1—Genomic DNA was extracted from peripheral blood mononuclear cells using a commercial DNA extraction kit/procedure (Gentra Puragene Blood Kit, Qiagen Inc., Valencia CA). The Taqman 5' nuclease genotyping assay was used for *OPRM1* SNP analysis (Applied Biosystems (ABI), Foster City, CA).

The 10 µl reaction mixture consists of 5 µl of TaqMan® Genotyping Master Mix, 1 µl of 40X Assay Mix (ABI) (8 µM detection probe for each allele, 36 µM forward and reverse primer each), 10 ng of genomic DNA diluted in 2 µl of Tris EDTA (TE) pH 8.0 (Quality Biological, Inc; Gaithersburg, MD) and 2 µl of PCR Water. Amplification was performed with a StepOne™ Real-Time PCR System v1.0 (ABI) using 48-well plates and the following amplification profile: 60°C for 30 s and 95°C for 10 min, followed by 50 cycles of 92°C for 15 s and 60°C for 1 min. After amplification, endpoint fluorescence intensity was measured directly in the reaction plates, directly on the ABI StepOne™ System. Genotypes were determined using a StepOne™ Software v1.0 (ABI). Four genotyping signal clusters were identified, representing asn40 and asp40 homozygotes, asn40/asp40 heterozygotes and DNA-free-template controls. Subject samples were handled the same as at least 3 controls of each of the above genotypes in each assay. The fluorescence intensity of the subject sample was compared to that of the control clusters and visually identified and classified as to correct genotype.

Dopamine Transporter Gene (DAT-SCL6R3) dat genotyping—PCR amplification was carried out for DAT VNTR with primers 5'-TGT GGT GTA GGG AAC GGC CTG AG-3' and 5'-CTT CCT GGA GGT CAC GGC TCA AGG-3' (Invitrogen, Carlsbad, CA) in a total volume of 15 µL containing 120 ng of genomic DNA, 1.6 µL of 10× PCR buffer, 0.45 µL of 50 mM MgCl₂, 0.25 µL of 5 U/µL Platinum® Taq DNA Polymerase, 1.5 µL each of 10 µM forward and reverse primers, 0.3 µL each of 10 mM dNTPs (all reagents supplied by Invitrogen), and 3.55 µL of nuclease-free water. Amplification was performed via PCR StepOne™ using 48-well plates under the following conditions: 94°C for 3 minutes, followed by 35 cycles of 94°C for 15 seconds and 72°C for 1 minute and 40 seconds, and a final 10-minute incubation at 72°C. Electrophoresis was performed on 15 µL of each sample on 2.0% agarose gels in 1XTAE and visualized with ethidium bromide under UV light. Genotypes were scored by two people independently and rerun when necessary.

Genotyping Quality Control

For *OPRM1* genotyping, approximately the first 100 subjects were genotyped in both our lab and that of David Goldman (Neurogenetics Lab at NIAAA) with 100% agreement. Four samples each representing asn40 homozygotes as well as asp40asn hetero and asp40asp homozygotes, with identical identification in both labs were used as “positive controls” for all subsequent Taqman assays.

For the DAT VNTR assay at least 2 of each 9,9 VNTR and 10,10 VNTR samples were sequenced as follows: after electrophoresis on a 2.0% agarose gel, samples were isolated (QIAquick® Gel Extraction Kit, QIAGEN Sciences) and sequenced in both directions using an ABI377 automated sequencer at the MUSC Nucleic Acid Analysis Facility (Charleston, SC). Results of the sequence were checked against published sequences for the specific SCL3R6 VNTR sequences. Once confirmed, these samples were used as positive controls for all subsequent genotyping to identify the appropriate bands on agarose gels indicating DAT genotypes of 9,9; 9,10; or 10,10 VNTR's.

Medication Compliance Measurement

At the end of the study, and once analysis of the main outcome variables were conducted, the medication assignment blind was broken and all day-6 urines of those assigned to take naltrexone as well as a random selection (n=10) assigned to take placebo were analyzed for naltrexone/6beta-naltrexol levels by LC-MS/MS (Laboratory of Peter Jatlow M.D., Yale University School of Medicine) to assure proper medication assignment and compliance. All subjects assigned to the naltrexone group had evidence of urine naltrexone/6beta-naltrexol while the placebo group had no measureable levels.

