

Initial Evidence of an Association Between OPRM1 and Adolescent Alcohol Misuse

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Background: Considerable research efforts have attempted to identify genes associated with alcoholism among adults, yet few studies have examined adolescents. Identifying genes associated with alcohol misuse in youth is important given that the relative contribution of genetic and environmental influences on alcoholism varies across development. The purpose of this study was to examine the association between a polymorphism of the μ -opioid receptor gene (OPRM1) and alcohol misuse in a sample of youth and to test whether heightened sensitivity to the reinforcing effects of alcohol mediated this relationship.

Methods: Adolescents ($n = 187$; mean age = 15.4 years; 47.6% female) were genotyped for A118G (rs1799971), a single-nucleotide polymorphism (SNP) of the OPRM1 gene, and assessed for alcohol use disorder (AUD) diagnoses and other psychopathology. Alcohol misuse was also measured continuously to maximize detection of drinking problems in youth. Drinking motives were used to capture the extent to which youth consumed alcohol to enhance positive affect.

Results: AUD groups differed significantly in terms of allelic distributions of the A118G SNP, such that 51.9% of youth with an AUD carried at least one copy of the G allele compared to 16.3% of non-AUD controls. Those who carried the G allele endorsed drinking to enhance positive affect more strongly than those who were homozygous for the A allele and drinking to enhance positive affect mediated the association between OPRM1 and alcohol-related problems.

Conclusions: These data build on findings from adult studies and provide the first evidence that a polymorphism of the OPRM1 receptor gene is associated with the development of early-onset alcohol-related problems during adolescence, in part, by heightening sensitivity to the reinforcing effects of alcohol.

Key Words: Alcoholism, Genetics, OPRM1, Adolescents, Drinking Motives.

ALCOHOLISM IS HIGHLY heritable with symptom onset typically occurring during adolescence or young adulthood (Liu et al., 2004). Efforts to identify specific genes that confer liability for alcoholism have relied to a great extent on a candidate gene approach whereby genes of putative etiological relevance are studied in a case-control design (Köhnke, 2008). Yet because scientific advances in this area are based almost exclusively on research with adults, the degree to which these findings generalize to adolescents remains unknown. By directly investigating the etiological relevance of candidate genes in youth, our understanding of adolescent alcohol misuse and the genes associated with alcoholism could be substantially enhanced. This is particularly important in light of evidence that the relative contribution of

genetic and environmental influences on alcoholism appears to vary across development (e.g., Kendler et al., 2008; Pagan et al., 2006).

The endogenous opioid system plays an integral role in the pathophysiology of alcohol and other drug dependence (Dackis and O'Brien, 2005). Alcohol consumption increases opioidergic activity, which inhibits gamma aminobutyric acid (GABA) neurotransmission and results in acute dopamine release from mesocorticolimbic neurons (Kreek, 1996). This acute dopamine release is critically involved in the rewarding and reinforcing effects of alcohol and other addictive substances (Weiss and Porrino, 2002). Given the essential role of the endogenous opioid system in the pharmacological effects of alcohol, the μ -opioid receptor gene (OPRM1) has received considerable attention as a candidate gene for alcoholism risk.

Of the myriad genetic variants identified in the OPRM1 gene, the single nucleotide polymorphism (SNP) A118G (rs1799971) is the most common and widely studied in relation to alcoholism. Across populations, most individuals are homozygous for an allelic variant of this SNP (A allele) that codes the amino acid asparagine in the receptor protein, while a minority of individuals carry a variant that results in a substitution of asparagine for aspartate in the receptor protein (G allele). The relative frequencies of these variants, however,

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Received for publication May 26, 2009; accepted August 30, 2009.

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DOI: 10.1111/j.1530-0277.2009.01073.x

differ considerably among distinct racial groups, such that approximately 15% of Caucasians carry at least one copy of the G allele compared to 35% of East Asians and only 1% of African Americans (Bergen et al., 1997; Bond et al., 1998; Crowley et al., 2003; Gelernter et al., 1999; Hernandez-Avila et al., 2003). Initial molecular research suggested that this allelic substitution affects receptor activity for endogenous ligand β -endorphin, such that the G allele variant is associated with significantly higher affinity for β -endorphin (Bond et al., 1998). But subsequent studies failed to replicate this finding (Befort et al., 2001; Beyer et al., 2004) and recent research found an association between the G allele and loss of μ -opioid receptor function via reduced messenger RNA and OPRM1 protein levels (Zhang et al., 2005). In addition, neuroimaging research similarly found lower availability of μ -opioid receptors in the ventral striatum of adult alcoholics with the G allelic variant as compared to those without this allele (Heinz et al., 2005). Although additional research is necessary to fully elucidate how variation in the OPRM1 receptor gene relates to the neurobiology of alcoholism, animal studies indicate that this polymorphism is central to the reinforcing effects of alcohol (e.g., Barr et al., 2007; Ghozland et al., 2005).

Despite neurophysiological and preclinical evidence implicating the A118G SNP in the pathogenesis of alcoholism, findings from research evaluating this association in adults are mixed (see Arias et al., 2006; van der Zwaluw et al., 2007). While some studies found a higher prevalence of the G allele variant among adults with alcohol dependence compared to nonalcohol dependent controls (e.g., Bart et al., 2005; Town et al., 1999), others did not find this association (e.g., Bergen et al., 1997; Franke et al., 2001; Kim et al., 2004a; Loh et al., 2004; Lou et al., 2003; Nishizawa et al., 2006; Rommelspacher et al., 2001; Zhang et al., 2006). Possible explanations for disparate findings include sample selection biases (e.g., overreliance on individuals in treatment), clinical heterogeneity across studies (e.g., variation in comorbid psychopathology), and insufficient specificity in the diagnostic phenotype of alcohol dependence (van der Zwaluw et al., 2007). Indeed, in research where no significant association between OPRM1 and alcohol dependence was found, carriers of the G allele consumed alcohol significantly more frequently than those who were homozygous for the A allele (Kim et al., 2004b). Moreover, recent laboratory studies using an intermediate phenotype approach found that individuals with the G allele report higher levels of alcohol cue-elicited craving and higher subjective feelings of intoxication, stimulation, and positive mood across rising levels of breath alcohol concentration, as compared to individuals who were homozygous for the A allele (Ray and Hutchison, 2004, 2007; van den Wildenberg et al., 2007). Similarly, an association between the A118G SNP and self-reported responses to acute alcohol effects was found in a sample of American Indians (Ehlers et al., 2008). Inasmuch as intermediate phenotypes, such as sensitivity to alcohol, afford a more sensitive test of gene-disorder associations than complex psychiatric diagnoses (Gottesman and Gould, 2003), these studies suggest that car-

riers of the G allele exhibit greater sensitivity to the reinforcing effects of alcohol, which in turn may influence their susceptibility to problematic alcohol use.

