A cis-eQTL in *OPRM1* is Associated with Subjective Response to Alcohol and Alcohol Use

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Background: A functional polymorphism within the μ-opioid receptor (*OPRM1*) gene, rs1799971 (A118G), previously has been associated with measures of alcohol use and sensitivity to its effects, but findings have been inconclusive. A recent study suggested that a second nearby variant within *OPRM1*, rs3778150, is robustly associated with heroin dependence and fully explained a smaller observed association with rs1799971. Given evidence that the rs3778150-C allele is associated with decreased *OPRM1* expression levels in the human brain, the current study sought to test the hypothesis that rs3778150 represents a causal variant within *OPRM1* that increases risk for a variety of alcohol use phenotypes.

Methods: Participants with genotype and phenotype data from a larger experimental study (N = 152) were assessed on measures of subjective response to alcohol and alcohol use. Measures included (i) the Self-Rating of the Effects of Alcohol and the Alcohol Sensitivity Questionnaire, (ii) the Biphasic Alcohol Effects Scale (BAES) and ratings of subjective intoxication, and (iii) average number of drinks per week in the past month.

Results: Compared to rs3778150-T homozygous individuals, carriers of the rs3778150-C allele exhibited significantly lower retrospective self-report levels of alcohol sensitivity. Carriers of the rs3778150-C allele also exhibited lower levels of BAES alcohol-related stimulation during an alcohol challenge and reported higher levels of drinking in the last 30 days. With the exception of lower levels of BAES alcohol-related sedation, the rs1799971 variant did not show consistent significant association with any of the alcohol phenotypes in the presence of rs3778150.

Conclusions: Results suggest that rs3778150 may be causally related to alcohol use phenotypes, and could potentially account for previously observed associations of rs1799971 with substance use phenotypes. Future studies may investigate potential causal relations among genetic variants in *OPRM1*, subjective response to alcohol, and drinking phenotypes to further delineate the effects of rs3778150.

Key Words: *OPRM1*, Genetics, Subjective Response to Alcohol, Alcohol Challenge.

GENETIC FACTORS ARE one of numerous components thought to contribute to the development of substance use disorders (Heath et al., 1997). Heavy and problematic alcohol use behaviors demonstrate moderate levels of heritability (Heath and Martin, 1994; Prescott et al., 1994, 1997), and findings from twin, family, and adoption studies indicate that genetic factors account for 48 to 66% of the variation in alcohol dependence (Agrawal et al., 2012). Despite evidence for a substantial genetic contribution to these traits, molecular genetic studies have had limited success in the identification of specific variants or genes contributing to these heritable influences. Alternative approaches have focused on intermediate phenotypes underlying risk for alcohol use disorders, including an individual's subjective response to alcohol (Ray et al., 2010, 2016). Alcohol produces a range of pharmacological responses largely related to its stimulating and pleasant effects, sedative and unpleasant effects, and tension reduction (Bujarski et al., 2015; Ray et al., 2009). Variability in a person's experience of these effects is thought to influence risk for problematic alcohol use and alcohol dependence (King et al., 2014; Schuckit and Smith, 1996). The subjective response to alcohol phenotype is also known to be heritable (Kalu et al., 2012; Viken et al., 2003) and therefore has been a focus of molecular genetic, pharmacogenetic, and neuroimaging studies seeking to elucidate its role in the development of problematic alcohol use and alcohol use disorders (Enoch, 2014; Ray et al., 2016).

Alcohol's mechanism of action includes its effects on the brain's "reward centers" in the ventral tegmental area (VTA) and nucleus accumbens via interactions with numerous neurotransmitter systems. As it relates to a person's subjective response to alcohol, the endogenous opioid system is thought to partially mediate the reinforcing effects of alcohol in these brain regions (Gianoulakis, 2004; Koob and Kreek, 2007; Mague and Blendy, 2010). Specifically, acute alcohol administration acts on μ-opioid receptors in the VTA and nucleus

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Received for publication October 3, 2016; accepted February 25, 2017.

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DOI: 10.1111/acer.13369

accumbens, thereby increasing extracellular levels of dopamine in the mesolimbic pathway. The gene encoding the μ-opioid receptor (*OPRM1*) has received considerable attention as a neurological target for exogenous opioid and non-opioid substances. Studies have identified a nonsynonymous single nucleotide polymorphism (SNP) in *OPRM1* (rs1799971; A118G) that causes the Asn40Asp substitution from asparagine to aspartic acid at a *N*-glycosylation site (Ray et al., 2012). Although findings from in vitro studies have been mixed (for a review, see Ray et al., 2012), the amino acid change brought on by the presence of the minor (Asp) G allele has been shown to triple the μ-opioid receptor's binding affinity for β-endorphin and reduce cell-surface receptor binding site availability (Bond et al., 1998; Kroslak et al., 2007), and has also shown association with reduced *OPRM1* messenger RNA (mRNA) expression (Zhang et al., 2005).

