ARTICLE

The *OPRM1* A118G polymorphism: converging evidence against associations with alcohol sensitivity and consumption

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The endogenous opioid system may be involved in the development and maintenance of alcohol use disorder (AUD) and is a target for existing AUD pharmacotherapies. A functional polymorphism of the mu-opioid receptor gene (*OPRM1* A118G, rs1799971) may alter the risk of developing AUD. Human laboratory studies have demonstrated that minor allele carriers self-administer more alcohol, show greater sensitivity to alcohol's effects, and exhibit increased alcohol-induced dopamine release. On the other hand, large genome-wide association studies and meta-analyses of candidate gene studies have not found an association between this genotype and alcohol dependence diagnosis. Given this discrepancy, the present study sought to verify whether *OPRM1* A118G was associated with alcohol self-administration, subjective response to alcohol, and craving in a sample of 106 social drinkers of European ancestry who completed an intravenous alcohol self-administration session. We found no relationship between *OPRM1* rs1799971 genotype and subjective response to alcohol or craving. *OPRM1* genotype was not associated with total alcohol exposure or likelihood of attaining a binge-level exposure (80 mg%) during the intravenous alcohol self-administration session. Analysis of 90-day Timeline Followback interview data in a larger sample of 965 participants of European ancestry found no relationship between *OPRM1* genotype and alcohol consumption in either alcohol dependent or non-dependent participants. These findings suggest that there may not be an association between *OPRM1* rs1799971 genotype and alcohol consumption or sensitivity in individuals of European ancestry.

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INTRODUCTION

The opioid system is centrally involved in hedonic responses [1] and may be implicated in the development and maintenance of alcohol use disorder (AUD) [2]. Alcohol has been shown to trigger endogenous opioid release in rodents and humans [3–5]. This has important treatment implications, as opioid antagonists such as naltrexone reduce relapse to heavy drinking [6]. Given the role of the opioid system in AUD treatment, a functional single nucleotide polymorphism in the mu-opioid receptor gene, *OPRM1* A118G (Asn40Asp, rs1799971), has received significant attention. This polymorphism decreases mu-opioid receptor expression [7], resulting in reduced receptor availability in human positron emission tomography studies [8, 9]. Retrospective analyses of clinical trials suggested that *OPRM1* A118G influences naltrexone response [10, 11], although a recent well-designed prospective study found no such effect [12]. There is also evidence that *OPRM1* A118G affects neurobiological response to alcohol, as healthy male G allele carriers show greater alcohol-induced striatal dopamine release than A/A homozygotes [13]. It is therefore possible that G allele carriers could have altered alcohol sensitivity or alcohol-related behaviors.

Human laboratory studies have largely focused on the relationship between *OPRM1* rs1799971 genotype and intermediate phenotypes related to AUD such as subjective response to alcohol. Intermediate phenotypes are thought to be more precise outcome measures than AUD diagnosis and their use is postulated to increase the power to detect the effects of single nucleotide

polymorphisms [14, 15]. Human laboratory studies have provided evidence that G allele carriers have altered alcohol-induced subjective effects [16, 17], increased cue-induced craving [18], and increased intravenous self-administration of ethanol [19, 20]. In contrast, a large genome-wide association study (GWAS) and several meta-analyses of candidate gene studies have found no link between *OPRM1* rs1799971 genotype and alcohol dependence diagnosis [21–23]. There are several possible reasons for this discrepancy. Existing human laboratory studies have been limited by small sample sizes, which increases the likelihood of detecting spurious associations. Furthermore, these studies may not have adequately accounted for the inclusion of participants from diverse ancestries given that the frequency of *OPRM1* rs1799971 varies by ancestry. On the other hand, the use of alcohol dependence as an outcome in both GWAS and candidate gene studies could also be limiting. Alcohol dependence is a heterogeneous cluster of symptoms and may therefore be less robustly associated with *OPRM1* genotype than the more circumscribed outcomes used in human laboratory studies.

The present study aims to clarify the role of *OPRM1* rs1799971 in human alcohol consumption. We examined associations between the *OPRM1* A118G polymorphism and subjective response to ethanol, craving, and self-administration. To accomplish this, we analyzed data from the largest intravenous alcohol self-administration sample collected to date ($N = 162$), a substantially larger sample than was used in the original studies demonstrating

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associations with alcohol sensitivity ($N=38$) [17] and intravenous alcohol self-administration ($N=38$) [19]. To avoid the confounding effects of differential allele distribution across ancestries, we restricted our analyses to participants of European ancestry ($N=106$), the predominant ancestry in our sample. Given the existing human laboratory literature, we hypothesized that G allele carriers would report greater ethanol-induced subjective effects and higher levels of craving, which would lead to greater self-administer of ethanol. To determine whether G allele carriers with and without alcohol dependence had higher levels of alcohol consumption outside of the laboratory, we also performed a confirmatory analysis in a sample of nearly a thousand participants of European ancestry who underwent a comprehensive assessment for AUD.

