A Polymorphism of the \(\mu\)-Opioid Receptor Gene (\(OPRM1\)) and Sensitivity to the Effects of Alcohol in Humans

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**Background:** Recent research has implicated the endogenous opioid system in the development of alcohol use disorders. The A118G polymorphism of the \(OPRM1\) gene has been shown to confer functional differences to \(\mu\)-opioid receptors, such that the G variant binds \(\beta\)-endorphin three times more strongly than the A variant. The goal of this study was to test whether the A118G polymorphism is associated with sensitivity to the effects of alcohol.

**Methods:** Participants who were either homozygous for the A allele (\(n = 23\)) or heterozygous (\(n = 15\)) received intravenous doses of alcohol designed to reach three target levels of breath alcohol concentration: 0.02, 0.04, and 0.06. The testing procedure consisted of measures of subjective intoxication, stimulation, sedation, and mood states at baseline and at each of the three target breath alcohol concentrations.

**Results:** The results suggested that individuals with the G allele reported higher subjective feelings of intoxication, stimulation, sedation, and happiness across trials as compared with participants with the A allele. Furthermore, participants with the G allele were almost three times more likely to report a positive family history of alcohol use disorders than participants with the A allele.

**Conclusions:** These findings may help to explain previous research suggesting that naltrexone is more effective among individuals with the G allele. A medication that reduces feelings of euphoria after alcohol consumption may be more successful among individuals with a genetic predisposition to greater feelings of euphoria after consuming alcohol.

**Key Words:** Alcohol, Sensitivity, Gene, Phenotype, \(OPRM1\).

ALCOHOLISM IS A complex disorder with a strong genetic component that may account for approximately half of the variability in risk (Heath and Phil, 1995). Over the past several years, considerable research efforts have focused on identifying genetic markers for alcohol abuse and dependence. The search for the genetic underpinnings of alcoholism has to a great extent relied on a candidate gene approach to examine genes of putative etiological relevance. The endogenous opioid system has been associated with the pathophysiology of substance dependence, including alcohol addiction (for review, see Bodnar and Hadjimarkou, 2003; Gianoulakis, 2001). Evidence to support this association comes from both the animal (De Waele et al., 1995) and human (Herz, 1997) literature. Hence, the gene coding for the \(\mu\)-opioid receptor (\(OPRM1\)) has received increased attention as a candidate gene for the development of alcohol use disorders.

The \(\mu\)-opioid receptor, which is encoded by the \(OPRM1\) gene, is the primary site of action for opiates with high abuse potential, such as morphine, heroin, and methadone (Pasternack, 1993). In addition, research findings have suggested that nonopioid drugs, such as cocaine and alcohol, may exert some of their effects through the activation of \(\mu\)-opioid receptors (Herz, 1997; Kreek, 1996). Specifically, the opioidergic system is thought to mediate drug-induced feelings of euphoria, analgesia, and withdrawal (Bond et al., 1998; Gianoulakis, 2001), thus playing an important role in the rewarding properties of several substances, including alcohol. The reinforcing properties that result from the activation of \(\mu\)-receptors are thought to be related to their interaction with the mesolimbic dopamine system, a pathway theorized to be associated with the rewarding effects of drugs (Gianoulakis, 2001). Furthermore, indirect support for the role of opioid receptors in the development and maintenance of alcohol dependence stems from pharmacological trials demonstrating the efficacy of naltrexone, an opioid antagonist, for the treatment of alcohol dependence (Anton et al., 1999; Balldin et al., 2003; Kiefer et al., 2003; Monti et al., 2001; O’Malley et al., 1992; Volpicelli et al., 1992).
Given the evidence that the $\mu$-opioid receptor gene (OPRM1) may play a role in the development of addictive behavior, several studies have attempted to identify functional polymorphisms within the OPRM1 locus that may account for these effects. One of the most commonly studied polymorphisms is +118A/G, located in the +118 position in exon 1, which codes for the Asn40/Asp substitution. This polymorphism has been shown to affect receptor activity for the endogenous ligand $\beta$-endorphin, such that the Asp40 variant binds $\beta$-endorphin three times more strongly than the Asn40 allele (Bond et al., 1998). As suggested by Bond et al., individuals with the G allele may display behavioral differences in responses mediated by $\beta$-endorphins at the more sensitive $\mu$-receptors. Specifically, individuals with the G allele may demonstrate differences in behavioral measures of drug-induced euphoria, analgesia, and withdrawal, which are functions that have been associated with $\mu$-receptor activity.