Given the potential functional relevance of rs1799971 to μ-opioid receptor activity, numerous investigations have examined the association of rs1799971 with alcohol-related behaviors and alcohol use and dependence. Individual studies have reported associations of rs1799971 with phenotypes such as alcohol cue-reactivity (Bach et al., 2015; Courtney et al., 2015; Ray et al., 2014b) and subjective response and sensitivity to alcohol's effects (Ray et al., 2013, 2014a; for a review, see Ray et al., 2012). More broadly, rs1799971 has been associated with measures of alcohol use, such as drinking levels (e.g., Pfeifer et al., 2015), alcohol self-administration concentrations (Hendershot et al., 2014), and dependence rates (van der Zwaluw et al., 2007), all of which correlate highly with subjective response to alcohol. However, findings are mixed with regard to the presence or direction of effect. Meta-analyses have been conducted to identify the extent of influence conferred by rs1799971 (Arias et al., 2006; Chen et al., 2012; Schwantes-An et al., 2016). Most recently, the largest of these investigations assessed both general substance dependence (as defined by a DSM-IV lifetime dependence diagnosis for any of 5 substances: alcohol, nicotine, cannabis, cocaine, and opioids), and the individual substance dependence diagnoses in European ancestry individuals. Findings demonstrated a protective relation between the rs1799971-G allele and the general substance dependence phenotype, and this allele showed a similar direction of effect but was not significantly associated with specific dependence diagnosis (Schwantes-An et al., 2016), potentially due to a lack of power. Although this meta-analysis provides promising insight into the role of rs1799971 in substance use more generally, rather than alcohol specifically, the long-standing interest in and theoretical underpinnings of the role of the endogenous opioid system in subjective response to alcohol warrants further research to elucidate the influence of genetic variation within *OPRM1*.

To provide a fuller examination of *OPRM1* variation in relation to substance use phenotypes, a recent study sought to narrow the focus to variants presumed to alter *OPRM1* mRNA expression in the brain (Hancock et al., 2015). Their findings suggested that a variant located in close proximity to rs1799971 within a highly conserved region of *OPRM1,* rs3778150, is robustly associated with heroin addiction and notably is in partial linkage disequilibrium (LD) with rs1799971. The rs3778150-C allele was associated with decreased *OPRM1* expression levels in the human brain, and further, the association with rs3778150 fully explained a smaller observed association with rs1799971 (Hancock et al., 2015). They concluded that rs3778150 may represent a causal variant within *OPRM1* that increases risk for heroin addiction, thus providing a potential explanation for the mixed results reported in the broader substance use literature regarding the latter variant.

The current study sought to test this hypothesis, and in this way, extend the findings from Hancock and colleagues (2015) to evaluate the relations of genetic variation in *OPRM1* with alcohol use and measures of subjective response to alcohol. Given that rs1799971 was not significantly associated with heroin addiction in the absence of rs3778150, and rs3778150 was associated with both heroin addiction and reduced *OPRM1* mRNA expression in the brain (Hancock et al., 2015), including these variants in association studies could potentially clarify the effect of rs1799971 on alcohol use phenotypes. The association of rs3778150 with reduced *OPRM1* mRNA expression in the brain might reflect a genetic predisposition that reduces normal compensatory mechanisms in response to heroin exposure, requiring more of the substance to evoke a physiological effect and subsequently increased risk of addiction (Hancock et al., 2015). As such, we predicted that rs3778150 would be associated with lower subjective response to alcohol and higher levels of alcohol use, over and above any effects of rs1799971.

MATERIALS AND METHODS

Sample

Participants were individuals with both genotype data (i.e., saliva samples suitable for DNA extraction) and nonmissing phenotype data ($N = 428$) drawn from a larger experimental study investigating the acute effects of alcohol on measures of executive cognitive functioning. Participants were regular drinkers (i.e., consuming between 2 and 25 drinks per week on average) recruited from the Columbia, MO community. Inclusion criteria based on drinking habits ensured that the alcohol dose received in the study was comparable to participants' typical drinking experiences. Therefore, naïve drinkers (<2 drinks per week on average) and very heavy drinkers (≥25 or more drinks per week on average) were excluded from the study. Additional exclusion criteria included the endorsement of any condition contraindicated with alcohol administration (abstention; history of alcohol or drug abuse treatment or other serious mental or physical illness; deliberate attempts to cut down on drinking; prescription medication other than oral contraception; pregnancy) or that would prevent the successful completion of laboratory measures (color blindness; a primary language other than English). Exactly half of the sample was male ($n = 214$), with an average age of 23.2 (SD = 2.6) years, and participants reported drinking an average of 7.4 drinks per week (SD = 6.7). Descriptive statistics for individuals included in the analyses described below ($n = 282$) can be found in Table 1.

Table 1. Means (and SDs) of Demographic, Alcohol Sensitivity, and Drinking Characteristics as a Function of rs3778150 Genotype