Although a recent report indicated a nominal association between OPRM1 receptor gene variation and antisocial drug dependence among treatment-seeking adolescent Caucasian males (Corley et al., 2008), the association between OPRM1 and adolescent alcohol misuse remains untested. In light of the substantial neuronal remodeling that occurs during adolescence (Crews et al., 2007), which includes major maturational changes in mesocorticolimbic brain regions that govern the acute effects of alcohol, genetic influences on alcoholism may differ between adolescents and adults; large-scale quantitative genetic research provides support for this contention (Kendler et al., 2008; Pagan et al., 2006). Moreover, studies using animal models indicate that adolescent rodents respond differently to the acute effects of alcohol than adults, such that adolescents have lower sensitivity to the unpleasant effects of alcohol and greater sensitivity to certain reinforcing effects (Varlinskaya and Spear, 2000, 2006). This differential sensitivity to alcohol during adolescence may be particularly relevant when examining a genetic polymorphism thought to underlie sensitivity to the reinforcing effects of alcohol. Additionally, adolescents with alcohol-related problems have unique clinical characteristics. Compared to adults with alcohol dependence, adolescents drink less frequently, develop symptoms and meet criteria for dependence more quickly, and comprise a more homogeneous group in terms of age, duration of use, and comorbid psychopathology (Deas et al., 2000). Taken together, these findings illustrate the importance of testing the influence of candidate genes on alcohol misuse in adolescents.

In this study, we examined the association between the A118G SNP of the OPRM1 receptor gene and alcohol misuse in a sample of adolescents and tested whether heightened sensitivity to the reinforcing effects of alcohol mediated this relationship. First, adolescents who carried the G allele variant were predicted to have a higher likelihood of meeting diagnostic criteria for an alcohol use disorder (AUD), as defined by the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV-TR; American Psychiatric Association, 2000), than youth who were homozygous for the A allele. Second, in light of empirical evidence that AUD diagnostic thresholds may be inadequate for adolescents (Martin et al., 2006), we also examined the association between the A118G SNP and severity of alcohol-related problems using a well-established continuous measure to maximize our ability to capture problematic alcohol use in youth. Researchers have recognized that continuous measures may afford more sensitive tests of genetic effects on alcohol misuse than dichotomous diagnostic classification schemes, which have restricted variability (e.g., Dick et al., 2008; Hutchison et al., 2004; Irons et al., 2007). We use the term “alcohol misuse” to capture a broad spectrum of alcohol-related problems, which may or may not meet diagnostic threshold for an AUD. It was predicted that carriers of the G allele would experience more

severe alcohol-related problems than their homozygous counterparts. Third, carriers of the G allele were expected to endorse drinking motives that involve alcohol-induced reward more strongly than youth who were homozygous for the A allele and reward-focused drinking motives were predicted to mediate the association between the G allele and problematic alcohol use. Fourth, in an effort to test the discriminative validity of the mediational hypothesis, we conducted exploratory analyses to evaluate whether other drinking motives (i.e., social facilitation, coping-focused) similarly explained the association between OPRM1 and alcohol-related problems.

METHODS

Participants

Participants ($n = 187$, 12 to 19 years of age [$M = 15.4$, $SD = 1.8$]) were a subset of a larger sample of adolescents who took part in a study of biobehavioral mechanisms relating antisocial behavior and problematic substance use in youth. All youth who enrolled in the larger project were given the opportunity to participate in the genetic segment of the study and 90.1% agreed ($n = 190$). Separate informed written consent/assent was obtained for DNA collection. Consent was obtained from participants ≥ 18 years and from the parents/legal guardians of minors prior to participation; assent was obtained from minors. Of those who consented to the genetics study, 3 individuals were excluded from analyses due to an inability to successfully genotype their DNA sample. All procedures were approved by the Brown University Institutional Review Board.

In an effort to oversample for youth who engage in antisocial behavior and problematic substance use, adolescents were recruited for the larger project primarily from disadvantaged neighborhoods and a local truancy court program. To be eligible, adolescents had no history of traumatic brain injury or hearing difficulties. Participants were also required to test negative on a urine toxicology screen on the day of assessment for the following substances: alcohol, amphetamines, barbiturates, benzodiazepines, cocaine, and opiates. Although youth who endorsed suicidal ideation or psychotic symptoms were ineligible, other forms of psychopathology, including substance use disorders, were not exclusionary. Adolescents who enrolled in the genetic portion of the study did not differ from those who declined in terms of age, education, racial background and ethnicity, sex, or alcohol use disorders (p values > 0.05).

Recruitment and Procedures

Interested volunteers telephoned the laboratory to learn about the larger project and underwent a brief screening interview to determine initial eligibility. Those who did not endorse exclusionary criteria were invited to the laboratory to obtain written informed consent/assent and to complete an in-person screening interview. Eligible youth participated in a 1-day assessment session that involved administration of semi-structured clinical interviews to assess for psychopathology along with other self-report measures. Participants' parents/legal guardians were invited to take part in the study by completing semi-structured interviews and paper-and-pencil measures regarding the adolescent's developmental history and psychiatric functioning. Although informed written consent was required from parents/legal guardians of adolescents younger than age 18 years, parents/legal guardians were not required to participate in the study. This approach was chosen to allow recruitment of youth whose parents/legal guardians were unavailable or unwilling to participate in the project. Parent data, typically provided by the adolescent's mother (91.3% of cases), were obtained for 49.2% of adolescents. The addition of collateral reports from parents/legal guardians did not change the diagnostic status, in terms of either

alcohol abuse or dependence, for any participant in either the case or control group. Participants and parents/legal guardians who took part in the study were compensated for their participation.

Domains of Assessment and Measures

Psychopathology and Alcohol Use Disorder Diagnoses. The Kiddie Schedule for Affective Disorders for School-Age Children (KSADS; Kaufman et al., 1997) is a clinician-administered semi-structured interview that was used to assess for DSM-IV-TR psychopathology, including substance use disorders. When a parent/legal guardian elected to participate in the study, the parent/legal guardian and adolescent were interviewed separately.

Information collected from these interviews was integrated using an algorithm that identified the presence of a disorder if sufficient criteria were endorsed by either the parent or adolescent (Henin et al., 2007). In cases where a parent was unavailable to complete the assessment battery, diagnostic determinations were based on the adolescent's report. Interviewers received systematic training in diagnostic assessment with adolescents to achieve a high level of inter-rater reliability ($\kappa > 0.90$) at the item severity level prior to conducting interviews independently. Individual symptom items for each disorder were scored according to severity (0 = not present, 1 = subthreshold, 2 = clinical threshold). Potential drift in item severity ratings was minimized by reviewing all cases at weekly case consensus meetings that were supervised by 2 licensed clinical psychologists (RM, AJ). All symptom severity level and diagnostic decisions were made by case consensus with the interviewer(s) and both psychologists present.

Alcohol-Related Problems. The Rutgers Alcohol Problem Index (RAPI; White and Labouvie, 1989) is a 23-item continuous measure of alcohol-related problems that was developed and validated for use with adolescents. Adolescents reported how many times they experienced each item during the past 3 months on a 5-point scale (0 = never, 1 = 1 to 2 times, 2 = 3 to 5 times, 3 = 6 to 10 times, 4 = > 10 times). Items include general consequences (e.g., "was told by a neighbor or friend to stop or cut down on drinking") as well as consequences particularly applicable to adolescents (e.g., "not able to do your homework or study for a test"). A total score was computed by summing the items (Cronbach's $\alpha = 0.92$). The RAPI was administered to youth only if they reported alcohol use in the past 3 months.