MATERIALS AND METHODS

Intravenous alcohol self-administration study

Participants. Social drinkers ($N=162$) between the ages of 21 and 45 participated in intravenous alcohol self-administration

sessions at the National Institutes of Health Clinical Center (for detailed demographic information, see Table 1). Ancestry was determined using a genetic panel of ancestry informative markers (AIMs). Participants who were not of European Ancestry (AIMs European ancestry score less than 0.5) were excluded without reference to *OPRM1* A118G genotype ($N=55$). One participant was excluded because no information was available about his level of alcohol consumption. The final sample consisted of 106 participants (27 G allele carriers). Subjects must have consumed at least 5 drinks on one occasion in their lifetime. Participants were excluded if they met any of the following criteria: (1) non-drinker as per the 90-day Timeline Followback (TLFB) interview [24], (2) lifetime history of alcohol dependence, (3) lifetime history of mood, anxiety, or psychotic disorders, or (4) lifetime history of other substance dependence diagnosis, based on SCID-IV interview [25], (5) recent illicit use of psychoactive substances (e.g., positive urine toxicology screen), (6) history of acute alcohol-related skin flush [26], (6) regular tobacco use (>20 uses/week, as ascertained during clinical assessment), (7) clinically significant

Table 1. Characteristics of participants in the intravenous alcohol self-administration sample

Measure	Male ($N=56$)		Female ($N=50$)	
	AA ($N=36$)	AG/GG ($N=20$)	AA ($N=43$)	AG/GG ($N=7$)
<i>Demographic variables</i>				
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Age	25.0 (5.0)	23.5 (5.0)	23.0 (6.0)	24.0 (2.0)
AUDIT score ^a	6.0 (3.0)	6.0 (3.5)	5.0 (4.5)	6.0 (5.0)
Total drinks ^b	78.0 (56.1)	76.5 (57.0)	61.0 (75.0)	75.0 (68.0)
Average drinks per drinking day ^b	3.3 (1.7)	3.0 (3.0)	2.5 (1.7)	2.2 (2.1)
Binge drinking days ^b	3.5 (7.8)	2.0 (8.8)	4.0 (9.0)	5.0 (10.0)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Delay discounting ^{a,c}	−5.0 (1.8)	−4.7 (2.1)	−4.8 (1.9)	−4.8 (1.3)
SRE first five ^d	4.8 (2.3)	4.3 (2.1)	3.5 (1.2)	4.6 (3.0)
	N (%)	N (%)	N (%)	N (%)
Family history positive	8 (22.2)	5 (25.0)	8 (18.6)	1 (14.3)
<i>Outcome variables</i>				
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Feel effects ^e	21.0 (28.0)	17.0 (16.5)	18.0 (30.0)	22.0 (28.0)
Like effects ^f	56.0 (11.8)	53.5 (12.0)	52.0 (12.0)	58.0 (16.0)
Feel high ^g	11.0 (29.5)	21.0 (28.5)	18.0 (31.0)	14.0 (24.0)
Feel intoxicated ^h	16.0 (29.0)	15.0 (28.5)	18.0 (33.0)	22.0 (22.0)
Sedation change ⁱ	2.0 (5.0)	2.0 (7.8)	1.0 (6.0)	0.0 (19.0)
Total alcohol exposure ^{j,k}	4360.0 (6601.4)	5662.5 (6268.6)	1514.3 (5034.4)	3102.9 (6059.7)
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Want more ^l	44.0 (20.5)	47.5 (24.6)	32.9 (20.7)	42.7 (15.6)
Post-prime craving ^m	3.3 (6.4)	4.2 (6.3)	2.4 (4.8)	2.1 (5.3)
Stimulation change ⁱ	−0.1 (9.8)	1.7 (6.5)	−1.6 (7.7)	−1.9 (6.5)
	N (%)	N (%)	N (%)	N (%)
Bingers ^{l,n}	15 (41.7)	9 (45.0)	10 (23.8)	2 (28.6)

^aMissing data for some participants: $N=9$ for delay discounting, $N=2$ for AUDIT scores. ^bTotal drinks, average drinks per drinking day, and binge drinking days over the last 90 days were obtained using the Timeline Followback interview. ^cValues are reported as the natural logarithm of the discounting constant. ^dTotal score from the Self-Rating of the Effects of Alcohol form (SRE) from the first five drinking occasions. ^eMeasured using the “do you feel any drugs effects” item on the Drug Effects Questionnaire (DEQ) following the priming dose of alcohol. ^fMeasured using the “do you like the effects you are feeling now” item on the DEQ following the priming dose of alcohol. ^gMeasured using the “do you feel high” item on the DEQ following the priming dose of alcohol. ^hMeasured using the “do you feel intoxicated” item on the DEQ following the priming dose of alcohol. ⁱBiphasic Alcohol Effects Scale (BAES), change measured as BAES stimulant or sedation subscale score following the priming dose of alcohol minus the corresponding baseline subscale score. ^j $N=105$. One female (genotype = A/A) was excluded from alcohol self-administration analyses as her session ended at minute 41.5 due to technical difficulties. ^kTotal alcohol exposure is defined as the area under the curve of the estimated blood alcohol concentration versus time plot. ^lMeasured using the “would you like more of what you received, right now” item on the DEQ following the priming dose of alcohol. ^mPost-prime craving measured as Alcohol Urge Questionnaire (AUQ) score following the priming dose of alcohol minus AUQ score at baseline. ⁿA binge during the IV alcohol self-administration session is defined as reaching a software-estimated blood alcohol concentration of 80 mg%

Table 2. Characteristics of subjects in the alcohol use disorder assessment sample