Variables	rs3778150 genotype		Mean comparisons
	TT (n = 189)	TC/CC (n = 93)	
Age	23.19 (2.6)	23.37 (2.7)	$t(279) = -0.54$, $p = 0.587$
Sex (% male)	47.6%	51.6%	
rs1799971-G minor allele (%)	30.2%	14.0%	
Alcohol quantity × frequency	6.71 (6.5)	8.69 (7.1)*	$t(269) = -2.26$, $p = 0.025$
SRE-Total	5.46 (2.0)	5.89 (2.2)	$t(265) = -1.63$, $p = 0.104$
SRE-first 5 drinking episodes	3.95 (1.7)	4.09 (1.9)	$t(260) = -0.61$, $p = 0.543$
SRE-Heavy	5.51 (2.1)	6.07 (2.3)	$t(261) = -1.97$, $p = 0.050$
SRE (past 3 months)	6.80 (2.7)	7.48 (3.0)	$t(259) = -1.85$, $p = 0.066$
ASQ-Total	4.80 (1.8)	5.22 (1.7)	$t(280) = -1.87$, $p = 0.063$
ASQ-Light	3.24 (1.3)	3.30 (1.1)	$t(280) = -0.34$, $p = 0.734$
ASQ-Heavy	7.87 (3.0)	9.17 (3.4)*	$t(270) = -3.20$, $p = 0.002$
BAES alcohol-related stimulation (baseline)	3.93 (1.7)	3.83 (1.5)	$t(278) = 0.50$, $p = 0.621$
BAES alcohol-related sedation (baseline)	2.16 (1.1)	2.10 (1.1)	$t(278) = 0.37$, $p = 0.712$
BAES maximum alcohol-related stimulation	5.12 (1.7)	4.50 (1.7)*	$t(150) = 2.16$, $p = 0.032$
BAES maximum alcohol-related sedation	4.70 (2.1)	4.90 (2.0)	$t(150) = -0.57$, $p = 0.568$
Subjective intoxication	5.25 (1.7)	5.16 (1.2)	$t(150) = 0.34$, $p = 0.735$

SRE, Self-Report of the Effects of Alcohol; BAES, Biphasic Alcohol Effects Scale; ASQ, Alcohol Sensitivity Questionnaire.
*Mean comparisons with $p < 0.05$.

Self-Report Measures

Self-Rating of the Effects of Alcohol Form. The 12-item Self-Rating of the Effects of Alcohol (SRE; Schuckit et al., 1997) was used to assess level of response to alcohol. The SRE asks respondents to estimate the number of standard drinks required to experience 4 different effects, including (i) to "begin to feel any different" (any effect), (ii) to "feel a bit dizzy or begin to slur your speech," (iii) to "begin stumbling or walking in an uncoordinated manner," and (iv) to "pass out, or fall asleep when you did not want to." Respondents provide drink estimates for these 4 effects according to 3 separate time frames: Their first 5 lifetime drinking episodes (*SRE-First5*), during the period of their heaviest drinking (*SRE-Heavy*), and over the past 3 consecutive months in which they drank (*SRE-3 month*). The SRE overall score (*SRE-Total*) is obtained by summing the number of drinks estimated for each time frame divided by the total number of endorsed (nonmissing) items, and higher overall scores indicate lower sensitivity to alcohol.

Given that an item can be included in the score only if the participant reports having experienced that effect from drinking alcohol, SRE scores (and, hence, sensitivity estimates) tend to be biased due to an inherent correlation between the number of items endorsed and number of drinks reported (i.e., lighter drinkers are less likely to experience effects associated with heavier doses). In other words, missing items are not missing at random. Although SRE scores have been shown to predict future alcohol consumption and problems after accounting for number of SRE items endorsed (Schuckit et al., 2006), this issue has also been addressed by alternative methods of scoring in previous studies (Schuckit et al., 2007). More recently, Lee and colleagues (2015) used data from the current study's full sample to propose 2 additional methods to correct for this bias, including standardized person-mean imputation in which item responses are converted to z-scores before averaging across nonmissing items, thereby achieving comparable item distributions across individuals. In the current data, *SRE-Total* scores were computed using both conventional scoring and standardized person-mean imputation. Both approaches produced summary scores that performed similarly in the current sample, and therefore, the results of analyses using the conventional person-mean imputation scores are reported to provide consistency with prior studies.

Alcohol Sensitivity Questionnaire. The 15-item Alcohol Sensitivity Questionnaire (ASQ; O'Neill et al., 2002) was also used to assess level of response to alcohol, and each item corresponds to a different alcohol-related effect. The ASQ addresses certain limitations of the SRE by including items that relate to the stimulating effects of alcohol or those associated with smaller doses of alcohol, in addition to sedative effects observed with higher doses of alcohol and typically more prominent during the descending limb of the blood alcohol concentration (BAC) curve. For each item endorsed, respondents are asked to estimate the *minimum* number of drinks required to experience up to 9 effects that correspond to lighter drinking, and the *maximum* number of drinks they could consume before experiencing up to 6 effects that correspond to heavier drinking. This results in an overall score (*ASQ-Total*), where higher scores indicate lower sensitivity to alcohol, as well as a score for the stimulating, low-dose effects of alcohol (*ASQ-Light*), and a score for the sedative, high-dose effects of alcohol (*ASQ-Heavy*). For further details on the individual items and this measure, see Fleming and colleagues (2016).

Alcohol Use. Using items adapted from the National Institute on Alcohol Abuse and Alcoholism Task Force recommendations (NIAAA, 2003), participants estimated the average number of drinking episodes per week and average number of drinks consumed per episode over the past 3 months. Typical levels of alcohol use (quantity-frequency; *AlcQF*) were estimated by creating a product of the number of average weekly drinking episodes by the average number of drinks consumed per episode.

Subjective Effects of Alcohol

Subjective Intoxication. The alcohol challenge portion of the study included periodic assessments during which participants were asked to rate how drunk they felt on a 10-point scale (1 = *not drunk at all*; 10 = *the most drunk I've ever been*). This single item is consistent with similar assessments in previous research (e.g., Earleywine and Erblich, 1996; Newlin, 1985), and participants' maximum rating of subjective intoxication across all measurement occasions following alcohol administration (*SubIntox*) was used in the current analyses.