Drinking Motives. Motivations for alcohol use were assessed using a 15-item measure validated for use with adolescents (Cooper, 1994; Cooper et al., 1992). Adolescents rated how often they drink alcohol for various reasons using a 4-point scale (almost never/never to almost always). Based on research showing that adults with the G allele of the OPRM1 gene exhibit greater sensitivity to the pleasant effects of alcohol (Ray and Hutchison, 2004, 2007), we were primarily interested in the enhancement motives subscale, which assesses drinking to enhance positive affect using 5 items: "Because you like the feeling," "Because it's exciting," "To get high," "Because it's fun," and "Because it makes you feel good." A total score was computed by adding the 5 items (Cronbach's $\alpha = 0.93$). The social and coping motivation subscales of this measure were also assessed (Cronbach's α values = 0.72 and 0.77, respectively) and used to test the discriminative validity of our mediational hypothesis. The social motives subscale items ($n = 5$) assess the extent to which the respondent drinks alcohol to facilitate social interactions. Items from the social motives subscale include: "Because it is what most of your friends do when you get together," "To be sociable," "As a way to celebrate," "Because it is customary on special occasions," and "Because it makes a social gathering more enjoyable." The coping motives subscale items ($n = 5$) measure how much the respondent uses alcohol to cope with negative affect and reduce tension. Items from the

coping motives subscale include: "To relax," "To forget your worries," "Because you feel more self-confident or sure of yourself," "Because it helps when you feel depressed or nervous," and "To cheer up when you're in a bad mood." Assessing drinking motives was a useful strategy for capturing adolescents' expectancies regarding the subjective effects of alcohol given that alcohol administration was not viable due to ethical concerns. Moreover, several studies have found that drinking motives are influenced by genetic factors (e.g., Agrawal et al., 2007; Prescott et al., 2004).

Genotyping. Genomic DNA was collected and isolated from buccal swabs using standard procedures (Freeman et al., 1997; Walker et al., 1999). The A118G SNP in the OPMR1 gene was assayed using a commercially available instrument and TaqMan assays (Applied Biosystems, Foster City, CA). To ensure accurate data, genotypes for all participants were determined by 2 independent laboratory technicians blinded to participants' characteristics. In addition, a randomly selected subset of samples (10%) was re-assayed to assess reliability ($\kappa = 1.0$).

Statistical Analysis

Variables were examined for missing data and continuous variables were examined for distribution normality. Deviations of the observed genotypic distributions from Hardy-Weinberg equilibrium were tested with a chi square statistic (χ^2 ; Guo and Thompson, 1992). To compare genotypic distribution between the AUD and non-AUD adolescents we used chi-square. Evaluation of chi-square effect size magnitudes was based on the risk difference index, which was computed by subtracting the percent of G carriers with an AUD from the percent of adolescents who were homozygous for the A allele with an AUD. This indicator closely approximates the phi coefficient for all 2×2 contingency tables but is less influenced by special circumstances, such as small cell sizes (Rosenthal, 2001). Because the G allele frequency varies considerably across distinct racial groups, the analysis was repeated with Caucasians only to control for possible population stratification biases. Next, we used a logistic regression equation to examine whether genotype increased the likelihood of an early-onset AUD diagnosis beyond the influence of age and sex. The dependent variable was the presence of an AUD diagnosis (0 = no AUD, 1 = AUD). Predictor variables were entered into the regression equation in 2 steps. In the first step, the effects of age and sex (0 = male, 1 = female) were entered and in the second step genotype was entered as a categorical variable (0 = AA, 1 = AG or GG).

The next set of analyses evaluated the association between the candidate gene and a continuous measure of alcohol-related problems. We then tested the hypothesis that carriers of the G allele would endorse drinking motives that involve alcohol-induced reward more strongly than youth homozygous for the A allele and that these reward-focused motives would mediate the association between the G allele and alcohol misuse. We chose to use a continuous measure of alcohol-related problems as the dependent measure in these analyses to obtain finer gradations of alcohol-related problems than the dichotomous categorization afforded by AUD diagnoses. Additional support for this approach comes from evidence that DSM-IV-TR diagnostic thresholds for AUD diagnoses are inadequate for adolescents (Martin et al., 2006). Only adolescents who reported a history of alcohol use in the past 3 months were included in these analyses. Baron and Kenny's criteria for mediation were followed (Baron and Kenny, 1986). OPRM1 genotype served as the independent variable, drinking to enhance positive affect (i.e., reward-focused drinking motives) served as the potential mediating variable, and alcohol-related problems, as measured by the RAPI, served as the dependent variable. Using hierarchical linear regression equations we tested for: (i) evidence of a significant association between OPRM1 genotype (0 = AA, 1 = AG or GG) and alcohol-related problems; (ii) evidence of a significant association between OPRM1 genotype and the

potential mediator (i.e., reward-focused drinking motives); (iii) evidence of a significant association between the potential mediator and alcohol-related problems; and (iv) evidence that when the influence of the potential mediator is incorporated, OPRM1 genotype is no longer significantly associated with alcohol-related problems. To test the discriminative validity of the mediational hypothesis, we conducted exploratory analyses to evaluate whether other drinking motives similarly explained the association between OPRM1 and alcohol-related problems. Significance was based on variance accounted for by a variable by itself (R^2) or its addition to the model (ΔR^2), with critical $p < 0.05$; the direction of relationships between variables was determined by examining variable coefficients. All analyses were conducted using SPSS 14.0 (SPSS Inc., Chicago, IL).

RESULTS

Demographic and Clinical Characteristics

Participants identified themselves as Caucasian (57.2%), African American (36.4%), American Indian (2.7%), Asian (1.6%), Native Hawaiian (0.5%), or other (1.6%), and 21% of the sample was Hispanic; race and Hispanic ethnicity were not considered mutually exclusive. Fourteen percent of the sample met criteria for a DSM-IV-TR AUD diagnosis (6% abuse, 8% dependence). Consistent with the convention used in previous research (e.g., McGahey et al., 2006; Oslin et al., 2003; Tidey et al., 2008), OPRM1 status was categorized based on the presence of the G allele, such that carriers of the G allele (AG or GG; $n = 40$) were compared to those who were homozygous for the A allele (AA; $n = 147$). A test of Hardy-Weinberg equilibrium revealed a significant discrepancy between the observed and expected frequencies of the genotypes in our overall sample ($\chi^2 = 7.25$, df = 1, $p = 0.01$). No differences were found between participants with the G allele and participants homozygous for the A allele in terms of sex, age, or grade in school (p values > 0.47). But consistent with previous research, OPRM1 groups differed significantly in their racial compositions, such that 80% of carriers of the G allele were Caucasian compared to 51% of those homozygous for the A allele ($\chi^2 = 17.11$, $p < 0.01$). To examine whether the Hardy-Weinberg disequilibrium observed in the overall sample was due to the high frequency of the G allele in adolescents with AUD diagnoses and to differences in allelic distributions between Caucasian and non-Caucasian youth, we conducted a separate Hardy-Weinberg linkage equilibrium analysis for Caucasian non-AUD controls. Allele frequencies observed for this subgroup were consistent with the expected Hardy-Weinberg distribution ($\chi^2 = 3.79$, df = 1, $p > 0.05$). Demographic and clinical characteristics of participants by AUD diagnosis and genotype are presented in Table 1.