Characteristic	Non-dependent (N = 406)		Alcohol dependent (N = 559)	
	AA (N = 301)	AG/GG (N = 105)	AA (N = 419)	AG/GG (N = 140)
<i>Demographic variables</i>				
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
Age	27.2 (11.1)	26.1 (9.3)	43.0 (18.0)	46.6 (20.8)
Delay discounting ^a	−5.2 (2.1)	−4.9 (2.4)	−4.5 (3.0)	−4.1 (2.9)
FTND score ^a	0.0 (0.0)	0.0 (0.0)	2.0 (5.0)	2.0 (5.0)
	N (%)	N (%)	N (%)	N (%)
Sex (female) ^b	137 (45.5)	29 (27.6)	125 (29.8)	45 (32.1)
Family history positive ^a	68 (26.8)	24 (25.5)	259 (67.4)	83 (65.9)
Major depressive disorder	15 (5.0)	3 (2.9)	70 (16.7)	26 (18.6)
Anxiety disorder	11 (3.7)	4 (3.8)	142 (33.9)	53 (37.9)
Cocaine abuse/dependence	5 (1.7)	0 (0.0)	170 (40.6)	60 (42.9)
Cannabis abuse/dependence	15 (5.0)	5 (4.8)	229 (54.7)	70 (50.0)
<i>Outcome variables</i>				
	Median (IQR)	Median (IQR)	Median (IQR)	Median (IQR)
AUDIT score ^a	5.0 (5.0)	5.0 (5.0)	18.0 (13.5)	16.0 (14.0)
Total drinks ^a	63.0 (82.3)	77.0 (100.5)	789.8 (952.5)	810.0 (810.5)
Average drinks per drinking day ^a	2.5 (2.0)	2.6 (2.3)	12.1 (9.7)	11.9 (8.0)
Binge drinking days ^a	2.0 (9.0)	2.0 (12.0)	69.0 (54.0)	68.0 (48.5)

^aMissing data for some participants: N = 366 for delay discounting, N = 91 for Fagerström Test for Nicotine Dependence (FTND) Score, N = 107 for family history status, N = 537 for Alcohol Use Disorder Identification Test (AUDIT) score, and N = 116 for Timeline Followback interview. ^bFor non-dependent participants, there was a significant relationship between genotype and sex; $\chi^2(1) = 10.3$, $p = 0.001$

alcohol withdrawal (Clinical Institute Withdrawal Assessment for Alcohol Scale Revised score greater than 8) [27], (8) current or chronic medical conditions, including cardiovascular conditions, requiring inpatient treatment or frequent medical visits, or (9) use of medications that may interact with alcohol within the 2 weeks prior to the study. Females were excluded if they were breastfeeding, pregnant, or intended to become pregnant. Although not formal exclusion criteria, none of our participants displayed a heavy alcohol consumption pattern (>15 drinks per week for women or >20 drinks per week for men) and only one of our participants met criteria for alcohol abuse.

Genotyping. Genomic DNA was extracted from whole blood using the QIAmp DNA Blood Maxi Kit (Qiagen Hilden, Germany) and run on an Illumina OmniExpress BeadChip array (Illumina San Diego, California, USA). The average genotype reproducibility was more than 0.99994. AIMs [28] were obtained from the array data and ancestral proportions were calculated from extracted markers for all study participants. Ancestry assessment identified six ethnic factors: Africa, Europe, Asia, Far East Asia, Oceania, and Americas.

OPRM1 rs1799971 was genotyped using the assay-on-demand from Applied Biosystems (Foster City, California, USA). The alleles were discriminated by post-polymerase chain reaction plate read on an ABI Prism 7900HT Sequence Detection System. Our sample did not significantly deviate from Hardy-Weinberg equilibrium ($\chi^2 = 0.048$, $p = 0.826$).

Laboratory paradigm. Participants completed an intravenous alcohol self-administration session, a paradigm which has shown good test–retest reliability and external validity in prior studies [29, 30]. This paradigm eliminates the large variability in blood alcohol concentration (BAC) associated with oral alcohol intake [31, 32] by bypassing gastrointestinal absorption and first-pass metabolism and using an algorithm that adjusts for age, sex, height, and weight to account for differences in alcohol distribution and elimination [33].

As a result, each infusion increases alcohol levels by a standardized amount, allowing the infusion software to provide continuous estimates of arterial blood alcohol levels that closely track brain alcohol exposure [34] and breathalyzer readouts [35].

Participants were instructed not to drink alcohol in the 48 h prior to study procedures. Upon arrival, they provided a breathalyzer reading and a urine sample that was tested for illicit drugs (and pregnancy for females). These tests had to be negative to proceed with the study session. After eating a standardized 350 kilocalorie meal, an intravenous catheter was inserted into the antecubital vein in the forearm.

Intravenous alcohol self-administration was conducted using Computer-assisted Alcohol Infusion System software. The session consisted of a 25-min priming phase and a 125-min free access phase. During the first 10 min of the priming phase, participants were required to push a button four times at 2.5 min intervals. Each button press resulted in an alcohol infusion that raised BACs by 7.5 mg% in 2.5 min, such that participants achieved a peak concentration of approximately 30 mg% at minute 10. During the next 15 min, the button remained inactive while participants experienced the effects of alcohol. Starting at minute 25, participants could self-administer ad libitum, but they had to wait until one infusion was completed before initiating another. Breath alcohol concentration was also obtained via breathalyzer at 15-min intervals to confirm the software-calculated estimates; these readings were entered into the software to provide the model feedback and the infusion rate was automatically adjusted accordingly. Blood alcohol concentration was estimated continuously by the software based on infusion rate and model-estimated metabolism. Software estimates of BAC were used to determine cumulative alcohol exposure, defined as the area under the curve of the estimated BAC versus time plot, and whether a participant reached binge-level exposure, defined as achieving an estimated BAC greater or equal to 80 mg% [36]. A limit was imposed such that estimated BAC could not exceed 100 mg% to prevent adverse events due to intoxication.