The A118G polymorphism has also been associated with a differential response to opioid antagonists (Hernandez-Avila et al., 2003; Oslin et al., 2003). In particular, the A118G polymorphism has been associated with individuals’ responses to both naltrexone (Oslin et al., 2003) and naloxone (Hernandez-Avila et al., 2003). The relationship is such that individuals with the G allele demonstrate enhanced hypothalamic-pituitary-adrenal axis dynamics in response to an opiate blockade (Wand et al., 2001). Specifically, individuals with the G allele demonstrate an enhanced cortisol response and a reduced agonist effect after treatment with naloxone (Hernandez-Avila et al., 2003). Thus, recent studies suggest that this single nucleotide polymorphism (SNP) is functional on a cellular level as well a behavioral level. Taken together, these findings establish a strong theoretical background for implication of the $\mu$-opioid receptor gene, particularly the A118G functional polymorphism, in the development of alcohol addiction.

Many recent studies have tested the relationship between the A118G SNP of the OPRM1 gene and substance use disorders, particularly alcoholism and opioid dependence (Bergen et al., 1997; Crowley et al., 2003; Franke et al., 2001; Gelernter et al., 1999; Kim et al., 2004; Loh et al., 2004; Luo et al., 2003; Schinka et al., 2002; Shi et al., 2002; Szeto et al., 2001; Tan et al., 2003; Town et al., 1999). In addition, Kranzler et al. (1998) studied an intronic polymorphism within the OPRM1 gene. The results, however, are inconsistent, and although some investigations have found support for the association between the A118G SNP and alcohol or opioid dependence (Schinka et al., 2002; Szeto et al., 2001; Tan et al., 2003; Town et al., 1999), others have failed to replicate these findings (Bergen et al., 1997; Crowley et al., 2003; Franke et al., 2001; Gelernter et al., 1999; Loh et al., 2004; Luo et al., 2003; Shi et al., 2002). In addition, among the studies that have found support for an association between the A118G SNP and alcohol or opioid dependence, the nature of the relationship remains unclear. Specifically, some studies have reported a lower prevalence of the G allele among the dependent group (Schinka et al., 2002; Tan et al., 2003; Town et al., 1999), whereas others have found the opposite, such that the G allele either is more prevalent among the dependent group (Szeto et al., 2001) or is associated with a heavier drinking pattern (Kim et al., 2004).

The aforementioned association studies share a common methodological limitation whereby they rely on the diagnostic criteria of alcohol or opiate dependence as the behavioral marker or phenotype. A potential drawback of using such broad and heterogeneous behavioral categories (i.e., based on diagnostic criteria) is that it makes it extremely difficult to detect differences related to genetic variations. This difficulty is magnified in the case of complex genetic disorders such as alcohol dependence. An alternative strategy for dealing with the heterogeneity of diagnostic criteria is to use more focused trait markers or endophenotypes (Gottesman and Gould, 2003). A good phenotype must be narrowly defined, readily identifiable, and related to the disorder of interest (Hutchison et al., 2002). In addition, a good phenotype must be theoretically associated with the genetic variable of interest. For example, $\mu$-opioid receptors have been known to affect drug-induced feelings of analgesia, euphoria, and withdrawal (Bond et al., 1998; Gianoulakis, 2001). Therefore, behavioral measures of subjective responses assessing alcohol-induced feelings of euphoria, analgesia, and withdrawal may represent powerful intermediate markers against which to test the association between the OPRM1 gene and sensitivity to the effects of alcohol. The subjective response to alcohol, in turn, has been shown to be both heritable and associated with the larger phenotype of alcohol dependence (Schuckit, 1988; Schuckit and Smith, 1996; Viken et al., 2003). In summary, prior research findings provide theoretical support for the conceptualization of sensitivity to the effects of alcohol as a potential endophenotype for alcohol use disorders.