Association Between OPRM1 Genotype and Adolescent Alcohol Misuse

We used chi square analysis to test whether having an AUD diagnosis was associated with carrying the G allele. As illustrated in Table 2, AUD groups differed significantly in

Table 1. Sample Characteristics by Alcohol Use Disorder (AUD) Diagnosis and OPRM1 Genotype

Variable	Non-AUD		AUD	
	AA (n = 134)	AG/GG (n = 26)	AA (n = 13)	AG/GG (n = 14)
Demographic variables				
Gender (% Male)	53.7	65.4	23.1	42.9
Age (M, SD)	15.2 (1.8)	14.6 (1.6)	16.7 (1.3)	17.4 (1.0)
Race (% Caucasian)	50.0	81.0	61.5	78.6
Years of education (M, SD)	9.4 (2.9)	9.3 (1.7)	10.5 (3.5)	9.9 (3.8)
Psychopathology (%)				
Cannabis use disorder	9.0	11.5	46.2	64.3
Attention-deficit/hyperactivity disorder	17.9	26.9	53.8	28.6
Conduct/oppositional defiant disorder	11.2	11.5	30.8	21.4
Mood disorder	1.5	3.9	23.1	0.0
Anxiety disorder	15.7	11.5	15.4	21.4
Parent interview completed (%)	53	69.2	0	21.4

terms of allelic distributions of the A118G SNP, such that 51.9% of youth with an AUD carried at least one copy of the G allele compared to 16.3% of non-AUD controls. In addition, because the frequency of the G allele varies considerably across distinct racial groups, we repeated the chi square analysis with Caucasians only to control for possible population stratification biases. The pattern of results was similar, such that 57.9% of Caucasian youth with an AUD carried at least one copy of the G allele compared to 23.9% of Caucasian non-AUD youth. Next, we used a logistic regression equation to examine the effect of genotype on AUD diagnostic status while taking into consideration the influence of age and sex. Predictor variables were entered in 2 steps. In the first step, the effects of age and sex (0 = male, 1 = female) were entered. In the second step, the effect of genotype (0 = AA, 1 = AG or GG) was entered. The Wald chi-square test statistic was used to evaluate the incremental contribution of each predictor (Cohen et al., 2003). Results from Step 1 indicated that age and sex were significant and unique predictors, such that older adolescents [Wald χ^2 (N = 187) = 20.00, $p < 0.001$] and females [Wald χ^2 (N = 187) = 5.72, $p < 0.05$] were more likely to meet diagnostic criteria for an AUD diagnosis (Nagelkerke R^2 = 0.31). Results from Step 2 indicated that genotype significantly increased the likelihood of an adolescent-onset AUD diagnosis beyond the influence of age and sex [Wald χ^2 (N = 187) = 15.71, $p < 0.001$, Nagelkerke R^2 = 0.45].

Due to the relatively small number of participants with an AUD diagnosis and because AUD diagnostic criteria may not adequately capture pathological alcohol use in adolescents, we tested the association between OPRM1 genotype and a continuous measure of alcohol-related problems during the past 3 months in the subgroup of adolescents who consumed alcohol during that timeframe ($n = 53$). Allele frequencies observed for this subgroup were in Hardy-Weinberg equilibrium ($\chi^2 = 2.76$, df = 1, $p > 0.05$). One-way analysis of variance (ANOVA) revealed a significant association between genotype and alcohol-related problems, such that carriers of the G allele ($n = 17$) had more alcohol-related problems [$M = 0.94$ ($SD = 0.51$)] than adolescents who were homozygous for the A allele variant [$n = 36$; $M = 0.62$ ($SD = 0.44$)], with a medium magnitude effect size [$F(1, 52) = 5.32$, $p < 0.05$, $f = 0.31$]. Cohen's f was used to determine effect size magnitudes, as it is appropriate for ANOVA (Cohen, 1988). The analysis was repeated with participants' racial background, (Caucasian vs. non-Caucasian) included as a dichotomous covariate to control for possible population stratification biases—a recommended approach (e.g., Liu et al., 2006; Sinha et al., 2006; Tang et al., 2005), although not without argument (e.g., Burnett et al., 2006). Results were similar when racial background was included in the analysis, with a similar magnitude effect size [$F(1, 52) = 4.40$, $p < 0.05$, $f = 0.29$]. Moreover, the relationship between genotype and alcohol-related problems remained significant

Table 2. Distributions of OPRM1 Genotype Variants

Sample	Group	Genotypes (n)			Chi-square ^a	p value	Risk difference
		GG	AG	AA			
Full sample (n = 187)	Case	3	11	13	17.41	0.000	0.26
	Control	4	22	134			
Caucasians only (n = 107)	Case	3	8	8	8.63	0.003	0.23
	Control	4	17	67			

^aChi-square tests compared genotypic distribution frequencies (AA vs. AG or GG) between cases and controls; Case = AUD diagnosis present; Control = AUD diagnosis not present; Risk difference refers to the percent of adolescents who were homozygous for the A allele who had an AUD diagnosis minus the percent of G allele carriers who had an AUD diagnosis.

when statistically controlling for age and sex (p values < 0.05 , f values = 0.32 and 0.35, respectively).

Alcohol Reinforcement as a Mediator of Genetic Influences

Next, we tested whether drinking to enhance positive affect mediated the association between OPRM1 genotype and alcohol-related problems during the past 3 months in the subgroup of adolescents who consumed alcohol during that time-frame ($n = 53$). As illustrated in Table 3, carrying at least one copy of the G allele predicted greater alcohol-related problems and stronger endorsement of drinking for mood enhancement, and drinking for mood enhancement predicted more alcohol-related problems. When drinking for mood enhancement was entered with OPRM1 genotype, however, the association between OPRM1 genotype and alcohol-related problems was no longer significant. These findings are consistent with the hypothesis that drinking to enhance positive affect mediates the association between OPRM1 genotype and alcohol-related problems. To provide a more direct test of the mediating role of drinking for enhancement a Sobel test was performed (Sobel, 1982) and results indicated that drinking for enhancement was a significant mediator of the association between OPRM1 and alcohol-related problems ($z = 2.64$, $p < 0.01$). This set of analyses was repeated with adolescents' racial background (Caucasian vs. non-Caucasian) included in the regression equation to control for possible population stratification biases. All results were consistent with the initial findings: the G allele predicted alcohol-related problems and reward-focused drinking motives ($R^2 = 0.17$, $p < 0.05$, $\beta = 0.42$; $R^2 = 0.14$, $p < 0.05$, $\beta = 0.41$, respectively); reward-focused drinking motives predicted alcohol-related problems ($R^2 = 0.18$, $p < 0.01$, $\beta = 0.45$); the G allele no longer predicted alcohol-related problems when reward-focused drinking motives were entered with genotype ($p > 0.10$, $\beta = 0.28$).