Stimulation and Sedation. Ratings of participants' feelings of stimulation and sedation during the alcohol challenge portion of the study were obtained with the 14-item Biphasic Alcohol Effects Scale (BAES; Martin et al., 1993). This self-report questionnaire was

administered at each measurement occasion along with the item querying subjective intoxication. The BAES is composed of a 7-item sedation subscale and a 7-item stimulation subscale. Subscale items correspond to different effects experienced during each state (e.g., down, sluggish vs. up, exited) rated on a 10-point scale. Respondents' maximum rating of sedation and stimulation across all measurement occasions following alcohol administration (*SedMax* and *StimMax*, respectively) was used in the current analyses.

Procedure

Participants attended a preliminary laboratory session during which they completed all retrospective self-report measures (i.e., SRE, ASQ, and alcohol consumption items). The alcohol challenge protocols were administered during a second laboratory session approximately 1 to 3 weeks later (M = 19.1 days). Participants were instructed to eat a light meal 2 to 4 hours prior to their appointment time. Upon arrival, all individuals provided informed consent and either took a urine stream pregnancy test (women) or were asked to empty their bladder (men). Participants then completed a baseline assessment of subjective effects of alcohol, which included the BAES for stimulation and sedation, as well as a subjective intoxication rating. This assessment was followed by random assignment to receive an active placebo (diluted [10-proof] vodka and tonic water; 0.04 g/kg ethanol [EtOH]) or alcohol beverage (100-proof vodka and tonic water; 0.80 g/kg EtOH for men [0.72 g/kg for women]) with target peak breath alcohol concentration (BrAC) of 0.09%. The contents of the active placebo or alcohol beverage were divided into 3 equivalently sized drinks to be consumed over 8 minutes per drink.

A 5-minute absorption period followed beverage administration, and participants then completed BrAC measurements and assessment of subjective effects every 5 to 6 minutes until BrAC reached 0.065% for alcohol participants (or after 1 BrAC measurement for placebo participants). At this time, a battery of cognitive tasks relating to the larger study was administered, and BrAC, BAES, and subjective intoxication assessments were re-administered after every other cognitive task (approximately every 20 minutes). Upon completion of the cognitive tasks, BrAC and subjective effects were assessed every 5 minutes until BrAC descended from peak to 0.075%, at which time the cognitive task battery was completed again; as during the ascending limb, BrAC, BAES, and subjective intoxication were assessed after every other task.

Genotyping

As noted, this study was part of a larger effort examining genetic influences on cognition, and thus, participants were genotyped on a genomewide microarray as described below. The genomewide data were used in the quality control steps described below; however, the analysis focused solely on the 2 described *OPRM1* variants in order to address the study aims.

Quality Control. The Affymetrix Axiom Biobank Genotyping Array (Affymetrix, Inc., Santa Clara, CA) was used for rare and common variant genotyping and analysis, capturing variants with a minor allele frequency (MAF) >0.005. Assays and genotype calls were made according to protocols provided by Affymetrix. A number of standard genotyping quality control steps were conducted (Anderson et al., 2010) using PLINK 1.07 (Purcell et al., 2007) to assess genotyping sample quality and accuracy, as well as sample identities for the original data set of 428 individuals and 628,679 single nucleotide variants (SNVs). Degree of relatedness estimations identified 2 first-degree siblings. Given that analyses of alcohol challenge data would be restricted to individuals in the alcohol beverage condition, the sibling who had been assigned to the active placebo condition was removed in order to ensure that the sample was composed of unrelated individuals. Gender checks of sample identities were evaluated, and 7 individuals with unresolved discrepant sex codes were excluded.

One individual and 20,847 SNVs were removed due to low genotype call rates (individuals or SNVs with call rates <95%), and 190,855 monomorphic SNVs were excluded. Following tests for deviations from Hardy–Weinberg equilibrium, 1,028 SNVs with a p-value <1e-07 were removed. All duplicate markers, mitochondrial markers, and markers on sex chromosomes were excluded (n = 13,005 SNVs). Cross-referencing allele frequencies with the European sample for the 1000 Genomes Project (The 1000 Genomes Project Consortium, 2012) resulted in the exclusion of 381 SNVs, whose allele frequencies differed more than 0.20 from this reference panel. Following the completion of standard quality control procedures, 497 individuals and 393,812 SNVs remained in the data set. To capture nontyped genetic variation, genotype prephasing was conducted using the SHAPEIT2 program, followed by genomewide imputation using the 1000 Genomes Project reference haplotype panels with IMPUTE2 (Howie et al., 2009). The latter programs do not allow for the inclusion of indels, and therefore, all simple and complex indels (n = 8,751) were excluded prior to genotype prephasing and imputation. Finally, ancestry estimations were calculated from variants with a MAF $\geq$ 0.01 using principal components analysis (Price et al., 2006), as implemented in the Genomewide Complex Trait Analysis software (Yang et al., 2011). The resulting ancestry estimates were used as covariates in subsequent association tests to control for possible population substructure.