Riboflavin, as a measure of compliance (available on 76 individuals) was measured with fluorometric assay based on standard curves of weighed-in riboflavin (Anton, 1996). Compliance was defined as either a day-6 urine riboflavin over 1500 ug/ml or a doubling from pre-study baseline to day-6. There were 88% (Ntx/asn40), 83% (Ntx/asp40), 75% (placebo/asn40), and 90% (placebo/asp40) compliant individuals that did not differ significantly between medication groups ($X^2=0.110$, $p=0.74$) or genotype ($X^2=0.531$, $p=0.47$).

Mean peak blood alcohol concentrations (BAC) done after the priming drink targeted at 20-30mg% (equivalent to 2 standard drinks) in fact ranged from 21-26mg% for the four *OPRM1* gene by medication groups and was not statistically significant.

Primary Outcome Variables and Data Analysis

The two primary outcome variables identified a priori were 1) drinking during the 5-day natural drinking period (based on TLFB collected on Day 6) and 2) number of drinks consumed after the standard/priming drink in the bar-lab on day 7. In order of importance based on our past work the secondary outcome variables were identified as the BAES stimulation and sedation scales after the priming drink, as well as the SHAS and AUQ scale scores after the priming drink.

The analytic plan called for the initial analysis to be the main effects of the medication group and *OPRM1* genotype and, most importantly, their interaction on the primary outcome variables using two-way ANOVA. Subsequent exploratory analysis evaluated the role of DAT genotype alone, or interacting, with the *OPRM1* genotype and medication group (three-way ANOVA). A similar plan was applied to other secondary variables and other variables of interest (SHAS, AUQ, BAES, riboflavin levels) where appropriate.

RESULTS

Study Subjects

Salient subject demographics, alcohol consumption, severity and craving parameters by medication and *OPRM1* group are provided in table 1. The groups were generally well-balanced on all key variables with few significant differences. There was about 2:1 males to females with on average several years of college education. Overall, subjects drank heavily in a bit over 50% of the days prior to randomization and had 8-10 drinks per drinking day on average with ADS and OCDS scores in the mild to moderate range. In general this reflects the demographics of the wider population who were screened for study inclusion.

Genotyping Results and Assignments

The *OPRM1* genotyping was successful with 100% of the genotypes being completed in 265 individuals with 61 individuals having at least one asp40 allele, for an asp40 carrier rate of 23% which is consistent with Hapmap data for European Caucasians and exactly the rate reported for treatment-seeking alcoholics in the COMBINE Study (Anton et al., 2008).

Genotype frequency did not deviate from HWE ($X^2(2)=2.14$, $p=.344$). Forty-three of the asp40 carriers met further inclusion/exclusion criteria and were randomized to naltrexone ($n=19$) or placebo ($n=24$) while 40 individuals with an asn40asn genotype who were closely matched to the asp40 individuals were also randomized to naltrexone ($n=19$) or placebo ($n=21$).

DAT genotyping occurred in 265 individuals and 113 individuals had at least one 9 VNTR (43%) while 152 individuals had a 10/10 VNTR genotype (57%) consistent with other reports (Van der Zwaluw et al., 2009). Genotype frequency did not deviate from HWE ($X^2(2)=2.66$, $p=.26$). For randomized individuals ($N=83$) the frequency of the *OPRM1* by DAT genotype combinations were as follows: asp40 *OPRM1*/at least one 9 DAT VNTR ($n=19$), asp40 *OPRM1*/10,10 DAT VNTR ($n=24$), asn40asn *OPRM1*/at least one 9 DAT VNTR ($n=11$) and asn40asn *OPRM1*/10,10 DAT VNTR ($n=29$). Figure one shows the distribution (n) of these variants among medication groups.

Medication by OPRM1 genotype

Natural Drinking—The interaction of naltrexone with *OPRM1* genotype on subject drinking under normal environmental conditions during the five days prior to experimental procedures was not significantly different either for drinks per day or percent heavy drinking days (table 2). There were no significant main effects of *OPRM1* genotype ($F(1,81)=0.70$, $p=0.40$) or medication ($F(1,81)=2.15$, $p=.15$) on drinks per day and no significant main effects of *OPRM1* genotype ($F(1,81)=.32$, $p=0.57$) or medication ($F(1,81)=.545$, $p=.46$) on percent heavy drinking days. Adding covariates of age, ADS score, or baseline drinking did not substantially alter the findings. Sex did not have an effect on this analysis.