This study was designed to advance knowledge in the field by (1) using behavioral markers of sensitivity to the effects of alcohol instead of the broader phenotype of alcohol dependence and (2) applying an intravenous alcohol administration paradigm to reduce the experimental variability known to be caused by individual differences in the pharmacokinetics of alcohol (Li et al., 2001). In this context, this study was designed to test the association between the A118G SNP and measures of alcohol-induced sedation, stimulation, subjective response, and mood alterations after an acute infusion of alcohol. It was hypothesized that individuals with the Asp40 variant would display enhanced sensitivity to the effects of alcohol, consistent with the notion that the A to G substitution leads to increased efficiency in receptor binding.

**METHODS**

**Sample**

Participants were 388 subjects (188 females) at the University of Colorado whose ages ranged from 21 to 29 years. Inclusion criteria were the
following: (1) a score of 8 or higher on the Alcohol Use Disorders Identification Test, indicating a moderate or heavier drinking pattern (Allen et al., 1997); (2) no history of problems with alcohol or attempts to quit; (3) self-reported drinking frequency of three or more drinks (two for women) at least twice per week; (4) no history of adverse reactions to needle puncture; and (4) successful completion of a physical health examination. In addition, all female subjects tested negative for pregnancy before the alcohol administration, and all subjects were required to have a breath alcohol concentration (BAC) of zero before each session.

Procedure and Measures

After completing a telephone questionnaire, which included the Alcohol Use Disorders Identification Test, eligible participants were invited to the laboratory for a screening session. Upon arrival at the laboratory, participants read and signed an informed consent form, provided a saliva sample for DNA analyses, completed a series of self-report measures of personality and drinking behavior, and responded to an interview assessing for a family history of alcohol problems. Participants also completed the Rutgers Alcohol Problems Index, a measure designed to assess drinking problems in adolescents and college samples (White and Labouvie, 1980).

On the basis of the results from DNA analyses, participants were invited to the alcohol-infusion session. Specifically, participants were selected on the basis of their allele status, such that groups were balanced on the A118G SNP. Furthermore, before participating in the alcohol infusion session, subjects were asked to attend a physical examination at the General Clinical Research Center at the University of Colorado. The purpose of the medical visit was to ensure that participants were in good physical health and that they were medically eligible to take part in the alcohol-infusion procedure. A total of 76 participants (38 females) were screened in the laboratory; 42 attended the physical examination, 38 of whom were invited to the infusion session. Of the 76 participants screened, 52 (68.4%) were homozygous for the A allele, 22 (28.9%) had a copy of the G allele, and 2 (2.6%) were homozygous for the G allele. Only participants who were heterozygous (n = 15) or homozygous for the A allele (n = 23) completed the experimental portion of the study. The allele frequencies observed in this study were in conformity with Hardy-Weinberg equilibrium expectations ($\chi^2(2) = 0.03; p > 0.05$).

During the experimental session, participants were seated in a recliner chair, and the intravenous line was placed in their nondominant arm. Experimenters and the nursing staff were kept blind to genotype. Participants were asked to complete a baseline assessment packet before they received any alcohol. After completing the baseline assessment, participants received intravenous doses of alcohol, as described below. Participants then completed the same assessment measures at each of the following points in the ascending curve of the breath alcohol level: 0.02, 0.04, and 0.06. After the infusion procedure was finished, participants were debriefed, given a meal, and asked to stay in the laboratory until their BAC was less than 0.02. The following measures were used to test the relationship between the A to G substitution of the OPRM1 gene and sensitivity to the effects of alcohol.

**Subjective High Assessment Scale.** The Subjective High Assessment Scale was used to assess subjective feelings of alcohol intoxication. This measure was adapted by Schuckit (1984) and has since been used extensively in alcohol challenge studies.