Data Analysis

Individuals' typed genotypes at rs1799971 were obtained from the Affymetrix exome genotyping microarray. Genotypes at rs3778150 were imputed and converted to the most likely genotype, assigning genotype calls below 0.80 to missing for 1 individual. The info score from IMPUTE2 (Howie et al., 2009) for rs3778150 was 0.994, indicating that this SNP had been imputed with high certainty. The MAF for rs1799971 and rs3778150 was 0.14 and 0.16, respectively. Given the small number of individuals homozygous for the minor allele at both markers, genotypes at rs1799971 and rs3778150 were coded for the presence or absence of at least 1 minor G or C allele, respectively (rs1799971 genotypes: [AA: n = 302, AG/GG: n = 119]; rs3778150 genotypes: [TT: n = 295, CT/CC: n = 132]). Given that the study aims focused solely on these 2 variants, which are moderately correlated (r^2 = 0.03), in relation to a set of phenotypes that were themselves moderately correlated, no corrections for multiple testing were made for any of the described analyses. Analyses conducted with the retrospective self-report measures included participants in both active placebo and alcohol beverage conditions (n = 282), whereas only those individuals assigned to the alcohol beverage condition (n = 152) were retained for analyses of subjective response to alcohol during the alcohol challenge. Means and standard deviations for the above self-report and subjective effects of alcohol measures as a function of rs3778150 genotype are displayed in Table 1.

RESULTS

Square-root transformations of *AlcQF*, *SRE-Total*, *SRE-3 month*, *SRE-Heavy*, and *ASQ-Light* were employed to achieve normality. In order to assess the relations of rs1799971 and rs3778150 with these and the other measures of subjective response to alcohol and alcohol use, hierarchical multiple regression analyses composed of 3 steps were conducted. Age, age-squared (to account for linear and

quadratic effects of age), sex, and the first 3 eigenvectors generated from the principal components analysis designed to control for population stratification were entered into the model first to control for their effects before including rs1799971 and rs3778150 in the second and third steps, respectively. In this way, results could be evaluated to assess whether rs3778150 was significantly associated with any of the alcohol sensitivity and alcohol use phenotypes after accounting for the effects of rs1799971 genotype. Standardized regression coefficients are provided in the text below, and unstandardized regression coefficients and standard errors are reported in Tables 2–4.

Retrospective Measures of Alcohol Sensitivity and Alcohol Use

SRE Form. Carriers of the rs3778150-C allele exhibited significantly lower levels of overall alcohol sensitivity compared to rs3778150-T homozygous individuals, as measured by higher *SRE-Total* scores, $\beta = 0.12$, $t(244) = 2.11$, $p = 0.036$; $\Delta R^2 = 0.014$, lower levels of alcohol sensitivity during the most recent consecutive 3-month period in which they drank, that is., higher *SRE-3 month* scores: $\beta = 0.13$, $t(237) = 2.18$, $p = 0.030$; $\Delta R^2 = 0.016$, and lower levels of alcohol sensitivity during the period of heaviest drinking in their lives, that is., higher *SRE-Heavy* scores: $\beta = 0.13$, $t(244) = 2.21$, $p = 0.028$; $\Delta R^2 = 0.015$. The rs3778150 variant was not significantly associated with levels of alcohol sensitivity during their first 5 drinking episodes (*SRE-First5*), nor did the rs1799971 genotype show significant association with any of the SRE measures (Table 2).

Alcohol Sensitivity Questionnaire. Similar to the results based on the SRE, carriers of the rs3778150-C allele exhibited significantly lower levels of overall alcohol sensitivity compared to rs3778150-T homozygous individuals, as measured by higher *ASQ-Total* scores, $\beta = 0.14$, $t(258) = 2.61$, $p = 0.010$; $\Delta R^2 = 0.019$. Carriers of the rs3778150-C allele

Table 2. Results of Hierarchical Multiple Regression Analyses Predicting SRE Scores

Phenotype	Regression block	Variable	*b*	SE (*b*)	*p*	ΔR^2
SRE-Total	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.01	0.06	0.849	0.000
	Step 3	rs1799971	0.01	0.57	0.853	–
		rs3778150	0.11	0.05	0.036*	0.014*
SRE-3 month	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.06	0.07	0.394	0.002
	Step 3	rs1799971	−0.03	0.06	0.660	–
		rs3778150	0.14	0.06	0.030*	0.016*
SRE-Heavy	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.04	0.06	0.565	0.001
	Step 3	rs1799971	−0.01	0.06	0.869	–
		rs3778150	0.12	0.06	0.028*	0.015*
SRE-First5	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.01	0.06	0.843	0.000
	Step 3	rs1799971	−0.01	0.06	0.903	–
		rs3778150	0.02	0.05	0.703	0.001

SRE, Self-Report on the Effects of Alcohol.
*$p < 0.05$.

Table 3. Results of Hierarchical Multiple Regression Analyses Predicting ASQ Scores

Phenotype	Regression block	Variable	*b*	SE (*b*)	*p*	ΔR^2
ASQ-Total	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.20	0.21	0.337	0.003
	Step 3	rs1799971	−0.10	0.21	0.652	–
		rs3778150	0.49	0.19	0.010*	0.019*
ASQ-Light	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.07	0.04	0.123	0.007
	Step 3	rs1799971	−0.07	0.05	0.142	–
		rs3778150	0.01	0.04	0.809	0.000
ASQ-Heavy	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.07	0.07	0.276	0.003
	Step 3	rs1799971	−0.03	0.07	0.686	–
		rs3778150	0.24	0.06	0.000**	0.042**

ASQ, Alcohol Sensitivity Questionnaire.
*$p < 0.05$, **$p < 0.001$.