Bar Lab Drinking—The interaction of naltrexone with *OPRM1* genotype on the amount of drinks consumed after the priming drink in the bar-lab (table 2) was also not significant ($F(1,79)=1.20$, $p=.28$). There was also no main effect of *OPRM1* genotype ($F(1,81)=0.01$, $p=.99$) or medication ($F(1,81)=.01$, $p=.99$) on the amount of drinks consumed in the bar lab.

Alcohol Stimulation and Sedation—As measured by the BAES after the priming drink there was no significant interaction of medication by *OPRM1* genotype on stimulation ($F(1,79)=0.271$, $p=0.60$) or sedation ($F(1,79)=.008$, $p=0.93$) nor was there a main effect of medication on stimulation ($F(1,81)=1.08$, $p=.30$) or sedation ($F(1,81)=1.12$, $p=.29$). There was a trend ($F(1,81)=3.81$, $p=0.054$) for the asn40 allele subjects to have more alcohol stimulation (BAES peak score 12.9 ± 11.3 for asn40 and 8.3 ± 9.8 for asp40) but no significant difference on sedation ($F(1,81)=0.542$, $p=.46$). Peak blood alcohol level as a covariate had no material effect on these analyzes.

Alcohol induced craving (AUQ), high (SHAS)—There were no significant main effects or interactions of medication group and *OPRM1* genotype on alcohol-induced craving (p values > 0.3 for main effects and interaction) or high (p values > 0.4 for main effects and interaction) after the priming drink. Peak blood alcohol level as a covariate had no material effect on these analyzes.

Medication by OPRM1 and DAT genotypes

Natural drinking—During the 5-day natural environment drinking period there was a three way interaction ($F(1,75)=6.83$, $p=0.011$) between medication group, *OPRM1* genotype and DAT genotype on the number of drinks per day (figure 1). Decomposing this three-way interaction showed that when there was at least one DAT 9 VNTR present, naltrexone reduced alcohol consumption only when the subject also had the asn40asn genotype ($p=0.006$) but when there was no DAT 9 VNTR (i.e. 10/10 genotype) there was no

medication by *OPRM1* genotype interaction. The same three-way interaction was present with percent heavy drinking days but at a trend level ($F(1,75)=3.43$, $p=0.068$). The three-way interaction for number of drinks per day was similar but became even more significant for heavy drinking days when age, ADS score, and baseline drinking were used as covariates in the analyses ($p=0.015$ for drinks per day and $p=0.039$ for percent heavy drinking days).

Bar Lab Drinking—There were no significant main effects of DAT genotype ($F(1,81)=2.14$, $p=0.15$) or interactions with *OPRM1* ($F(3,79)=0.77$, $p=0.52$) or medication ($F(1,79)=0.01$, $p=0.92$) on alcohol consumed after the priming drink in the bar lab.

Alcohol Stimulation and Sedation—There was no three-way interaction between medication group, *OPRM1* genotype and DAT genotype on post alcohol priming drink stimulation ($F(1,75)=1.52$, $p=0.22$). Although, there was no significant two-way interaction ($F(1,79)=0.021$, $p=0.89$) of genotypes (figure 2) there was a main effect of *OPRM1* genotype ($F(1,79)=5.13$, $p=0.028$) and a main effect of DAT genotype ($F(1,79)=0.032$) on alcohol stimulation, such that those subjects with *OPRM1* asn40asn had more stimulation than asp40 subjects and those with at least one DAT 9 VNTR had more stimulation compared to those with no DAT 9 VNTR (i.e. 10/10 genotype). Of interest, the most alcohol stimulation was observed in those with both the *OPRM1* asn40asn genotype and having at least one DAT VNTR 9, the same group that had the lowest drinking while on naltrexone during the 5-day natural observation period. There were no significant interactions of medication by the two genotypes or main effects of genotype on alcohol induced sedation.