**Biphasic Alcohol Effects Scale.** The Biphasic Alcohol Effects Scale was used to collect information on self-reported feelings of stimulation and sedation after alcohol administration. This scale has been shown to be reliable and valid for investigating sensitivity to the effects of alcohol (Earleywine and Erblich, 1995; Martin et al., 1993) and for assessing medication effects (Swift et al., 1994).

**Profile of Mood States.** The short version of the Profile of Mood States (POMS) is a 40-item questionnaire and was used in this study to assess changes in affect after alcohol consumption (McNair et al., 1971). The following three subscales of the POMS were used in this study because of their theoretical association with the expected behavioral effects of the A118G SNP: vigor, tension, and happiness.

**Alcohol Administration.**

A number of studies have highlighted the importance of effectively controlling blood alcohol levels to reduce experimental variability (Li et al., 2001; O’Connor et al., 1998; Ramchandani et al., 1999). This is particularly important when testing participants’ sensitivity to the effects of alcohol. Therefore, our alcohol-administration paradigm consisted of delivering doses of alcohol intravenously, rather than relying on oral administration. The alcohol-infusion sessions took place at the General Clinical Research Center at the University of Colorado. The alcohol-administration procedures were performed by registered nurses under the direct supervision of a staff physician.

The infusion was performed with an intravenous 5% alcohol solution. An infusion nomogram was developed that took into account participants’ gender and weight. Male participants’ target infusion rates were determined by the following formula: 0.166 ml/min × weight in kilograms. In contrast, the following formula was used for female participants: 0.126 ml/min × weight in kilograms. Participants started the intravenous administration at half of their target infusion rate to ensure their safety and comfort during the procedure. After a few minutes, the infusion rates were increased to each individual’s target rate, and breath alcohol concentrations were monitored every 3 to 5 min. Target breath alcohol concentrations were as follows: 0.02, 0.04, and 0.06. Upon reaching each of the target levels of intoxication, participants’ infusion rates were reduced to half their maximum rate to maintain stable BACs during the testing procedure.

**DNA Analyses.**

DNA was collected by following published procedures (Freeman et al., 1997; Walker et al., 1999). Subjects swabbed their cheeks with three cotton swabs, followed by a rinse of the mouth with 10 ml of sucrose solution (4% in tap water). Genomic DNA was isolated from buccal cells by using a modification of published procedures (Lench et al., 1988; Spitz et al., 1996). An ABI Prism 7000 instrument was used to conduct 5’-nuclease (TaqMan) assays of the OPRM1 SNP by using assays commercially available from Applied Biosystems (Foster City, CA). This method involves allele-specific hybridization of oligonucleotide probes (Livak, 1999).

**RESULTS.**

A series of 2 × 3 mixed-design analyses of covariance were conducted in which OPRM1 genotype (AA versus AG allele) was a two-level between-subjects factor, trial was a three-level within-subjects factor (trial 1, BAC = 0.02; trial 2, BAC = 0.04; and trial 3, BAC = 0.06), and baseline measures were used as covariates. The primary goal of these analyses was to test for differences in measures of sensitivity to alcohol as a function of genotype. Specifically, we were interested in overall group differences in responses to acute doses of alcohol (main effects) and in group differences that emerged across levels of alcohol intoxication (group × trial interactions). Finally, we compared the OPRM1 groups on demographics variables and on measures of alcohol consumption, alcohol-related problems, and family history of alcohol pathology.
Breath Alcohol Concentration

Control over breath alcohol concentration was an important concern in this investigation. The following are the means and SDs for each of the target levels of intoxication: 0.02—mean, 0.0217; SD, 0.002; 0.04—mean, 0.0403; SD, 0.001; and 0.06—mean, 0.0605; SD, 0.002. These results suggest that the alcohol-infusion design yielded highly controlled levels of alcohol intoxication at all three trial points.

Sensitivity to Alcohol by the OPRM1 Variable

As described previously, a series of $2 \times 3$ mixed-design analyses of covariance were performed. The dependent measures examined were subjective feelings of alcohol intoxication, alcohol-induced feelings of stimulation and sedation, and mood alterations after alcohol intake.