Table 3. Summary of Hierarchical Regression Analyses Testing Mood Enhancement Drinking Motives as a Mediator of the Association Between OPRM1 and Alcohol-Related Problems

Analysis	Total ($n = 53$)		
	ΔR^2	Final B	df
Eq. 1: Alcohol problems on OPRM1	0.09 [†]		1, 51
OPRM1		0.31 [†]	
Eq. 2: Enhancement motives on OPRM1	0.15 [†]		1, 51
OPRM1		0.38 [†]	
Eq. 3: Alcohol problems on enhancement motives	0.31 [‡]		1, 51
Enhancement motives		0.56 [‡]	
Eq. 4: Alcohol problems on OPRM1 and enhancement motives	0.32 [‡]		2, 50
OPRM1		0.11	
Enhancement motives		0.52 [‡]	

[†] $p < 0.01$; [‡] $p < 0.001$.

To explore the discriminative validity of drinking for enhancement as a mediator of the association between the OPRM1 gene and alcohol-related problems compared to other drinking motives, we examined whether social drinking motives similarly mediated this association. In contrast to the enhancement motives subscale, which reflects an internal motivation to drink to experience pleasurable effects, the social motives subscale reflects externally motivated drinking aimed toward facilitation of social activities and interactions (Cooper et al., 1992). Previous research with adolescents found these scales to be empirically distinct (e.g., Cooper, 1994), and in the present study the correlation between these scales was modest and not significant ($r = 0.25$, $p > 0.05$). As expected, results indicated that social drinking motives were associated with greater alcohol-related problems ($R^2 = 0.18$, $p < 0.01$, $\beta = 0.42$). But OPRM1 genotype was unrelated to social motives ($R^2 = 0.09$, $p = 0.53$, $\beta = 0.09$) and when social motives were entered with OPRM1 genotype, the association between OPRM1 genotype and alcohol-related problems remained significant ($R^2 = 0.25$, $p < 0.05$, $\beta = 0.27$). Although the coping motives subscale shared a robust positive correlation with drinking for enhancement ($r = 0.60$, $p < 0.05$)—a finding consistent with other studies of drinking motives in youth—we tested coping motives as a separate potential mediator in order to provide a full depiction of the data. Similar to our findings for drinking to enhance positive mood, drinking to reduce negative affect was associated with greater alcohol-related problems ($R^2 = 0.14$, $p < 0.01$, $\beta = 0.37$) and OPRM1 genotype ($R^2 = 0.09$, $p < 0.05$, $\beta = 0.30$). When coping motives were entered with OPRM1 genotype, the association between OPRM1 genotype and alcohol-related problems was not significant ($R^2 = 0.18$, $p > 0.10$, $\beta = 0.22$). Sobel test results, however, indicated that the mediational pathway between OPRM1 and alcohol-related problems via drinking to attenuate negative affect was not significant ($z = 1.7$, $p = 0.09$). Taken together, it appears that the association between OPRM1 and alcohol-related problems is mediated, at least in part, by drinking motives that involve manipulating mood states (i.e., enhancing positive affect or attenuating negative affect). And this effect was stronger for positive mood enhancement than for negative mood reduction, as the indirect effect of coping motives on alcohol misuse was weak.

DISCUSSION

In this study, the association between a polymorphism of the OPRM1 receptor gene and adolescent alcohol misuse was tested. Our findings provide the first evidence that the A118G SNP of the OPRM1 gene is associated with the development of AUD diagnoses during adolescence as well as with a greater number of alcohol-related problems among adolescent drinkers. Specifically, adolescents who met DSM-IV-TR criteria for an AUD diagnosis had a higher prevalence of the G allele (51.9%) than non-AUD youth (16.3%), and this

disproportionate distribution remained significant when analyses were restricted to Caucasian youth (57.9% vs. 23.9%, respectively). In addition, the G allele accounted for 9% of the variance in alcohol-related problems experienced by youth in the past 3 months, with a medium magnitude effect size ($f = 0.31$). These findings coincide with an adult study in terms of the nature and magnitude of the association between OPRM1 and alcoholism (Bart et al., 2005). Adult carriers of the G allele had a higher prevalence of alcohol dependence (23.1%) than nonalcoholic controls (13.5%); the risk attributable to genotype was 11.1%. Nonetheless, it is important to recognize that a number of adult studies did not find this association (e.g., Bergen et al., 1997; Franke et al., 2001; Kim et al., 2004a; Loh et al., 2004; Lou et al., 2003; Nishizawa et al., 2006; Rommelspacher et al., 2001).

Although inconsistent findings could accurately indicate that variation in the OPRM1 receptor gene does not confer liability for alcoholism, limitations of existing work justify the need for additional research before making this determination—particularly as it pertains to adolescents (van der Zwaluw et al., 2007). Although it may be likely that similar genetic mechanisms confer liability for alcohol misuse in adults and adolescents, adolescents undergo substantial neuronal remodeling that appears to alter their sensitivity to alcohol and possibly impact if and how genes influence adolescent alcohol misuse. While human data on alcohol sensitivity in adolescents are scarce due to ethical issues around administering alcohol to youth, animal models of adolescence indicate that youth are less sensitive than adults to most of the negative effects of alcohol (e.g., alcohol-induced motor impairment, social impairment, dysphoria, sedation, hangover effects; e.g., Varlinskaya and Spear, 2000). In addition, adolescent animals appear more sensitive to some of the reinforcing effects of alcohol, especially alcohol-induced social facilitation (e.g., Varlinskaya and Spear, 2006). These findings coincide with one early study of human adolescents that found 8- to 15-year-old boys did not show behavioral signs of intoxication when given a large dose of alcohol (Behar et al., 1983). Given these data, we cannot assume that the genetic influences on alcoholism observed in adults will generalize to adolescents. As such, this study is an important first step toward filling this significant gap in the literature.

Findings from this study provide initial evidence that altered sensitivity to the reinforcing effects of alcohol may be a mechanism by which OPRM1 confers risk for alcohol-related problems among adolescents. We found that adolescent drinkers who carried at least one copy of the G allele endorsed drinking to enhance positive affect more strongly than adolescents who were homozygous for the A allele and that drinking to enhance positive affect mediated the association between OPRM1 genotype and alcohol-related problems. Exploratory analyses supported the discriminative validity of this effect by showing that drinking motivated by social facilitation, rather than by mood alteration, was not related to OPRM1 and did not influence the relationship between genotype and alcohol-related problems. These results

extend findings from alcohol-administration research, which indicate that young adult carriers of the G allele exhibit greater dose-dependent responsiveness to the reinforcing effects of alcohol (i.e., higher subjective feelings of intoxication, stimulation, and positive mood across rising levels of breath alcohol concentration) than those homozygous for the A allele (Ray and Hutchison, 2004, 2007; van den Wildenberg et al., 2007).