Alcohol induced craving (AUQ), high (SHAS)—There was no significant main effects of genotypes or interaction of medication group, *OPRM1*, and DAT genotypes on alcohol induced craving ($p>0.5$ for main effects and interaction) or high ($p>0.5$ for main effects and interaction) after the priming drink.

DISCUSSION

This clinical experiment attempted to evaluate the importance of the putative *OPRM1* asn40asp genetic polymorphism in predicting naltrexone effects on alcohol in a group of individuals meeting criteria for alcohol dependence, but who were not seeking treatment. A number of studies had suggested that the asparagine to aspartate substitution at the 40 position on the mu opiate receptor protein resulted in a different response to alcohol, a greater mitigation of alcohol effects by naltrexone, or a greater treatment response to naltrexone (Ray et al., 2011). This study utilized a previously validated sub-acute naltrexone dosing and bar-lab strategy to further evaluate the role of this single SNP *OPRM1* difference under controlled conditions. In addition, since the brain opioid and dopamine systems interact, and likely play a role in reward and reinforcement, we also explored interaction of the *OPRM1* asp40 SNP with the putative dopamine transporter VNTR.

We did not find any significant interaction of naltrexone with the *OPRM1* genotypes of interest in this study. Surprisingly, there was also no significant main effect of naltrexone despite such an effect being evident in similar previous studies (O'Malley et al., 2002; Drobes et al., 2003; Anton et al., 2004) and no main effect of *OPRM1* genotype on alcohol consumption despite reports to the contrary (Ray and Hutchinson, 2004; Ray and Hutchinson, 2007). The reasons for this are unclear, but might include slight differences in study population, the a priori genetic analysis and selection influencing expectancies, or just a type II error (an a priori power calculation predicted 80% chance of finding a main effect of naltrexone and a 80% chance of detecting a naltrexone by *OPRM1* interaction with this sample size). Given this lack of “main effect” of naltrexone, the lack of an interaction

between genotype and medication might be considered less meaningful. On the other hand, it is also possible that the *OPRM1* gene by naltrexone interaction might not be as salient in this younger, less severe, and lower drinking population, compared to more severely affected treatment seekers. For instance, one might speculate that during the onset of alcohol dependence, reward salience might be more important than “compulsive” drinking. This could hypothetically mean that dopamine gene effects might be more salient in early-stage dependence, and only in later stages of dependence would opioid gene effects, and their interaction with naltrexone, become more salient and important. In general, this study population was less severe in amount of alcohol consumption and dependence symptoms, and their OCDS scores were much lower than in clinical trials where the asp40 SNP was associated with naltrexone response (e.g. Anton et. al. 2008). This might suggest that “habit” or “compulsive drinking” might be more influenced by the opioid system and moderated by naltrexone, especially in those where naltrexone might be more pharmacologically powerful (*OPRM1* asp40 carriers).

While only exploratory, we did find that a naltrexone effect on natural drinking was predicted by an interaction of DAT VNTR with *OPRM1* genotypes. Those with a least one DAT 9 VNTR who also had the asn40asn *OPRM1* genotype had a lower alcohol intake while taking naltrexone. The same individuals (DAT 9 VNTR/*OPRM1* asn40asn), who responded best to naltrexone, also had the highest stimulation to alcohol in the bar lab setting. While, in this study, we did not find an effect of naltrexone on this genetic interaction, in the past we have found that naltrexone actually broke the link between alcohol stimulation and further drinking (Anton et al., 2004). However, since the number of individuals in each DAT/*OPRM1* group is small, especially when split by medication assignment, these results require cautious interpretation.

Nevertheless, the fact that the DAT genotype might influence naltrexone response or interact with the *OPRM1* receptor has intuitive appeal. It has been speculated, and partially supported, that the dopamine transporter coded by the genotype with at least one 9 VNTR is less effective/functional than the one coded by the 10/10 VNTR genotype (Fuke et al., 2001). Since the dopamine transporter removes dopamine from the synaptic cleft, a “loss-of-function” genotype would allow more dopamine availability after pre-synaptic release. Theoretically, this should produce more reward and reinforcement and, perhaps, more stimulation. It has been reported that individuals with at least one DAT 9 VNTR genotype have a greater brain reward system response to reward anticipation and receipt during fMRI paradigms (Dreher et al., 2009; Forbes et al., 2009) and also a greater ventral striatal responses to smoking cues (Franklin et al., 2009; Franklin et al., 2011). To our knowledge this has not been explored in alcoholics and there has not been an attempt to evaluate the combined effects of DAT and *OPRM1* genetic differences simultaneously.

