Priority Communication

Biological Psychiatry

# A Pharmacogenetic Determinant of Mu-Opioid Receptor Antagonist Effects on Alcohol Reward and Consumption: Evidence from Humanized Mice

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**ABSTRACT**

**BACKGROUND:** It has been proposed that therapeutic responses to naltrexone in alcoholism are moderated by variation at the mu-opioid receptor gene locus (*OPRM1*). This remains controversial because human results vary and no prospectively genotyped studies have been reported. We generated humanized mice carrying the respective human *OPRM1* A118G alleles. Here, we used this model system to examine the role of *OPRM1* A118G variation for opioid antagonist effects on alcohol responses.

**METHODS:** Effects of naltrexone on alcohol reward were examined using intracranial self-stimulation. Effects of naltrexone or nalmefene on alcohol intake were examined in continuous access home cage two-bottle free-choice drinking and operant alcohol self-administration paradigms.

**RESULTS:** Alcohol lowered brain stimulation reward thresholds in 118GG mice in a manner characteristic of rewarding drugs, and this effect was blocked by naltrexone. Brain stimulation reward thresholds were unchanged by alcohol or naltrexone in 118AA mice. In the home cage, increased alcohol intake emerged in 118GG mice with increasing alcohol concentrations and was 33% higher at 17% alcohol. At this concentration, naltrexone selectively suppressed alcohol intake in 118GG animals to a level virtually identical to that of 118AA mice. No effect of naltrexone was found in the latter group. Similarly, both naltrexone and nalmefene were more effective in suppressing operant alcohol self-administration in 118GG mice.

**CONCLUSIONS:** In a model that allows close experimental control, *OPRM1* A118G variation robustly moderates effects of opioid antagonism on alcohol reward and consumption. These findings strongly support a personalized medicine approach to alcoholism treatment that takes into account *OPRM1* genotype.

*Keywords:* Alcohol, Intracranial self-stimulation (ICSS), Mouse, Mu-opioid receptor, Pharmacogenetics, Reward

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Alcohol abuse remains a major global disease burden, causing a large need for effective therapies (1). While acamprosate, a previously suggested functional glutamate antagonist recently shown to exert its effect predominantly via calcium (2), and the opioid receptor antagonist naltrexone are currently approved for alcoholism treatment and meta-analyses support their efficacy, their average effect size is limited and prescription rates low (3–5). The small average effect sizes may reflect limited clinical utility but may alternatively result from heterogeneity of treatment responses. Identifying predictors of treatment response would have significant clinical impact by allowing delivery of effective treatment to responsive patient populations. Genetic variation is a potential source of individual variation in treatment responses, and understanding its role may enable development of personalized medicine approaches to alcoholism treatment (6,7).

Alcohol intake triggers release of endogenous opioids (8,9). The mu-opioid receptor (MOP), encoded by the *OPRM1* locus, mediates, in part, rewarding properties of alcohol and is the target for naltrexone. MOP deletion in mice results in attenuation or elimination of alcohol self-administration (10,11), while opioid antagonists suppress alcohol intake in numerous species (12). Functional genetic variation at the *OPRM1* locus may moderate therapeutic responses to opioid antagonists such as naltrexone. A single-nucleotide polymorphism (SNP) A118G exists in *OPRM1* exon 1 and encodes an Asn40Asp substitution in the extracellular N-terminal loop of the MOP, resulting in loss of a glycosylation site (13). The precise molecular consequences of this mutation remain unclear, but findings in animal models, human laboratory studies, and some but not all clinical trials indicate that the *OPRM1* 118G allele confers elevated alcohol reward and enhanced therapeutic response to naltrexone [reviewed in (5,6,14,15)]. A pharmacogenetic role of this variant is of particular interest among individuals of European or Asian ancestry, where its frequency is 15% to 30% and 40% to 50%, respectively, while 118G allele frequency is low (1% to 3%) among individuals of African or Hispanic ancestry (16,17).

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Similar to other drugs of abuse, alcohol reward is, in part, mediated through dopamine (DA) release in the ventral striatum. Using positron emission tomography and displacement of the D2/3-receptor ligand [$^{11}$C]-raclopride, we demonstrated that alcohol-induced DA release in social drinkers is enhanced in the ventral striatum of 118G-allele carriers (18). We also generated humanized mouse lines in which mouse *Oprm1* exon 1 was replaced with the human sequence on a C57BL/6 background, with or without introduction of the 118G SNP through site-directed mutagenesis. This approach is in contrast with the mouse line (19) in which an orthologous SNP (A112G) was introduced into the murine exon 1. In both lines, presence of the minor allele leads to a deletion of an extracellular glycosylation site; however, the murine 112A isoform has four N-terminal extracellular glycosylation sites and the 112G isoform has three, while the humanized 118A isoform has five sites and the 118G has four. This difference may partially account for differing results between the two lines. Using in vivo microdialysis to measure alcohol-induced DA release in the ventral striatum of these mice, we found enhanced DA responses to alcohol in the h/m*OPRM1*-118GG line compared with h/m*OPRM1*-118AA mice (18). This allowed us to isolate the influence of A118G variation from potential confounds of other variants that may be in linkage disequilibrium with it or from ethnic stratification. Our findings demonstrated that introduction of the 118G mutation is sufficient to replicate the functional consequences of human *OPRM1*-A118G variation for a key response subserving alcohol reward and validated these humanized mouse lines as a reverse-translational tool.