Measures. To assess subjective response to alcohol prior to the development of tolerance, we used the first five drinking occasion items on the self-rating of the effects of alcohol form (SRE) [37]. This asks participants how many drinks it took for them to feel different, to feel dizzy, to begin stumbling, and to pass out during the first five drinking occasions. The final score represents the mean of the number of drinks needed to achieve each outcome, with a higher number of drinks indicating a lower level of response to alcohol.

During the intravenous alcohol self-administration session, participants were also assessed for subjective response to alcohol and craving using a series of questionnaires administered at baseline, after receiving the priming dose of alcohol (minute 20), and every 15 min during the free access phase. Measures included: (1) The Drug Effects Questionnaire (DEQ) [38]: Our DEQ employs a 100 mm visual analog scale that allows subjects to identify the degree to which they experience five potential outcomes of alcohol intoxication: Feel ("do you feel any drug effects?"), Like ("do you like the effects you are feeling now?"), More ("would you like more of what you received, right now?"), High ("do you feel high?"), and Intoxicated ("do you feel intoxicated?"). (2) The Biphasic Alcohol Effects Scale (BAES): The BAES contains 14 items rated on an 11-point Likert scale that collectively measure acute alcohol-induced stimulation and sedation [39]. Seven-item stimulant and sedative subscales were used in our analyses. (3) The Alcohol Urge Questionnaire (AUQ): The AUQ contains eight items rated on a 7-point Likert scale to assess alcohol craving [40].

AUD assessment study

Participants. A total of 1836 participants were recruited under three NIH Institutional Review Board-approved screening and evaluation protocols. Participants were excluded if they were less than 18, pregnant, or were unable to provide informed consent. Prisoners were also excluded. These protocols serve to extensively phenotype both healthy controls and individuals with AUD in order to (1) collect a common set of measures for all research participants at our institute and (2) determine eligibility to participate in other studies. Thus, all of the alcohol self-administration participants completed the screening and evaluation protocol prior to being enrolled in the self-administration study. Alcohol self-administration participants were selected from the larger group using the inclusion and exclusion criteria specified in the intravenous alcohol self-administration study section.

To avoid potential confounds from unequally distributed allele frequencies across ancestries, the analysis was limited to individuals of European ancestry ($N=1002$). Among subjects of European ancestry, those with missing SCID data were also excluded ($N=37$). The final sample was 965 participants. The participants were divided into those without a current or past diagnosis of alcohol dependence (control group, $N=406$) and participants with a current or past diagnosis of alcohol dependence (AD group, $N=559$, Table 2).

Measures. All subjects who participated in the intravenous alcohol self-administration study also participated in the screening and evaluation protocol. Thus, measures obtained during this protocol were also used in analyses restricted to the self-administration sample.

Alcohol consumption was determined using 90-day TLFB interviews, a method of retrospectively estimating daily drinking using a calendar (Sobell et al. 1992). We looked at three outcome measures: total drinks, total number of drinking days (days in which participants consumed at least one drink), and total number of binge days over the 90-day period. We used the National Institute on Alcohol Abuse and Alcoholism's definition of a binge: four or more drinks for women and five or more drinks for men in a given day [36]. TLFB interviews were conducted with 88.0% of the final sample ($N=849$). Participants also completed the Alcohol Use Disorder Identification Test (AUDIT), a 10-item questionnaire

that assesses problematic alcohol use in the past year [41]. A cut-off score of 8 was used as a marker of risky drinking. Alcohol dependence and abuse diagnoses were determined using the Structured Clinical Interview for DSM-IV axis I disorders (SCID-IV) [25]. Nicotine use was assessed using the Fagerström Test for Nicotine Dependence [42].

Several additional measures obtained during the screening and evaluation visit have been shown to affect intravenous alcohol self-administration [43] and were included as covariates in some analyses. Family history of alcoholism was determined using a two-step process. Participants first completed the Family Tree Questionnaire to identify first-degree relatives and second-degree relatives who may have had alcohol-related problems [44]. Participants subsequently completed the family history assessment plus individual assessment modules of the Semi-Structured Assessment for the Genetics of Alcoholism for all identified relatives to confirm alcohol-related problems [45]. A family history density score was calculated by dividing the number of relatives with alcohol problems by the total number of first-degree relatives and second-degree relatives. A delay discounting task was used to measure behavioral impulsivity [46]. During this task, participants choose between smaller immediate hypothetical rewards or larger hypothetical rewards received after a delay (e.g., \$90 now or \$100 in 7 days). The degree of discounting delayed rewards, k , is a measure of impulsivity that can be calculated using the equation developed by Mazur [47].