Of note, and consistent with this hypothesis, naltrexone has been shown to block the release of dopamine in the nucleus accumbens in animals (Gonzales and Weiss, 1998; Middaugh and Bandy, 2000; Ramchandani et al., 2011) and also ventral striatal activation to alcohol cues in man (Myrick et al., 2008). If a person also has a loss-of-function dopamine transporter gene i.e. at least one 9 VNTR, then it would be those very people that might respond most robustly to the dopamine reducing effects of naltrexone. They would be the most “reward sensitive” to alcohol and would get a reciprocal down regulation in dopamine-induced alcohol stimulation while taking naltrexone, translating into less stimulation and less drinking. Why this should not occur more strongly in asp40 *OPRM1* genotype individuals (the ones that had been thought to be most naltrexone responsive) is not clear. However, as stated previously, there might be either developmental issues, and/or brain regional effect differences etc. that are not completely understood at this time.

The field of pharmacogenetics is evolving and as knowledge accumulates, more gene-gene and gene-environment interactions are likely to evolve as well. This layered on top of the growing appreciation that alcohol dependence is a pathophysiological process that changes over time, will make the simultaneous evaluation of genes in different salient brain systems more important. In addition, more specificity in the definition of alcohol using and dependent populations is likely to assume a greater importance for alcohol and/or medication by gene interactions. To the extent that the data presented here reinforce that concept, their importance transcends their immediate relevancy.

Acknowledgments

The authors wish to acknowledge the assistance of the following people. Patricia Latham, PhD, RN, Tara Wright, MD, Scott Stewart, MD, and Derrick Vergne, MD, helped support the recruitment and assessment of subjects. Gabor Oroszi, MD, PhD, Garrick Klaybor, BS, and Melanie McMillan, MS helped with genotyping. Maggie Wilkes MD assisted in data management and analysis. Travis Poole, BS provided editorial assistance and graphic display. David Goldman MD, PhD, (NIAAA lab for Neurogenetics) consulted on genotyping measurement and provided *OPRM1* genotyping assistance.

Supported by NIAAA grants P50 AA010761 and K05 AA017435.

REFERENCES

- Anton RF. New methodologies for pharmacologic treatment trials for alcohol dependence. *Alcohol Clin Exp Res*. 1996; 20:3A–9A. [PubMed: 8651457]
- Anton RF, Drobos DJ, Voronin K, Durazo-Avizu R, Moak D. Naltrexone effects on alcohol consumption in a clinical laboratory paradigm: Temporal effects of drinking. *Psychopharmacology*. 2004; 173:32–40. [PubMed: 14722705]
- Anton R, Moak D, Latham P. The obsessive compulsive drinking scale (OCDS): A new method of assessing outcome in alcoholism treatment studies. *Arch Gen Psychiatry*. 1996; 53:225–231. [PubMed: 8611059]
- Anton RF, Oroszi G, O'Malley SS, Couper DJ, Swift R, Pettinati HM, Goldman D. An evaluation of μ -opioid receptor (*OPRM1*) as a predictor of naltrexone response in the treatment of alcohol dependence: Results from the combined pharmacotherapies and behavioral interventions for alcohol dependence (COMBINE) study. *Arch Gen Psychiatry*. 2008; 65:135–144. [PubMed: 18250251]
- APA. *Diagnostic and Statistical Manual of Mental Disorders*. American Psychiatric Press; Washington, DC: 1994.
- Barr CS, Chen SA, Schwandt ML, Lindell SG, Sun H, Suomi SJ, Heilig M. Suppression of alcohol preference by naltrexone in the rhesus macaque: a critical role of genetic variation at the micro-opioid receptor gene locus. *Biol Psychiatry*. 2010; 67:78–80. [PubMed: 19748082]
- Barr CS, Schwandt M, Lindell SG, Chen SA, Goldman D, Suomi SJ, Higley JD, Heilig M. Association of a Functional Polymorphism in the {micro}-Opioid Receptor Gene With Alcohol Response and Consumption in Male Rhesus Macaques. *Arch Gen Psychiatry*. 2007; 64:369–376. [PubMed: 17339526]
- Bohn MJ, Krahn DD, Staehler BA. Development and initial validation of a measure of drinking urges in abstinent alcoholics. *Alcohol Clin Exp Res*. 1995; 19:600–606. [PubMed: 7573780]
- Dreher JC, Kohn P, Kolachana B, Weinberger DR, Berman KF. Variation in dopamine genes influences responsivity of the human reward system. *Proc Natl Acad Sci U S A*. 2009; 106:617–622. [PubMed: 19104049]
- Drobos DJ, Anton RF, Thomas SE, Voronin K. A clinical laboratory paradigm for evaluating medication effects on alcohol consumption: naltrexone and nalmefene. *Neuropsychopharmacology*. 2003; 28:755–764. [PubMed: 12655322]
- First, MB.; Spitzer, RL.; Gibbon, M.; Williams, JBW. *Structured Clinical Interview for DSM-IV Axis I Disorders - Patient Edition*. New York State Psychiatric Institute; New York, NY: 1997.