Table 4. Results of Hierarchical Multiple Regression Analyses Predicting Past-Month Alcohol Use Levels and Alcohol Challenge Responses

Phenotype	Regression block	Variable	*b*	SE(*b*)	*p*	ΔR^2
QF	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.09	0.14	0.515	0.001
	Step 3	rs1799971	−0.01	0.14	0.962	–
		rs3778150	0.38	0.12	0.002**	0.032**
StimMax	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	0.08	0.33	0.804	0.000
	Step 3	rs1799971	−0.07	0.33	0.839	–
		rs3778150	−0.67	0.29	0.021*	0.037*
SedMax	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.23	0.09	0.015*	0.039*
	Step 3	rs1799971	−0.22	0.10	0.025*	–
		rs3778150	0.06	0.09	0.471	0.003
SubIntox	Step 1	Covariates	–	–	–	–
	Step 2	rs1799971	−0.22	0.30	0.458	0.004
	Step 3	rs1799971	−0.23	0.31	0.459	–
		rs3778150	−0.02	0.27	0.942	0.000

QF = average alcohol quantity × frequency per week in the past 3 months. StimMax = maximum Biphasic Alcohol Effects Scale (BAES) alcohol-related stimulation. SedMax = maximum BAES alcohol-related sedation. SubIntox = maximum self-reported subjective intoxication.
*$p < 0.05$, **$p < 0.01$.

also exhibited significantly lower levels of alcohol sensitivity to the sedating effects of alcohol compared to rs3778150-T homozygous individuals, as measured by higher *ASQ-Heavy* scores, $\beta = 0.21$, $t(254) = 3.90$, $p < 0.001$; $\Delta R^2 = 0.042$. However, the rs3778150 genotype was not significantly associated with sensitivity to the stimulating effects of alcohol (i.e., *ASQ-Light*), nor did the rs1799971 genotype show significant association with any of the ASQ measures (Table 3).

Alcohol Use. Carriers of the rs3778150-C allele exhibited significantly higher drinking levels, compared to rs3778150-T homozygous individuals, $\beta = 0.18$, $t(260) = 3.14$, $p = 0.002$, $\Delta R^2 = 0.032$. The association of rs1799971 with *AlcQF* was not significant (Table 4).

Experimental Measures of Alcohol Sensitivity

Subjective Intoxication. Although carriers of the rs3778150-C allele reported lower levels of maximum subjective intoxication ($M = 5.16$, SD = 1.2) compared to rs3778150-T homozygous individuals ($M = 5.27$, SD = 1.6), this difference was not statistically significant. In addition, the rs1799971 genotype did not show a significant association with *SubIntox* (Table 4).

Stimulation and Sedation. Carriers of the rs3778150-C allele reported significantly lower levels of maximum alcohol-related stimulation, as measured by lower *StimMax* scores, $\beta = -0.20$, $t(133) = -2.34$, $p = 0.021$; $\Delta R^2 = 0.037$. In contrast, carriers of the rs3778150-C allele did not differ from rs3778150-T homozygous individuals in their ratings of maximum alcohol-related sedation. Furthermore, although the rs1799971 genotype was not significantly associated with *StimMax*, the association of rs1799971 with *SedMax* remained significant in the presence of rs3778150, with carriers of the rs1799971-G allele reporting significantly lower levels of alcohol-related sedation, compared with rs1799971-A homozygous individuals, $\beta = -0.21$, $t(134) = -2.50$, $p = 0.015$. The addition of rs1799971 accounted for an increase of 4.5% in explained variance explained for *SedMax*, over and above the effects of covariates entered in Step 1. When rs3778150 was entered instead at Step 2, the addition of rs1799971 into the model at Step 3 accounted for an increase of 4.0% of explained variance in *SedMax* (Table 4).

DISCUSSION

The endogenous opioid system has been hypothesized to partially mediate the reinforcing properties of alcohol, and there has been a long-standing interest in how variation in the gene encoding the μ-opioid receptor (*OPRM1*) relates to measures of alcohol consumption and dependence. Numerous studies have tested genetic variation in *OPRM1* for association with these phenotypes, but have yielded few replicable, robust findings. Much of the existing research has focused on a missense variant in *OPRM1*, rs1799971; however, recent evidence suggests that a second *OPRM1* variant, rs3778150, may be accounting for the mixed findings observed for heroin and other opioid use (Hancock et al., 2015). In the aforementioned study, the rs3778150-C allele was only observed in the presence of the rs1799971-A allele. This haplotype was significantly associated with liability for heroin addiction across European American and African American individuals, as well as in a meta-analysis; however, in the absence of rs3778150-C, the rs1799971-A allele showed no association with heroin addiction in either ancestral group or in a meta-analysis. Therefore, their findings suggested that the association of rs1799971 with heroin addiction was dependent on the presence of the rs3778150-C risk allele. The current study sought to extend this hypothesis to alcohol use phenotypes, and thus examined the relations of

these 2 *OPRM1* variants, rs1799971 and rs3778150, with measures of subjective response to alcohol and alcohol consumption.