Statistical analysis

Craving and subjective response to alcohol. To ensure that participants experienced alcohol-induced subjective effects and craving following the priming dose of alcohol, we performed Wilcoxon Signed-Rank Tests comparing DEQ and AUQ measures before and after the alcohol prime. To examine whether *OPRM1* rs1799971 was associated with craving and subjective response to alcohol, we used linear regression controlled for age and sex. Log-transformations and Box-Cox transformations [48] were employed when necessary in order to meet model assumptions. When the assumptions could not be met, we also analyzed the data using Student's t -tests or Mann-Whitney tests split by sex (given that genotype and sex were significantly related in the intravenous self-administration sample, $\chi^2(1)=6.6$, $p=0.010$). Subjective response to alcohol was also analyzed in a subgroup of risky drinkers (AUDIT score ≥ 8) as this group has been evaluated in

Fig. 1 *OPRM1* genotype and alcohol-induced subjective response in the intravenous alcohol self-administration sample. Following the administration of a priming dose of alcohol, subjects reported how intoxicated they felt ("Feel Intoxicated") and how much they liked the effects of alcohol ("Like Effects"). The horizontal line in the middle of each box indicates the median, while the bottom and top borders of the box represent the 25th and 75th percentile values, respectively. No significant differences were observed between genotype groups

Table 3. Associations between *OPRM1* genotype and subjective response to alcohol

	BAC ^a = 25 mg% (N = 106)		BAC = 50 mg% (N = 47)		BAC = 80 mg% (N = 36)	
	Coefficient (95% CI)	p	Coefficient (95% CI)	p	Coefficient (95% CI)	p
<i>Model 1: DEQ like effects^b</i>						
<i>OPRM1</i> ^c	-2.73 (-8.30, 2.84)	0.334	-1.92 (-12.04, 8.19)	0.703	-7.31 (-16.28, 1.66)	0.107
Sex ^d	3.58 (-1.29, 8.44)	0.148	1.89 (-7.96, 11.73)	0.701	5.64 (-3.14, 14.41)	0.200
Age ^e	-0.61 (-1.12, -0.11)	0.018	-0.27 (-1.32, 0.79)	0.612	3676869 (505685, 6848054)	0.024
<i>Model 2: DEQ feel intoxicated^b</i>						
<i>OPRM1</i>	0.15 (-8.43, 8.74)	0.972	4.63 (-12.75, 22.00)	0.594	-10.98 (-30.74, 8.78)	0.266
Sex	-2.44 (-9.93, 5.06)	0.521	3.18 (-13.73, 20.09)	0.706	13.18 (-6.19, 32.55)	0.175
Age	0.31 (-0.47, 1.09)	0.433	1.14 (-0.66, 2.95)	0.208	-1.46 (-3.94, 1.03)	0.241
<i>Model 3: DEQ want more</i>						
<i>OPRM1</i>	5.49 (-4.12, 15.10)	0.260	-0.99 (-11.30, 9.32)	0.847	-8.85 (-19.24, 1.53)	0.092
Sex	9.94 (1.55, 18.33)	0.021	-3.04 (-13.07, 6.99)	0.544	-1.99 (-12.15, 8.17)	0.692
Age ^e	-0.58 (-1.45, 0.29)	0.189	-0.95 (-2.02, 0.12)	0.081	4307487 (635611, 7979363)	0.023
<i>Model 4: DEQ feel effects^b</i>						
<i>OPRM1</i>	-4.22 (-13.74, 5.30)	0.381	5.36 (-10.94, 21.66)	0.510	-9.41 (-27.22, 8.41)	0.290
Sex	1.11 (-7.21, 9.42)	0.793	3.44 (-12.42, 19.30)	0.664	1.35 (-16.12, 18.81)	0.876
Age	0.19 (-0.67, 1.05)	0.660	1.28 (-0.41, 2.97)	0.135	-0.45 (-2.69, 1.79)	0.685
<i>Model 5: DEQ feel high^b</i>						
<i>OPRM1</i>	2.40 (-6.28, 11.08)	0.585	8.61 (-9.62, 26.85)	0.346	-9.38 (-30.24, 11.49)	0.367
Sex	-1.75 (-9.33, 5.83)	0.648	9.40 (-8.34, 27.14)	0.291	11.31 (-9.14, 31.76)	0.268
Age	0.46 (-0.32, 1.25)	0.246	1.59 (-0.31, 3.48)	0.098	-0.59 (-3.21, 2.03)	0.652
<i>Model 6: BAES stimulation</i>						
<i>OPRM1</i>	1.14 (-2.61, 4.90)	0.547	-0.87 (-10.63, 8.88)	0.857	-5.35 (-16.24, 5.53)	0.324
Sex	2.00 (-1.29, 5.29)	0.231	4.70 (-4.80, 14.19)	0.324	5.60 (-5.07, 16.27)	0.293
Age ^e	514454 (-703709, 1732617)	0.404	-0.36 (-1.37, 0.65)	0.477	-0.94 (-2.31, 0.43)	0.171
<i>Model 7: BAES sedation^f</i>						
<i>OPRM1</i>	1.01 (-2.03, 4.05)	0.510	-4.71 (-10.17, 0.74)	0.088	-4.39 (-12.15, 3.38)	0.258
Sex	-0.44 (-3.09, 2.21)	0.743	5.92 (0.62, 11.23)	0.030	7.89 (0.28, 15.50)	0.043
Age	0.05 (-0.23, 0.32)	0.730	-0.06 (-0.62, 0.51)	0.840	-0.45 (-1.43, 0.52)	0.350