If replicated, the present findings would improve our understanding of the biobehavioral mechanisms underlying alcohol misuse in adolescents. Individual differences in sensitivity to the acute effects of alcohol are heritable in both animals and humans (McBride and Li, 1998; Schuckit and Smith, 1996). And this differential sensitivity is central to most contemporary conceptual models of alcoholism (e.g., Corbin et al., 2006; Newlin and Thomson, 1990; Ray et al., 2009; Schuckit et al., 2007; Schuckit et al., 2008). Empirical support for this notion comes from prospective research showing that initial responsiveness to the acute effects of alcohol early in life is a robust predictor of subsequent heavy drinking and alcohol-related problems in adulthood (Schuckit et al., 2007). For example, adolescents who report low sensitivity to alcohol's effects, typically characterized by blunted responsiveness to the sedating and unpleasant effects during the descending limb of the blood alcohol curve, experience greater liability for developing drinking problems (Schuckit, 1994). Likewise, heightened sensitivity to the stimulatory and reinforcing effects of alcohol during the ascending limb of the blood alcohol curve also appears to confer risk for alcoholism (Corbin et al., 2008; King et al., 1997; Papineau et al., 1998). This confluence of insensitivity to alcohol's unpleasant effects and heightened sensitivity to its pleasurable effects may promote heavy drinking during adolescence by making alcohol more reinforcing in the absence of normal signals to moderate intake (Newlin and Thomson, 1990). Indeed, the clinical characteristics of adolescent problem drinkers coincide with this notion; although teens drink less often than adults, they develop symptoms of alcohol dependence more quickly (Deas et al., 2000). Our findings provide additional data to support an association between individual differences in drinking motives centered on the reinforcing aspects of alcohol ingestion (e.g., enhancing positive affect) and alcohol-related problems, and provide initial evidence that this liability is, in part, genetically driven in youth.

This study may also have important implications for developing more effective treatment strategies for adolescents, particularly in terms of pharmacological interventions. Each year more than 1 million youth in the U.S. develop a sufficient spectrum of alcohol-related problems to warrant a diagnosis of alcohol dependence and alcohol addiction remains a major impetus for substance abuse treatment admissions among teenagers [Substance Abuse and Mental Health Services Administration (SAMHSA), Office of Applied Studies, 2007, 2008]. Yet despite the clinical demand for effective alcohol interventions for youth, less than one-third experience

sustained benefit from existing psychosocial treatments (e.g., Brown et al., 2001). While researchers have advanced pharmacotherapy for adults, medication development research with adolescents remains at a nascent stage. Studies of healthy adults and a variety of clinical populations consistently find that carriers of the G allele exhibit altered physiological and subjective responses to pharmacological agents that affect opioid receptor activity (e.g., Somogyi et al., 2007). In terms of alcohol research, laboratory studies show that opioid receptor antagonists (e.g., naltrexone hydrochloride) blunt the reinforcing effects of alcohol ingestion and this effect is more pronounced in individuals who carry at least one copy of the G allele (e.g., Ray and Hutchison, 2007). Moreover, clinical trials indicate that G allele carriers benefit more from naltrexone treatment than those without the allele (e.g., Anton et al., 2008; Oroszi et al., 2009; Oslin et al., 2003), although this finding is not consistent across studies (Gelernter et al., 2007). Although double-blind, placebo-controlled clinical trials with adolescents are almost nonexistent, 2 case reports, one open-label study, and one small double-blind randomized trial studied naltrexone in adolescents. Despite methodological limitations, these reports suggest that opioid receptor antagonists hold promise for reducing alcohol use in youth. Identifying genetic influences on adolescent alcohol misuse may help advance medication development research with youth, in part, by shedding light on the types of youth most likely to benefit from particular pharmacological treatments.

Despite the potential importance of these findings, the limitations of this study should be considered. A test of Hardy–Weinberg equilibrium revealed a significant discrepancy between the observed and expected frequencies of the genotypes in our overall sample. There are several possible reasons for this disequilibrium, such as inaccurate genotyping, population admixture (i.e., frequencies of the marker allele and target disorder differ by racial group), and assortative mating (i.e., individuals select mates in a nonrandom manner based on shared characteristics). Erroneous genotyping was an unlikely explanation in this study. Genotypes for all participants were determined by 2 independent technicians blinded to participants' characteristics. In addition, a randomly selected subset of samples (10%) was re-assayed with a high level of reliability ($\kappa = 1.0$). To mitigate potential effects of admixture, we reanalyzed the data with Caucasian youth only and results were consistent with our overall findings. In terms of assortative mating, there is significant spousal concordance for AUDs (Grant et al., 2007). Insofar as this study recruited a selective sample of youth, nonrandom mating may have resulted in offspring with relatively elevated frequencies of alleles that confer risk for alcoholism. Notably, allele frequencies observed among Caucasian non-AUD controls were consistent with the expected Hardy–Weinberg distribution, suggesting that the disequilibrium observed in the overall sample was due to the high frequency of the G allele in adolescents with early-onset AUD diagnoses and to differences in allelic distributions across racial groups. Nonetheless, while one or more of these factors may explain the disequilibrium

of allele frequencies observed in our overall sample, the specific sources of this discrepancy cannot be determined. Examination of these factors in a larger, unselected sample of youth is warranted.

We must also acknowledge that, given the young age of participants in this study, some youth classified as controls may develop future drinking problems. Therefore, the most we can say from our cross-sectional data, which were collected from youth during a specific window of development, is that this SNP appears to be associated with alcohol misuse among teenagers. This limitation precludes the ability to infer the degree to which the findings generalize to older youth and young adults. Additional research is necessary to answer this question.

Another consideration was the use of drinking motives as a proxy for a direct assessment of the subjective responses to alcohol. Studies of acute alcohol effects in adults typically rely on human laboratory paradigms that involve alcohol administration. Important advantages of such paradigms include capturing the subjective effects of alcohol in close temporal proximity to drinking and the careful assessment of subjective effects across specific blood alcohol concentration levels. But because alcohol cannot be administered to adolescents due to important legal and ethical concerns, drinking motives were assessed to capture the degree to which youth use alcohol for its social and mood altering properties. An important limitation of this approach is the reliance on retrospective reports, which may be imprecise due to inaccurate recall or social desirability biases. Finally, the emergence of alcohol problems during adolescence is commonly associated with certain psychiatric conditions, such as disruptive behavior and mood disorders. It is therefore possible that the observed association between OPRM1 and alcohol misuse resulted from shared liability with psychiatric or other risk factors for alcoholism. Although the sample size in this study restricted our ability to explore these interactions, this is an important target for future research.

Despite these limitations, this study represents an important first step toward elucidating genetic influences on adolescent alcohol misuse. Moreover, this study has a number of strengths that should be considered in balance with its limitations. These strengths include empirical attention on an understudied population, careful characterization of the sample using well-validated and developmentally appropriate measures, the inclusion of both male and female adolescents, and examination of empirically derived mechanisms underlying genetic influences. An important goal for future research is to replicate these findings in a larger sample of youth and to identify protective environmental factors that reduce genetic risk for alcoholism.