Consistent with the hypothesis that rs3778150 would be associated with retrospective measures of alcohol sensitivity, individuals carrying the rs3778150-C allele reported lower sensitivity to alcohol, as demonstrated by higher *SRE-Total* scores, higher *SRE-3 month* scores, and higher *SRE-Heavy* scores, compared to individuals homozygous for the rs3778150-T allele. In addition, rs3778150 was associated with higher *ASQ-Total* scores, and higher scores on the *ASQ-Heavy* subscale, such that carriers of the rs3778150-C allele reported lower overall subjective response to alcohol, as well as lower subjective response to the sedative effects associated with heavier doses of alcohol. The significant relation of rs3778150 with *ASQ-Total* and *ASQ-Heavy* is consistent with the robust effects observed for rs3778150 with the majority of SRE subscales, given that the range of effects assessed by the SRE mainly correspond to sedative effects associated with higher doses of alcohol, similar to the items included in the *ASQ-Heavy* subscale. Finally, the presence of at least 1 rs3778150-C allele was also associated with significantly higher levels of alcohol use. Thus, these findings suggest that rs3778150 is related to measures of alcohol sensitivity and higher levels of alcohol use.

Additionally, associations of rs3778150 extended in part to measures of experimental measures of alcohol sensitivity. Specifically, carriers of the rs3778150-C allele reported significantly lower levels of BAES alcohol-related stimulation (*StimMax*) during an alcohol challenge session, but not with self-reported levels of alcohol-related sedation (*SedMax*) during the alcohol challenge or subjective intoxication, despite being strongly related to overall measures of alcohol sensitivity and sensitivity to the sedative-like effects assessed by the ASQ and SRE. Mean comparisons for rs3778150 genotype groups indicated that carriers of the rs3778150-C allele reported higher levels of alcohol-related sedation (Table 1), although this difference was not significant, even when included with other covariates in the hierarchical multiple regression analyses. It may be that power issues contributed to the lack of total replication across retrospective and alcohol challenge measures of subjective response for rs3778150.

In contrast to previous research, rs1799971 was not significantly associated with any measure of alcohol sensitivity or alcohol use, with the exception of lower levels of maximum alcohol-related sedation reported during the alcohol challenge session. The rs1799971 variant has more frequently shown association with measures of alcohol-induced stimulation, and these studies have found little support for the association of rs1799971 with reduced alcohol-related sedation (Bujarski and Ray, 2014; Ray and Hutchison, 2004; Ray et al., 2013, 2014a). Thus, given both the general lack of association for rs1799971 with alcohol-induced sedation in previous studies, and with any of the other measures of subjective response in the current study, the association of rs1799971 with levels of BAES alcohol-related sedation should be interpreted with caution and warrants further investigation. Nonetheless, this finding does raise the possibility that both variants may be relevant to alcohol sensitivity and consumption phenotypes.

This is the first study to evidence a relation between rs3778150 and measures of alcohol sensitivity and consumption. These findings tentatively suggest that the rs3778150-C allele is associated with a low subjective response to alcohol. It is important to note that rs3778150 and rs1799971 are in modest LD—that is, their genotypes are modestly correlated and not independent. In the study by Hancock and colleagues (2015), the rs3778150-C minor allele was observed only in the presence of the rs1799971-A allele, which was also the case in the present study. Their findings demonstrated that rs1799971-A was associated with increased risk for heroin addiction only when tested together with rs3778150-C. Similarly, a recent meta-analysis reported a protective effect for rs1799971-G on general substance dependence and DSM-IV alcohol dependence, although the test of the latter phenotype did not achieve statistical significance and did not account for any effects of rs3778150 (Schwantes-An et al., 2016). Although the direction of effect for the associated allele is consistent across these 2 studies, previous findings implicating rs1799971 in alcohol sensitivity and alcohol use may have inadvertently been capturing the effects of rs3778150 and likely other *OPRM1* intron 1 variants, as has been suggested in past research (Levran et al., 2011; Zhang et al., 2006). As such, rs3778150 may account, at least in part, for the mixed relations previously observed for rs1799971 and alcohol use phenotypes.

It is worth noting, however, that the mixed relations of rs1799971 with measures of subjective response to alcohol in past studies may also extend to rs3778150. The associations of rs3778150 with the sedative effects of alcohol based on retrospective self-report measures (i.e., SRE, ASQ) in the current study did not generalize to the alcohol challenge; rs3778150 was instead related to lower levels of maximum alcohol-related stimulation. Numerous factors could account for these inconsistent associations of rs3778150 with different facets of subjective response. The retrospective self-report measures instruct individuals to estimate the number of drinks needed to experience specific effects related to stimulation or sedation for different time periods. In contrast, alcohol sensitivity is assessed during the alcohol challenge by having participants rate the extent to which they are experiencing different states that tap stimulation or sedation, but items are worded such that these effects are not wholly attributable to alcohol. Therefore, subjective response is captured in different ways (i.e., drink estimates vs. ratings of effects experienced) for the retrospective measures and the alcohol challenge, which could decrease the likelihood that effects will generalize across both modalities. In addition, the retrospective measures require an individual to reference an entire episode of drinking, compared to the repeated momentary estimations provided over the course of a drinking episode

during the alcohol challenge and the subsequent conclusion of subjective response assessments before an individual's BrAC returns to zero (upon reaching 0.02%). Finally, important differences in components such as the rapidity of intoxication and peak BAC achieved are likely to impact a person's subjective response to alcohol. As such, these findings warrant further investigation of this genomic region and its relation to alcohol use phenotypes.