BAC blood alcohol concentration. ^aFor Models 1 to 5 BAC = 25 mg%: the dependent variable is the DEQ measure taken after the priming dose of alcohol; BAC = 50 and 80 mg%: the dependent variable is the DEQ measure taken at the time point most proximal to achieving the target blood alcohol concentration. For Models 6 and 7 BAC = 25 mg%: the dependent variable is change in BAES subscale score following the priming dose of alcohol; BAC = 50 and 80mg%: the dependent variable is the change in BAES subscale score from baseline to the time point most proximal to achieving the target blood alcohol concentration. ^bFor all models testing DEQ measures at BAC= 25 mg% except for the DEQ Want More model, the residuals were not normally distributed. This may affect the accuracy of the above estimates. Therefore, the relationship between genotype and the distribution of these four DEQ measures at BAC = 25 mg% also analyzed using Mann-Whitney tests split by sex. These results are reported in the supplement. There were also outliers in the DEQ like effects models at BAC = 25 and 50mg%, which could have affected model fit. Reanalysis with outliers removed did not change the significance of our findings (BAC = 25 mg%, two outliers removed, *OPRM1* genotype $b = -1.21$, 95% CI -5.86 to 3.43, $p = 0.605$, BAC = 50 mg%, one outlier removed, *OPRM1* genotype $b = 1.94$, 95% CI -6.75 to 10.63, $p = 0.655$). ^c*OPRM1* genotype coded as a binary variable (1 = G allele carrier, 0 = A/A homozygote). ^dSex coded as a binary variable (1 = male, 0 = female). ^eAge is Box-Cox transformed (exponent -4.0) for DEQ like effects at BAC = 80 mg%, DEQ want alcohol at BAC = 80 mg%, and BAES stimulation at BAC = 25 mg %. ^fFor the models testing BAES sedation subscale at BAC = 25 and 50mg%, the assumptions of the model were violated, which may affect the accuracy of the above estimates. Therefore, the relationship between genotype and the distribution of change in sedation subscale scores was also analyzed using Mann-Whitney tests. These results are reported in the supplement. For the model testing BAES sedation at BAC = 80 mg%, there was an outlier. Reanalysis with this outlier removed did not change the significance of our findings (*OPRM1* genotype $b = -1.38$, 95% CI -5.77 to 3.01, $p = 0.527$)

previous studies assessing the effect of *OPRM1* A118G on alcohol sensitivity.

Intravenous alcohol self-administration. We plotted Kaplan-Meier Curves split by genotype in males and females to assess the association between genotype and rate of bingeing throughout the free access phase of the IV alcohol self-administration session. To further examine whether rs1799971 was a predictor of cumulative alcohol exposure (area under the curve of the breath alcohol

concentration versus time plot) and rate of bingeing, we used linear regression and Cox proportional hazards analysis respectively. For both analyses, the model included genotype, sex, age, delay discounting, and family history of alcohol dependence given that these other measures are associated with rate of consumption [43]. One participant (*OPRM1* genotype = A/A) terminated her intravenous alcohol self-administration session at minute 16.5 of the free access phase as a result of equipment malfunction. Because it is impossible to achieve a binge level of exposure

Fig. 2 Kaplan-Meier survival curves examining the effect of *OPRM1* genotype on rate of bingeing in the intravenous alcohol self-administration sample. Panels show the cumulative probability of achieving a binge-level exposure (estimated blood alcohol concentration of 80 mg%) during the intravenous alcohol self-administration session in males and females. There were no significant differences between genotype groups

within this timeframe, this participant's data was only used for the subjective response and craving analyses (thus $N = 105$ for the alcohol self-administration analyses).

Alcohol consumption. Mann-Whitney tests were used to detect any differences in the distribution of TLFB measures between the two genotype groups in both dependent and non-dependent participants.

Alcohol dependence diagnosis. The relationship between lifetime AD diagnosis and genotype in the AUD assessment sample was analyzed using binary logistic regression while controlling for sex, age, and the most prevalent psychiatric comorbidities in our sample (cocaine and cannabis dependence or abuse, any anxiety disorder, and major depressive disorder).

Given the strong evidence that correcting for multiple comparisons reduces false positive results in candidate gene studies [49], we planned to perform a Benjamini-Hochberg false discovery rate procedure [50] for analyses with multiple outcomes (e.g. DEQ subscales). Uncorrected p -values are reported given that none of these analyses reached statistical significance prior to correction.

RESULTS

Intravenous alcohol self-administration results

***OPRM1* genotype and subjective response to alcohol.** In the intravenous alcohol self-administration sample, we used the SRE to assess the effect of *OPRM1* rs1799971 genotype on subjective response to alcohol during the first five drinking occasions. Linear regression with age (Box-Cox transformed), sex, and genotype as independent variables revealed no effect of genotype on this measure ($b = -0.005$, 95% CI -0.879 to 0.869 , $p = 0.991$, Table S1).

During the intravenous infusion, subjective response was measured following a priming dose of alcohol (median peak BAC attained = 25.38 mg%, interquartile range = 5.92 mg%). The priming dose of alcohol led to a significant change in the distribution of all five DEQ measures from baseline ($Z_w < -3.0$, $p < 0.001$ for all tests). Mann-Whitney tests split for sex revealed no effect of *OPRM1* genotype on the distribution of liking alcohol or feeling intoxicated in males (like effects $U(36,20) = 305.0$, $p = 0.345$; feel intoxicated $U(36,20) = 338.0$, $p = 0.706$, Fig. 1) or females (like effects $U(43,7) = 126.0$, $p = 0.492$; feel intoxicated $U(43,7) = 143.0$, $p = 0.834$). Further analyses using the other DEQ items and the BAES stimulant and sedative subscales as dependent variables

found no effect of genotype on subjective response following alcohol priming (Table 3 and Supplemental Results). Results remained insignificant when the analyses were restricted to participants with AUDIT score ≥ 8 (Table S2).