- Forbes EE, Brown SM, Kimak M, Ferrell RE, Manuck SB, Hariri AR. Genetic variation in components of dopamine neurotransmission impacts ventral striatal reactivity associated with impulsivity. *Mol Psychiatry*. 2009; 14:60–70. [PubMed: 17893706]
- Franklin TR, Lohoff FW, Wang Z, Sciortino N, Harper D, Li Y, Jens W, Cruz J, Kampman K, Ehrman R, Berrettini W, Detre JA, O'Brien CP, Childress AR. DAT genotype modulates brain and behavioral responses elicited by cigarette cues. *Neuropsychopharmacology*. 2009; 34:717–728. [PubMed: 18704100]
- Franklin TR, Wang Z, Li Y, Suh JJ, Goldman M, Lohoff FW, Cruz J, Hazan R, Jens W, Detre JA, Berrettini W, O'Brien CP, Childress AR. Dopamine transporter genotype modulation of neural responses to smoking cues: confirmation in a new cohort. *Addiction Biology*. 2011; 16:308–322. [PubMed: 21299752]
- Fuke S, Suo S, Takahashi N, Koike H, Sasagawa N, Ishiura S. The VNTR polymorphism of the human dopamine transporter (DAT1) gene affects gene expression. *Pharmacogenomics J*. 2001; 1:152–156. [PubMed: 11911442]
- Gelernter J, Gueorguieva R, Kranzler HR, Zhang H, Cramer J, Rosenheck R, Krystal JH. Opioid receptor gene (OPRM1, OPRK1, and OPRD1) variants and response to naltrexone treatment for alcohol dependence: Results from the VA cooperative study. *Alcohol Clin Exp Res*. 2007; 31:555–563. [PubMed: 17374034]
- Gonzales RA, Weiss F. Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. *J Neurosci*. 1998; 18:10663–10671. [PubMed: 9852601]
- King AC, Houle T, De Wit H, Holdstock L, Schuster A. Biphasic alcohol response differs in heavy versus light drinkers. *Alcohol Clin Exp Res*. 2002; 26:827–835. [PubMed: 12068251]
- Kornet M, Goosen C, Van Ree JM. Effect of naltrexone on alcohol consumption during chronic alcohol drinking and after a period of imposed abstinence in free-choice drinking rhesus monkeys. *Psychopharmacology*. 1991; 104:367–376. [PubMed: 1924644]
- Kranzler HR, Van Kirk J. Efficacy of naltrexone and acamprosate for alcoholism treatment: A meta-analysis. *Alcohol Clin Exp Res*. 2001; 25:1335–1341. [PubMed: 11584154]
- Martin CS, Earleywine M, Musty RE, Perrine MW, Swift RM. Development and validation of the biphasic alcohol effects scale. *Alcohol Clin Exp Res*. 1993; 17:140–146. [PubMed: 8452195]
- Middaugh LD, Bandy A-LE. Naltrexone effects on ethanol consumption and response to ethanol conditioned cues in C57BL/6 mice. *Psychopharmacology*. 2000; 151:321–327. [PubMed: 11026738]
- Myrick H, Anton RF, Li X, Henderson S, Randall PK, Voronin K. Effect of naltrexone and ondansetron on alcohol cue-induced activation of the ventral striatum in alcohol-dependent people. *Arch Gen Psychiatry*. 2008; 65:466–475. [PubMed: 18391135]
- O'Brien CP. Prospects for a genomic approach to the treatment of alcoholism. *Arch Gen Psychiatry*. 2008; 65:132–133. [PubMed: 18250250]
- O'Malley SS, Krishnan-Sarin S, Farren C, Sinha R, Kreek MJ. Naltrexone decreases craving and alcohol self-administration in alcohol-dependent subjects and activates the hypothalamo-pituitary-adrenocortical axis. *Psychopharmacology*. 2002 see comment.
- Ooteman W, Naassila M, Koeter MW, Verheul R, Schippers GM, Houchi H, Daoust M, Van Den Brink W. Predicting the effect of naltrexone and acamprosate in alcohol-dependent patients using genetic indicators. *Addiction Biology*. 2009; 14:328–337. [PubMed: 19523047]
- Oslin DW, Berrettini W, Kranzler HR, Pettinati HM, Gelernter J, Volpicelli JR, O'Brien CP. A functional polymorphism on the μ -opioid receptor gene is associated with naltrexone response in alcohol-dependent patients. *Neuropsychopharmacology*. 2003; 28:1546–1552. [PubMed: 12813472]
- Ramchandani VA, Umhau J, Pavon FJ, Ruiz-Velasco V, Margas W, Sun H, Damadzic R, Eskay R, Schoor M, Thorsell A, Schwandt ML, Sommer WH, George DT, Parsons LH, Herscovitch P, Hommer D, Heilig M. A genetic determinant of the striatal dopamine response to alcohol in men. *Mol Psychiatry*. 2011; 16:809–817. [PubMed: 20479755]