The pattern of associations for rs3778150 with subjective response to alcohol and levels of alcohol use is consistent with prior observations that the rs3778150-C minor allele is associated with decreased *OPRM1* expression levels in the human brain and increased risk for heroin addiction (Hancock et al., 2015). In their study, Hancock and colleagues (2015) suggested that the usual compensatory mechanism to up-regulate *OPRM1* expression in response to heroin exposure is disrupted by the presence of the rs3778150-C allele, necessitating increased use to acquire similar effects and thus increasing liability for addiction. Given the hypothesis that the endogenous opioid system modulates the reinforcing effects of alcohol, the results from the current study suggest that rs3778150 may directly influence a person's subjective response to alcohol and subsequently increase risk for higher levels of alcohol use via similar mechanisms. Although rs1799971 did not show consistent association with any measure of subjective response to alcohol or alcohol consumption, this again replicates findings from Hancock and colleagues (2015) and lends further support to suggestions that specific haplotypes carrying noncoding regulatory variants may account for the lack of replicable associations observed for rs1799971 with substance use phenotypes (Levran et al., 2011; Zhang et al., 2006).

The findings from the current study are also largely consistent with theoretical arguments that low subjective response to alcohol constitutes a risk factor that contributes to problematic alcohol use and increased liability for alcohol dependence (low level of response model; Schuckit, 1994; Schuckit and Smith, 1996). Supplementary analyses were conducted in order to test other theoretical models that evaluate subjective response to alcohol and its rewarding and aversive effects in relation to liability for alcohol dependence. There was no evidence of differential sensitivity to alcohol's effects across ascending and descending BrAC limbs, as Newlin and Thomson's (1990) differentiator model might assume, nor evidence for increased sensitivity to the stimulant and sedative effects at peak BrAC, as the modified differentiator model might indicate (King et al., 2011).

Finally, the incentive sensitization theory proposes that heavy prolonged exposure to alcohol results in both increased salience of drug cues and neuroadaptations that sensitize brain circuits in regions specifically involved in motivation or "wanting" of the drug, but not necessarily hedonic "liking" of the drug (Robinson and Berridge, 1993). Findings from previous studies have suggested that individuals with a low subjective response to alcohol display increased neural responses reflecting enhanced incentive value of alcohol cues (Bartholow et al., 2007, 2010) as well as automatic approach tendencies to alcohol cues reflecting greater "wanting" (Fleming and Bartholow, 2014). This increased salience is thought to result from neuroadaptations that sensitize the mesolimbic dopamine pathway to specific drug effects and related stimuli. Evidence has shown that acute alcohol administration acts on μ-opioid receptors to produce the hedonic "liking" effects and subsequently modulate activity of dopaminergic neurons that produce the "wanting" effects. However, it is unclear to what extent these *OPRM1* variants (rs3778150 and rs1799971) might influence incentive sensitization processes and subsequent risk for problematic alcohol use, given that the current study was unable to evaluate their genetic effects in the context of this model. Genetic factors may be helpful to inform on etiology and differentiate between theoretical models of alcohol dependence, although it is unrealistic to expect any single variant to have a large effect or generalize across different complex behavioral models, such as those described above. Nonetheless, the results from the current study support arguments that genetic variation in *OPRM1* may act to lower subjective response to alcohol and thus increase risk for higher levels of alcohol use.

The findings from the current study should be considered in light of its limitations and strengths. Limitations include the numerous differences between retrospective self-report measures and assessments during the alcohol challenge as discussed above, such as the time period referenced to estimate different effects (i.e., an entire night vs. the past 20 minutes) and the influence of factors affecting subjective experiences (e.g., rapidity of intoxication, peak BAC achieved). These also include a relatively narrow scale with which to measure subjective response during the alcohol challenge. Participants in the current study used the 10-point scale from the BAES (Martin et al., 1993) to rate the extent of stimulation and sedation, and a 10-point scale to rate how drunk they felt. In contrast, other studies have used alternative measures such as the Subjective High Assessment Scale (e.g., Schuckit et al., 2000), which uses a 36-point visual analog scale to rate the extent of response and thus allows for a broader range of effect and greater variability for comparisons. In addition, the small sample size, although larger relative to past studies that have tested the effects of rs1799971 on measures of alcohol sensitivity and alcohol consumption, also represents a limitation of the current study. As effect sizes for any single variant will be small, future research should employ larger samples to characterize the extent of rs3778150 and rs1799971's influence on alcohol response and alcohol use. Furthermore, given that the sample was limited to moderate drinkers, it is unclear how these findings might generalize to lighter or heavier drinking individuals. Future studies might also evaluate how these variants relate to subjective response in the presence of higher or lower doses of alcohol. However, a major strength of the current study was the inclusion of rs3778150 in order to provide the first test of this variant in relation to alcohol sensitivity and alcohol use phenotypes. A related strength was the utilization of

numerous measures of alcohol response, and thus the ability to examine the relations of 2 putatively causal genetic variants in *OPRM1* with self-report and experimental indices of subjective response to alcohol.

These results provide further support suggesting that rs3778150 may be causally related to substance use phenotypes, including alcohol use, and could potentially account for previously observed associations of rs1799971 with substance use phenotypes. Furthermore, these findings provide additional clarification regarding the role of *OPRM1* and rs1799971. Future studies should investigate potential causal relations among genetic variants in *OPRM1*, subjective response to alcohol, and drinking phenotypes to further delineate the associations of rs3778150 with alcohol use and dependence.

FUNDING SUPPORT

This research was supported by grants P60 AA011998 and T32 AA013526 from the National Institute on Alcohol Abuse and Alcoholism. All authors report no conflicts of interest.

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