To ensure that a genotype effect would not be observed at higher blood alcohol levels, we examined subjective response to alcohol in subgroups of participants who reached BACs of 50 mg% ($N = 47$, 14G allele carriers) and 80 mg% ($N = 36$, 11 G allele carriers). Subjective response was measured at the time point most proximal to reaching the target BAC. Linear regression models with age, sex, and genotype as independent variables and each of the above DEQ and BAES measures as the dependent variable revealed no effect of genotype on subjective response at either of these BACs (Table 3).

***OPRM1* genotype and craving for alcohol.** We used the AUQ to examine whether *OPRM1* rs1799971 genotype affected baseline alcohol craving and change in craving following the priming dose of alcohol during the intravenous self-administration session. Linear regression with baseline craving (log-transformed) as the dependent variable and age (Box-Cox transformed), sex, and genotype as independent variables found no effect of genotype on baseline craving ($b = -0.034$, 95% CI -0.210 to 0.141 , $p = 0.700$, Table S3). The priming dose of alcohol produced a significant change in craving ($Z_w = -5.05$, $p < 0.001$). Student's t -tests split for sex revealed that mean alcohol-priming induced change in craving did not differ by genotype in males ($t(54) = -0.462$, $p = 0.646$) or females ($t(48) = 0.152$, $p = 0.880$).

***OPRM1* genotype and alcohol self-administration.** We plotted Kaplan-Meier survival curves by *OPRM1* rs1799971 genotype and sex to assess the effects of genotype on rate of bingeing, defined as achieving an estimated BAC ≥ 80 mg%. There was no difference between the two genotype groups (males: log-rank test = 0.044, $p = 0.834$, females: log-rank test = 0.127, $p = 0.721$, Fig. 2). We then employed a Cox proportional hazards model to determine whether genotype was associated with rate of bingeing throughout the session and a linear regression model to determine whether genotype was associated with total alcohol exposure (log-transformed) throughout the session. Age, sex, delay discounting, family history density, and *OPRM1* genotype were the independent variables in these models. There was no association between rate of bingeing throughout the session and genotype (Hazard ratio = 1.030, 95% CI 0.483–2.197, $p = 0.938$, Table S4) or total alcohol exposure and genotype ($b = 0.116$, 95% CI -0.347 to 0.579 , $p =$

Fig. 3 *OPRM1* genotype and binge drinking days in the alcohol use disorder assessment sample. Histograms depict the distribution of binge drinking days in participants without a lifetime history of alcohol dependence (**a**) and with a lifetime history of alcohol dependence (**b**). The distributions were not significantly different between genotype groups

0.621, Table S5). Our findings did not differ when the analysis was restricted to participants with AUDIT score ≥ 8 (Tables S4 and S5).

AUD assessment sample results

***OPRM1* genotype and alcohol consumption.** To determine whether there was an association between *OPRM1* rs1799971 and real-world alcohol consumption, consumption over the 90 days preceding assessment was analyzed in dependent and non-dependent participants in our AUD assessment sample. There was no effect of genotype on the distribution of total drinks consumed, number of drinking days, or number of binge drinking days in either dependent participants (total drinks $U(388,132) = 25297.5$, $p = 0.835$, drinking days $U(388,132) = 24319.5$, $p = 0.385$, binge drinking days $U(388,132) = 25156.5$, $p = 0.761$, Fig. 3) or non-dependent participants (total drinks $U(244,85) = 9703.5$, $p = 0.377$, drinking days $U(244,85) = 9857.0$, $p = 0.497$, binge drinking days $U(244,85) = 10364.5$, $p = 0.994$). Given that there was a relationship between sex and genotype in non-dependent participants (Table 2), we repeated this analysis split for sex, which did not alter the significance of our findings.

***OPRM1* genotype and alcohol dependence diagnosis.** *OPRM1* rs1799971 minor allele frequencies in alcohol dependent cases and non-dependent controls were 0.132 and 0.140 respectively. Logistic regression was used to investigate the effect of G allele carrier status on the probability of AD diagnosis after controlling for sex and the most common comorbid DSM-IV diagnoses in our sample. Genotype was not a significant predictor of lifetime alcohol dependence when controlling for these variables (OR = 0.954, 95% CI 0.643–1.417, $p = 0.816$, Table S6).

DISCUSSION

Our results provide a strong challenge to the hypothesis that *OPRM1* A118G is associated with alcohol consumption. We did not see any differences between genotype groups in either cumulative alcohol exposure or rate of bingeing during our laboratory paradigm, which provides continuous and accurate estimates of BAC. Furthermore, using a large sample of dependent and non-dependent participants, we demonstrated that there is no difference in total alcohol consumption, drinking days, binge drinking days, or likelihood of an alcohol dependence diagnosis between G allele carriers and A/A homozygotes, which accords with a similar analysis of several large Finnish cohorts [51].

In concordance with our consumption analyses, we found that *OPRM1* rs1799971 genotype did not affect subjective response to alcohol or craving. We used multiple measures of subjective response. Firstly, we looked at whether genotype affected alcohol sensitivity during the first five drinking occasions, a measure which is subject to recall bias but should not be confounded by alcohol exposure and eventual tolerance. Secondly, using our intravenous alcohol self-administration paradigm, we examined whether genotype affected subjective response following a fixed priming dose of alcohol, a measure which may be confounded by tolerance but is not subject to recall bias. Finally, to determine whether a genotype effect would only be observed at higher levels of alcohol exposure, we examined whether genotype predicted subjective response at blood alcohol levels of 50 and 80 mg%. These analyses provided convergent evidence against an effect of *OPRM1* rs1799971 genotype on subjective response to alcohol. We also found no evidence of differences between genotype groups in baseline or alcohol-induced craving.