A pharmacogenetic role of *OPRM1*-A118G variation for therapeutic naltrexone response in alcoholism remains controversial. Human data have been variable, and studies employing prospective genotyping and stratification by genotype are lacking. Here, we used the humanized *OPRM1* mouse model to evaluate the role of *OPRM1*-A118G variation in moderating naltrexone's effects on alcohol reward and intake. We evaluated effects of naltrexone on alcohol reward using intracranial self-stimulation (ICSS) and effects on consumption using home cage two-bottle free-choice drinking and operant self-administration paradigms. In the latter model, we also evaluated nalmefene, a mu-opioid receptor antagonist with partial agonistic activity at the kappa-opioid receptor demonstrated to be more effective than naltrexone in suppressing alcohol intake in rats (20) and recently approved for clinical use in a novel as-needed approach in the European Union (21).

## METHODS AND MATERIALS

### Animals

The generation of the h/m*OPRM1*-118AA and h/m*OPRM1*-118GG mice has been described (18). Briefly, two humanized mouse lines were generated on a C57BL/6 background. The mouse *Oprm1* exon 1 was replaced by the human sequence. One line, h/m*OPRM1*-118AA, was homozygous for the major human 118A allele. For h/m*OPRM1*–118GG, the same insert was used, but site-directed mutagenesis was first used to introduce a G in position 118. The lines are genetically identical, with the exception of the A → G substitution. The two lines were crossed and maintained through heterozygous breeding as a line carrying both alleles at the *OPRM1*-A118G site.

For all experiments, adult male (6 to 8 weeks at the beginning of the experiments) littermates were used. Animals were single-housed in standard cages at 21 ± 1°C and 50 ± 5% relative humidity on a 12-hour light/dark cycle, with lights on at 7:00 AM. Animals used for ICSS were housed on a 12-hour reverse light cycle (lights on at 8:00 PM, off at 8:00 AM). Animals had free access to standard chow and tap water. Experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (ICSS) or the European Union guidelines on the care and use of laboratory animals (home cage and operant drinking).

### Drugs

For the operant procedures and two-bottle choice drinking, alcohol (95% ethyl alcohol) was diluted in tap water to target concentration (vol/vol). For operant responding, sodium saccharin (Sigma Chemical Co., Schnelldorf, Germany) was initially added to water and the alcohol solutions to achieve .2% (wt/vol) and then faded out. For ICSS experiments, alcohol solutions were administered via oral gavage in a volume of 1 mL/100 g body weight. Naltrexone (Sigma/RBI, St. Louis, Missouri) and nalmefene (Enzo, Life Sciences, Lorrach, Germany), were dissolved in .9% saline and administered intraperitoneally at a volume of 10 mL/kg (doses of .1 to 1.0 mg/kg dependent on behavior tested).

### Intracranial Self-Stimulation

ICSS was performed as described (22). Briefly, mice were anesthetized with ketamine/xylazine (120/9 mg/kg, Sigma), stereotaxically implanted with insulated monopolar stainless steel electrodes (.28 mm diameter) (Plastics One, Roanoke, Virginia) to the right medial forebrain bundle in the lateral hypothalamus (coordinates: anterior-posterior −1.2, medial-lateral −1.0, dorsal-ventral −5.0), and trained to respond for brain stimulation reward (BSR).

During each testing session, mice responded during three consecutive series of 15 descending frequencies (.05 $\log_{10}$ steps). Daily baseline BSR thresholds ($\theta_0$) and maximum operant response rates (MAX) were calculated from the average of the second and third series. After baseline determinations, mice were removed from the conditioning chambers, treated with drug, returned to the chambers, and allowed to respond during one 15-minute series. In drug pretreatment studies, mice were removed from the conditioning chambers, injected with the first drug (intraperitoneal [IP]), returned to their home cages for 15 minutes, removed and gavaged with alcohol or water (by mouth [PO]), and returned to the conditioning chambers for one 15-minute response series.

In the first experiment, all mice received vehicle (water) or alcohol (.6 to 2.4 g/kg, PO). Two weeks after the conclusion of the first experiment, mice were assigned to one of two treatment groups: all mice in the first group received vehicle or naltrexone (.1 to 1.0 mg/kg IP; 118AA $n$ = 11, 118GG $n$ = 10), while all mice in the second group received alcohol (.6 g/kg PO) or vehicle (water PO) after vehicle (saline IP) or naltrexone (1.0 mg/kg IP) pretreatment (h/m*OPRM1*-118AA $n$ = 8, h/m*OPRM1*-118GG $n$ = 11). Drugs were administered