ACKNOWLEDGMENTS

This research was supported by a grant and Career Development Award from the National Institute on Drug Abuse (R21 DA016904, K01 DA017671), a Career Development

Award and training grant from the National Institute on Alcohol Abuse and Alcoholism (K23 AA014966, T32 AA07459), and by a Research Career Development Award and a Senior Research Career Scientist Award from the Department of Veterans Affairs. The authors thank Alexander Blanchard, Candace Shuman, Jamie Gainor, Chinatsu McGeary, Joshua Orabone, Kathryn Plummer, and Lance Swenson for their assistance completing this research and Linda Spear for comments on earlier drafts of this manuscript.

REFERENCES

- Agrawal A, Dick DM, Bucholz KK, Madden PAF, Cooper ML, Sher KJ, Heath AC (2007) Drinking expectancies and motives: a genetic study of young adult women. *Addiction* 103:194–204.
- American Psychiatric Association (2000) Diagnostic and Statistical Manual of Mental Disorders, 4th edn. Text Revision. American Psychiatric Association, Washington, DC.
- Anton RF, Oroszi G, O'Malley S, Couper D, Swift R, Pettinati H, Goldman D (2008) An evaluation of mu-opioid receptor (OPRM1) as a predictor of naltrexone response in the treatment of alcohol dependence: results from the Combined Pharmacotherapies and Behavioral Interventions for Alcohol Dependence (COMBINE) study. *Arch Gen Psychiatry* 65:135–144.
- Arias A, Feinn F, Kranzler HR (2006) Association of an Asn40Asp (A118G) polymorphism in the μ -opioid receptor gene with substance dependence: a meta-analysis. *Drug Alcohol Depend* 83:262–268.
- Baron RM, Kenny DA (1986) The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. *J Pers Soc Psychol* 51:1173–1182.
- Barr CS, Schwandt M, Lindell SG, Chen SA, Goldman D, Suomi SJ, Higley D, Heilig M (2007) Association of functional polymorphism in the μ -opioid receptor gene with alcohol response and consumption in male rhesus macaques. *Arch Gen Psychiatry* 64:369–376.
- Bart G, Kreek MJ, Ott J, LaForge KS, Proudnikov D, Pollak L, Heilig M (2005) Increased attributable risk related to a functional μ -opioid receptor gene polymorphism in association with alcohol dependence in central Sweden. *Neuropsychopharmacology* 30:417–422.
- Befort K, Filliol D, Decaillot FM, Gaveriaux-Ruff C, Hoehe M, Kieffer BL (2001) A single nucleotide polymorphic mutation in the human μ -opioid receptor severely impairs receptor signaling. *J Biol Chem* 176:3130–3137.
- Behar D, Berg CJ, Rapoport JL, Nelson W, Linnoila M, Cohen M, Bozovich C, Marshall T (1983) Behavioral and physiological effects of ethanol in high-risk and control children: a pilot study. *Alcohol Clin Exp Res* 7:404–410.
- Bergen AW, Kokoszka J, Peterson R, Long JC, Virkkunen M, Linnoila M, Goldman D (1997) μ opioid receptor gene variants: lack of association with alcohol dependence. *Mol Psychiatry* 2:490–494.
- Beyer A, Koch T, Schröder H, Schulz S, Höllt V (2004) Effect of the A118G polymorphism on binding affinity and agonist-mediated endocytosis, desensitization, and resensitization of the human μ -opioid receptor. *J Neurochem* 89:553–560.
- Bond C, LaForge KS, Tian M, Melia D, Zhang S, Borg L, Gong J, Schluger J, Strong JA, Leal SM, Tischfield JA, Kreek M, Yu L (1998) Single-nucleotide polymorphism in the human mu opioid receptor gene alters beta-endorphin binding and activity: possible implications for opiate addiction. *Proc Natl Acad Sci USA* 95:9608–9613.
- Brown SA, D'Amico EJ, McCarthy DM, Tapert SF (2001) Four-year outcomes from adolescent alcohol and drug treatment. *J Stud Alcohol* 62:381–388.
- Burnett MS, Strain KJ, Lesnick TG, de Andrade M, Rocca WA, Maraganore DM (2006) Reliability of self-reported ancestry among siblings: implications for genetic association studies. *Am J Epidemiol* 163:486–492.
- Cohen J (1988) Statistical Power Analysis for the Behavioral Sciences, 2nd edn. Erlbaum, Hillsdale, NJ.
- Cohen J, Cohen P, West S, Aiken L (2003) Applied Multiple Regression/Correlation Analysis for the Behavioral Sciences, 3rd edn. Erlbaum, Mahwah, NJ.
- Cooper ML (1994) Motivations for alcohol use among adolescents: development and validation of a four-factor model. *Psychol Assess* 6:117–128.
- Cooper ML, Russell M, Skinner JB, Windle M (1992) Development and validation of a three-dimensional measure of drinking motives. *Psychol Assess: J Consult Clin Psychol* 4:123–132.
- Corbin WR, Fromme K, Bergeson SE (2006) Preliminary data on the association among the serotonin transporter polymorphisms, subjective alcohol experiences, and drinking behavior. *J Stud Alcohol* 67:5–13.
- Corbin WR, Gearhardt A, Fromme K (2008) Stimulant alcohol effects prime within session drinking behavior. *Psychopharmacology* 197:327–339.
- Corley RP, Zeiger JS, Crowley T, Ehringer MA, Hewitt JK, Hopfer CJ, Lessem J, McQueen MB, Rhee SH, Smolen A, Stallings MC, Young SE, Krauter K (2008) Association of candidate genes with antisocial drug dependence in adolescents. *Drug Alcohol Depend* 96:90–98.
- Crews F, He J, Hodge C (2007) Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav* 86:189–199.
- Crowley JJ, Oslin DW, Patkar AA, Gottheil E, DeMaria PA Jr, O'Brien CP, Berettini WH, Grice DE (2003) A genetic association study of the mu opioid receptor and severe opioid dependence. *Psychiatr Genet* 13:169–173.
- Dackis C, O'Brien O (2005) Neurobiology of addiction: treatment and public policy ramifications. *Nat Neurosci* 8:1431–1436.
- Deas D, Riggs P, Langenbucher J, Goldman M, Brown S (2000) Adolescents are not adults: developmental considerations in alcohol users. *Alcohol Clin Exp Res* 24:232–237.
- Dick DM, Aliev F, Wang JC, Grucza RA, Schuckit M, Kuperman S, Kramer J, Hinrichs A, Bertelsen S, Budde JP, Hesselbrock V, Porjesz B, Edenberg HJ, Bierut LJ, Goate A (2008) Using dimensional models of externalizing psychopathology to aid in gene identification. *Arch Gen Psychiatry* 65:310–318.
- Ehlers CL, Lind PA, Wilhelmsen KC (2008) Association between single nucleotide polymorphisms in the mu opioid receptor gene (OPRM1) and self-reported responses to alcohol in American Indians. *BMC Med Genet* 9:35.
- Franke P, Wang T, Nethen MM, Knapp M, Neidt H, Albrecht S et al. (2001) Nonreplication of association between mu-opioid-receptor gene (OPRM1) A118G polymorphism and substance dependence. *Am J Med Genet* 105:114–119.
- Freeman B, Powell J, Ball D, Hill L, Craig I, Plomin R (1997) DNA by mail: an inexpensive and noninvasive method for collecting DNA samples from widely dispersed populations. *Behav Genet* 27:251–257.
- Gelernter J, Gueorguieva R, Kranzler HR, Zhang H, Cramer J, Rosenheck R, Krystal JH (2007) Opioid receptor gene (OPRM1, OPRK1, and OPRD1) variants and response to naltrexone treatment for alcohol dependence: results from the VA Cooperative Study. *Alcohol Clin Exp Res* 31:555–563.
- Gelernter J, Kranzler H, Cubellis J (1999) Genetics of two mu opioid receptor gene (OPRM1) exon 1 polymorphisms: population studies, and allele frequencies in alcohol- and drug-dependent subjects. *Mol Psychiatry* 4:476–483.
- Ghozland S, Chu K, Kieffer BL, Roberts AJ (2005) Lack of stimulant and anxiolytic-like effects of ethanol and accelerated development of ethanol dependence in mu-opioid receptor knockout mice. *Neuropharmacology* 49:493–501.
- Gottesman II, Gould TD (2003) The endophenotype concept in psychiatry: etymology and strategic intentions. *Am J Psychiatry* 160:636–645.
- Grant JD, Heath AC, Bucholz KK, Madden PAF, Agrawal A, Statham DJ, Martin NG (2007) Spousal concordance for alcohol dependence: evidence for assortative mating or spousal interaction effects? *Alcohol Clin Exp Res* 31:717–728.
- Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. *Biometrics* 48:361–372.
- Heinz A, Reimold M, Wräse J, Hermann D, Croissant B, Mundt G, Dohmen BM, Braus DF, Schumann G, Machulla HJ, Bares R, Mann K (2005) Correlation of stable elevations in striatal μ -opioid receptor availability in