Our findings contrast with several prior studies that have reported positive associations between *OPRM1* rs1799971 genotype and subjective response to alcohol. These studies have assessed subjective response to alcohol using a variety of measures including the Biphasic Alcohol Effects Scale, the DEQ, and the Profile of Mood States. Many items and subscales derived from these measures are only weakly correlated (for correlations between our subjective response measures following the priming dose of alcohol, see Table S7), and looking at items on a range of scales raises the risk of finding spurious associations due to multiple comparisons. Moreover, *OPRM1* rs1799971 genotype has not reliably predicted changes in the same subjective measures across studies. For example, one study found that compared to A/A homozygotes, G allele carriers report greater alcohol-induced sedation but similar alcohol-induced vigor [17], whereas another found that they experience greater alcohol-induced vigor but similar alcohol-induced sedation [16]. Given these limitations and inconsistencies, publication of additional well-powered studies followed by careful meta-analysis of reported findings will be needed to resolve whether an association truly exists between *OPRM1* rs1799971 genotype and subjective response to alcohol.

It is possible that *OPRM1* A118G may interact with other polymorphisms to influence alcohol sensitivity or consumption. For example, several analyses suggest that *OPRM1* A118G's effects on alcohol sensitivity may be influenced by an interaction with a

40-base pair variable number tandem repeat of the dopamine transporter gene (*SLC6A3* or *DAT1*) [52–54]. In these studies, samples were divided into those homozygous for the 10-repeat allele (A10) and those carrying at least one allele with 9 repeats or less (A9). However, findings to date have been inconsistent. In one study, G allele carriers with two A10 alleles showed steeper alcohol-induced changes in the BAES stimulant subscale than other genotype groups [53]. In contrast, another study found that A/A homozygotes who were A9 carriers demonstrated the greatest alcohol-induced stimulation after consuming a single drink [52], while a third found study found no evidence of an *OPRM1* by *DAT1* interaction on this subscale [54]. Using the subjective high assessment scale, which predominantly measures negative subjective effects, one study found that G allele carriers with the A9 allele had higher scores compared to the other three genotype groups [54], whereas another did not observe any genotype interaction [52]. These studies had important differences with regards to participant characteristics, dose of alcohol administered, and route of administration. Further studies are therefore necessary to determine whether interactions between *OPRM1* A118G and other genetic variants could explain the variable effects on alcohol sensitivity and consumption observed in prior studies.

The effects of *OPRM1* A118G genotype in different ancestries remains to be explored. Unlike larger genome wide association studies which are stratified by ancestry, many published human laboratory studies recruit participants from diverse ancestries. This may lead to confounding as minor allele frequency varies substantially by ancestry. Genotype effects on alcohol consumption may also vary by ancestry. For example, there is meta-analytic evidence that Asian but not Caucasian G allele carriers may be at higher risk of alcohol dependence [55]. These possible ancestry-specific effects should be considered in future studies.

There are several limitations to the current study. This is a retrospective analysis of the effects of *OPRM1* A118G genotype on consumption and sensitivity; studies that recruit by genotype may produce different results. Nonetheless, analyses were carefully controlled for confounding variables to reduce the possibility that sampling affected our conclusions. Intravenous alcohol administration differs from oral alcohol consumption in that it eliminates alcoholic beverage-related cues such as appearance, smell, and taste. There is evidence that *OPRM1* A118G influences cue-induced craving [18], which would not be detected by our paradigm. However, if *OPRM1* A118G causes greater cue-induced craving, one would expect that this should translate to greater real-world alcohol consumption, which we did not observe in our AUD assessment sample. Our intravenous self-administration analyses were also limited to social drinkers and the findings may not extend to heavier drinkers. However, we did not observe any genotype effects in exploratory analyses of our participants with AUDIT scores greater or equal to 8. Furthermore, in our AUD assessment sample, we did not find any genotype effects on alcohol consumption in dependent or non-dependent participants. This suggests that there are not differential genotype effects in social versus heavy drinkers. Finally, it has been suggested that G allele carriers may have a slight reduction in overall liability for substance use disorders [23]. Such a small effect would not be detectable in our sample. We were also underpowered to examine whether individuals homozygous for the G allele differed from heterozygotes as there were only 17 G/G homozygotes in our AUD assessment sample. Larger samples will be required to address this question.

The *OPRM1* A118G polymorphism has important physiological consequences including decreased expression of mu-opioid receptors and altered dopaminergic response to alcohol. However, these physiological alterations did not translate into altered subjective response to alcohol, increased craving, or increased alcohol consumption in our sample. This suggests that *OPRM1*

A118G may not contribute to the development and maintenance of AUD in individuals of European ancestry.

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AUTHOR CONTRIBUTIONS

MES and VAR conceptualized the manuscript. MES and TDK conducted the data analysis and drafted the manuscript. JLG assisted with drafting the introduction and with figure preparation. HS genotyped participants for *OPRM1* rs1799971. BLS supervised the collection of intravenous alcohol self-administration data. JES assisted with data analysis. All authors revised the manuscript, critically reviewed content, and approved the final version for publication. MES and TDK contributed equally to this manuscript.

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ADDITIONAL INFORMATION

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