Subjective Feelings of Intoxication

Analyses revealed a significant main effect of genotype such that the AG group reported, on average, higher levels of subjective intoxication compared with the AA group after controlling for baseline assessment [$F(1,34) = 13.20; p < 0.001$]. There was also a significant group $\times$ trial interaction [$F(2,68) = 4.71; p < 0.05$]. As can be seen in Fig. 1, the AG group reported greater feelings of intoxication across trials as compared with the AA group.

Alcohol-Induced Stimulation and Sedation

There was a significant main effect of OPRM1 on self-reported feelings of sedation, such that the AG group reported higher levels of alcohol-induced sedation than did the AA group [$F(1,33) = 7.65; p < 0.01$; Fig. 2]. There was no genotype $\times$ trial interaction. In addition, for the stimulation subscale, there was no main effect of OPRM1 [$F(1,33) < 1.00$; not significant]. There was, however, a significant group $\times$ trial interaction, wherein individuals with the G allele reported higher increases in alcohol-induced stimulation across trials than individuals with the A allele [$F(2,66) = 3.81; p < 0.05$; Fig. 3].

Profile of Mood States

There was no main effect of OPRM1, and there were no genotype $\times$ trial interactions for either the tension or the vigor subscales of the POMS. There was, however, a group $\times$ trial interaction in the happiness subscale of the POMS [$F(2,66) = 4.08; p < 0.05$], such that the AG group reported higher increases in state happiness across trials than the AA group (Fig. 4).

Demographic, Family History, and Alcohol Consumption Differences

Subsequent analyses compared the AA and AG groups on demographic, family history, and drinking outcome vari-
able (Table 1). There were no significant differences in demographic variables between the two groups. Furthermore, the groups did not differ on measures of alcohol problems (Rutgers Alcohol Problems Index), frequency of drinking episodes, and average quantity of drinks per episode. These results suggest that neither demographic nor drinking variables accounted for the differences in sensitivity to alcohol observed between the two groups. With regard to family history of alcohol use disorders, however, there was a significant difference between groups \( \chi^2(1) = 6.31; p = 0.01 \), such that individuals with the G allele were almost three times more likely to report a positive family history of alcohol use disorders than participants with the A allele. These results suggest that the A/G substitution may be more prevalent among children of alcoholics.

**Family History × Genotype Interactions**

Given that individuals with the G allele were more likely to report a family history of alcohol use disorders, post hoc analyses were performed to rule out the possibility that the effects of the 118A/G SNP on alcohol sensitivity were due to family history. Results revealed that even after controlling for family history, participants with the G allele scored higher on subjective intoxication \( F(1,27) = 8.82; p < 0.01 \), alcohol-induced sedation \( F(1,27) = 4.90; p < 0.05 \), stimulation across trials \( F(2,54) = 4.17; p < 0.05 \), and positive affect \( F(1,26) = 4.29; p < 0.05 \). Furthermore, there were no significant interactions between genotype and family history in predicting subjective feelings of intoxication \( p = 0.94 \), alcohol-induced stimulation \( p = 0.51 \), and sedation \( p = 0.18 \). There was, however, a significant three-way interaction such that family history moderated the relationship between genotype and changes in positive affect across trials \( F(2,52) = 3.36; p < 0.05 \). Specifically, individuals with a copy of the G allele were more likely to report increases in positive affect across trials if they had a negative family history of alcohol use disorders. In short, post hoc analyses suggested that the effects of the A118G SNP on alcohol sensitivity could not be explained by family history.

**DISCUSSION**

This study used an intravenous alcohol-administration paradigm to investigate the association between sensitivity to the effects of alcohol and the A118G polymorphism of the OPRM1 gene, which codes for \( \mu \)-opioid receptors. It was predicted that sensitivity to alcohol would be higher among participants with the G allele, given that the Asp40 variant binds \( \beta \)-endorphin three times more strongly than the Asn40 allele (Bond et al., 1998). Results confirmed this hypothesis. The AG group scored significantly higher than the AA group on the following measures of sensitivity to the effects of alcohol: (1) subjective feelings of intoxication; (2) self-reported stimulation and sedation after alcohol consumption; and (3) increases in positive mood, as measured by the happiness subscale of the POMS. In summary, individuals with the G allele demonstrated higher sensitivity to the effects of alcohol than participants with the A allele.