- Ray LA, Barr CS, Blendy JA, Oslin D, Goldman D, Anton RF. The Role of the Asn40Asp Polymorphism of the Mu Opioid Receptor Gene (OPRM1) on Alcoholism Etiology and Treatment: A Critical Review. *Alcohol Clin Exp Res*. 2011 In Press.
- Ray LA, Hutchison KE. Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response: A double-blind placebo-controlled study. *Arch Gen Psychiatry*. 2007; 64:1069–1077. [PubMed: 17768272]
- Ray LA, Hutchison KE. A polymorphism of the mu-opioid receptor gene (*OPRM1*) and sensitivity to the effects of alcohol in humans. *Alcohol Clin Exp Res*. 2004; 28:1789–95. [PubMed: 15608594]
- Schuckit M. Subjective responses to alcohol in sons of alcoholics and control subjects. *Arch Gen Psychiatry*. 1984; 41:879–884. [PubMed: 6466047]
- Skinner HA, Allen BA. Alcohol dependence syndrome: measurement and validation. *J Abnorm Psychol*. 1982; 91:199–209. [PubMed: 7096790]
- Sobell LC, Sobell MB, Leo GI, Cancilla A. Reliability of a timeline method: assessing normal drinkers' reports of recent drinking and a comparative evaluation across several populations. *Br J Addict*. 1988; 83:393–402. [PubMed: 3395719]
- Streeton C, Whelan G. Naltrexone, a relapse prevention maintenance treatment of alcohol dependence: a meta-analysis of randomized controlled trials. *Alcohol Alcohol*. 2001; 36:544–552. [PubMed: 11704620]
- Sullivan JT, Sykora K, Schneiderman J, Naranjo CA, Sellers EM. Assessment of alcohol withdrawal: the revised clinical institute withdrawal assessment for alcohol scale (CIWA-Ar). *Br J Addict*. 1989; 84:1353–1357. [PubMed: 2597811]
- Thomas SE, Drobos DJ, Voronin K, Anton RF. Following alcohol consumption, nontreatment-seeking alcoholics report greater stimulation but similar sedation compared with social drinkers. *J Stud Alcohol*. 2004; 65:330–335. [PubMed: 15222589]
- Vallender EJ, Ruedi-Bettschen D, Miller GM, Platt DM. A pharmacogenetic model of naltrexone-induced attenuation of alcohol consumption in rhesus monkeys. *Drug Alcohol Depend*. 2010; 109:252–256. [PubMed: 20153935]
- Van Der Zwaluw CS, Engels RC, Buitelaar J, Verkes RJ, Franke B, Scholte RH. Polymorphisms in the dopamine transporter gene (SLC6A3/DAT1) and alcohol dependence in humans: a systematic review. *Pharmacogenomics*. 2009; 10:853–866. [PubMed: 19450132]
- Voronin K, Randall P, Myrick H, Anton R. Aripiprazole effects on alcohol consumption and subjective reports in a clinical laboratory paradigm--possible influence of self-control. *Alcohol Clin Exp Res*. 2008; 32:1954–1961. [PubMed: 18782344]
- Watson, TE. Total body water and alcohol levels: updating the fundamentals. In: BATT, CA., editor. *Human Metabolism of Alcoholism*. CRC Press; Boca Raton: 1989.