Furthermore, participants with the G allele were almost three times more likely to report a positive family history of alcohol use disorders than participants with the A allele. This finding indicates that the A to G substitution may be more prevalent among children of alcoholics.

### Table 1. Pretest Differences Between Participants With AA Versus AG Alleles

<table>
<thead>
<tr>
<th>Variable</th>
<th>AA allele ((n = 23)^a)</th>
<th>AG allele ((n = 15)^a)</th>
<th>Test for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (% male)</td>
<td>47.83</td>
<td>60.0</td>
<td>( \chi^2(1) &lt; 1, NS )</td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>96.0</td>
<td>91.30</td>
<td>( \chi^2(1) &lt; 1, NS )</td>
</tr>
<tr>
<td>Family history of alcohol problems (% family history positive)</td>
<td>25.0</td>
<td>69.23</td>
<td>( \chi^2(1) = 6.31, p = .01 )</td>
</tr>
<tr>
<td>Age (years)</td>
<td>22.39 (2.27)</td>
<td>21.60 (0.74)</td>
<td>( t(36) = -1.55, NS )</td>
</tr>
<tr>
<td>Alcohol problems in past year (RAPI) (possible range of scale: 0–92)</td>
<td>18.35 (13.34)</td>
<td>25.13 (17.18)</td>
<td>( t(36) = -1.37, NS )</td>
</tr>
<tr>
<td>Frequency of drinking episodes in past year (possible range of scale: 0–11; 6 = twice a week)</td>
<td>6.26 (1.84)</td>
<td>6.07 (1.44)</td>
<td>( t(36) &lt; 1, NS )</td>
</tr>
<tr>
<td>Average number of drinks per drinking occasion (in the last year)</td>
<td>4.06 (1.66)</td>
<td>4.40 (1.75)</td>
<td>( t(36) &lt; 1, NS )</td>
</tr>
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</table>

NS, not significant; RAPI, Rutgers Alcohol Problems Index.

* Standard deviations appear in parentheses below the means of continuous variables.
pharmacological treatment option for alcoholism. Specifically, it has been demonstrated that individuals with the G allele have higher success rates than individuals with the A allele when treated with naltrexone (Oslin et al., 2003). Furthermore, prior research has shown that individuals treated with naltrexone report a lower subjective high upon exposure to alcohol than placebo-treated individuals (Volpicelli et al., 1995). It was hypothesized that the treatment effects of naltrexone are obtained through blockade of the positive experiences produced by alcohol (Volpicelli et al., 1995). Results from this study indicated that participants with the G allele show greater subjective feelings of intoxication and greater overall sensitivity to the effects of alcohol. Thus, a medication that reduces feelings of euphoria after alcohol consumption may be more successful among individuals with a genetic predisposition to greater feelings of euphoria when consuming alcohol. In addition, the simple fact that individuals with the G allele may have more efficient binding of naltrexone to μ-opiate receptors may convey a superior response to a given dose of naltrexone. In either case, the results of this study support the previously reported findings that naltrexone is more effective among individuals with the G allele (Oslin et al., 2003).

To directly test these follow-up questions, future research should examine whether the A to G substitution may modulate the effects of naltrexone on reducing feelings of “high” and overall pleasurable experiences during alcohol consumption. More specifically, future research should examine whether the A to G substitution influences the dose-response curve of naltrexone with respect to treatment response, as well as the effects of naltrexone on acute responses to alcohol. In addition, future studies using a similar intravenous paradigm should add a saline control condition or collect baseline data after the intravenous line has been placed. Finally, further research is needed to examine whether the A to G substitution is truly more common among children of alcoholics.

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REFERENCES