Figure 1. Drinking (drinks/day) under natural conditions in non-treatment seeking alcoholics while taking naltrexone and placebo by *OPRM1* and DAT genotypes. There was a significant three-way interaction of medication group by *OPRM1* by DAT (see text for detail). Those with at least one DAT 9 VNTR and who were *OPRM1* asn40 homozygotes had the greatest response to naltrexone. Sample size (n) for each group is given in insert boxes within each column.

Figure 2.

Alcohol-induced stimulation after a priming drink based on *OPRM1* and DAT genotypes. There were main effects of *OPRM1* genotype (asn40 more than asp40) as well as DAT genotype (9 VNTR carriers more than 10 VNTR homozygotes). See text for detail.

Table 1

Demographics, drinking history, and severity of randomized non-treatment seeking alcohol dependent individuals. Asp40 indicates either heterozygous (asn40asp) or homozygous (asp40asp) at the *OPRM1* 118 locus.

	Naltrexone		Placebo		Medication		Genotype		Gene x Med	
	Asn40	Asp40	Asn40	Asp40						
N = 83	19	19	21	24						
Gender: male/female	13/6	12/7	14/7	15/9	X ²	P	X ²	P	X ²	P
Age	31 +/- 7	29 +/- 10	23 +/- 10	28 +/- 10	F (df 1,79)	P	F (df 1,79)	P	F (df 1,79)	P
Education (years)	15 +/- 1	14 +/- 2	15 +/- 2	15 +/- 2	3.98	0.05	0.548	0.46	2.43	0.12
ADS score	13 +/- 3	10 +/- 4	13 +/- 4	10 +/- 4	0.076	0.78	1.37	0.26	1.66	0.20
OCDS score	15 +/- 4	16 +/- 6	17 +/- 6	16 +/- 6	0.386	0.54	8.19	0.005	0.047	0.83
Drinks/day	6.1 +/- 2.0	5.0 +/- 2.8	6.5 +/- 2.9	5.7 +/- 2.8	0.163	0.69	0.016	0.90	0.082	0.78
Drinks/drinking day	9.7 +/- 3.4	8.0 +/- 4.7	9.5 +/- 4.8	9.6 +/- 4.7	0.879	0.35	2.21	0.14	0.103	0.75
% Heavy Drinking days	54 +/- 14	50 +/- 19	57 +/- 19	51 +/- 19	0.497	0.48	0.644	0.43	0.709	0.40
					0.288	0.59	1.29	0.26	0.027	0.87

Alcohol consumption during the 5-day natural observation period and after the priming drink in the bar-lab based on *OPRM1* genotype and medication group. Asp40 indicates either heterozygous (asn40asp) or homozygous (asp40asp) at the *OPRM1* 118 locus.

Table 2

	Naltrexone		Placebo		F (df 1,79)	P
	asn40asn	asp40	asn40asn	asp40		
N	19	19	21	24		
5 Day Drinking:						
Drinks/Day	4.7 ± 1.8	5.7 ± 3.2	6.2 ± 3.4	6.3 ± 3.7	0.44	0.51
Heavy Drinking Days (%)	44 ± 21	53 ± 27	53 ± 24	52 ± 27	0.85	0.36
Bar Lab Drinks	3.0 ± 2.9	3.8 ± 3.1	3.8 ± 2.8	3.1 ± 3.3	1.20	0.28