- detoxified alcohol patients with alcohol craving: a positron emission tomography study using carbon¹¹-labeled carfentanil. *Arch Gen Psychiatry* 62:57–64.
- Henin A, Mick E, Biederman J, Fried R, Wosniak J, Farone SV, Harrington K, Davis S, Doyle AE (2007) Can bipolar disorder-specific neuropsychological impairments in children be identified? *J Consult Clin Psychol* 75:210–220.
- Hernandez-Avila CA, Wand G, Luo X, Gelernter J, Kranzler HR (2003) Association between the cortisol response to opioid blockade and the Asn40Asp polymorphism at the mu-opioid receptor locus (OPRM1). *Am J Med Genet* 118B:60–65.
- Hutchison KE, Stallings M, McGeary J, Bryan A (2004) Population stratification in the candidate gene study: fatal threat or red herring? *Psychol Bull* 130:66–79.
- Irons DE, McGue M, Iacono WG, Oetting WS (2007) Mendelian randomization: a novel test of the gateway hypothesis and models of gene-environment interplay. *Dev Psychopathol* 19:1181–1195.
- Kaufman J, Birmaher B, Brent D, Rao U, Flynn C, Moreci P, Williamson D, Ryan N (1997) Schedule for affective disorders and schizophrenia for school-age children—present version and lifetime version (K-SADS-PL): initial reliability and validity data. *J Am Acad Child Adolesc Psychiatry* 36:980–988.
- Kendler KS, Schmitt E, Aggen SH, Prescott CA (2008) Genetic and environmental influences on alcohol, caffeine, cannabis, and nicotine use from early adolescence to middle adulthood. *Arch Gen Psychiatry* 65:674–682.
- Kim SG, Kim CM, Kang DH, Kim YJ, Byun WT, Kim SY, Park JM, Kim MJ, Oslin DW (2004b) Association of functional opioid receptor genotypes with alcohol dependence in Koreans. *Alcohol Clin Exp Res* 28:986–990.
- Kim SA, Kim JW, Song JY, Park S, Lee HJ, Chung JH (2004a) Association of polymorphisms in nicotinic acetylcholine receptor α_4 subunit gene (CHRNA4), μ -opioid receptor gene (OPRM1), and ethanol-metabolizing enzyme genes with alcoholism in Korean patients. *Alcohol* 34:115–120.
- King AC, Volpicelli JR, Frazer A, O'Brien CP (1997) Effect of naltrexone on subjective alcohol response in subjects at high and low risk for future alcohol dependence. *Psychopharmacology* 129:15–22.
- Kohnke MD (2008) Approach to the genetics of alcoholism: a review based on pathophysiology. *Biochem Pharmacol* 75:160–177.
- Kreek MJ (1996) Opioid receptors: some perspectives from early studies of their role in normal physiology, stress responsivity, and in specific addictive diseases. *Neurochem Res* 21:1469–1488.
- Liu I-C, Blacker DL, Xu R, Fitzmaurice G, Tsuang MT, Lyons MJ (2004) Genetic and environmental contributions to age of onset of alcohol dependence symptoms in male twins. *Addiction* 99:1403–1409.
- Liu XQ, Paterson AD, John EM, Knight JA (2006) The role of self-defined race/ethnicity in population structure control. *Ann Hum Genet* 70:496–505.
- Loh EW, Fann CSJ, Chang YT, Chang CT, Cheng ATA (2004) Endogenous opioid receptor genes and alcohol dependence among Taiwanese Han. *Alcohol Clin Exp Res* 28:15–19.
- Lou X, Kranzler HR, Zhao H, Gelernter J (2003) Haplotypes at the OPRM1 locus are associated with susceptibility to substance dependence in European-Americans. *Am J Med Genet* 120B:97–108.
- Martin CS, Chung T, Kirisci L, Langenbucher JW (2006) Item response theory analysis of diagnostic criteria for alcohol and cannabis use disorders in adolescents: implications for DSM-V. *J Abnorm Psychol* 115:807–814.
- McBride WJ, Li TK (1998) Animal models of alcoholism: neurobiology of high alcohol-drinking behavior in rodents. *Crit Rev Neurobiol* 12:339–369.
- McGeary JE, Monti PM, Rohsenow DJ, Tidey J, Swift R, Miranda R Jr (2006) Genetic moderators of naltrexone's effects on alcohol cue reactivity. *Alcohol Clin Exp Res* 30:1288–1296.
- Newlin DB, Thomson JB (1990) Alcohol challenge with sons of alcoholics: a critical review and analysis. *Psychol Bull* 108:383–402.
- Nishizawa D, Han W, Hasegawa J, Ishida T, Numata Y, Sato T, Kawai A, Ikeda K (2006) Association of μ -opioid receptor gene polymorphism A118G with alcohol dependence in a Japanese population. *Neuropsychobiology* 53:137–141.
- Oroszi G, Anton RF, O'Malley S, Swift R, Pettinati H, Couper D, Yuan Q, Goldman D (2009) OPRM1 Asn40Asp predicts response to naltrexone treatment: a haplotype-based approach. *Alcohol Clin Exp Res* 33:383–393.
- Oslin DW, Berrettini W, Kranzler HR, Pettinati H, Gelernter J, Volpicelli JR, O'Brien CP (2003) A functional polymorphism of the mu-opioid receptor gene is associated with naltrexone response in alcohol dependent patients. *Neuropsychopharmacology* 28:1546–1552.
- Pagan JL, Rose RJ, Viken RJ, Pulkkinen L, Kaprio J, Dick D (2006) Genetic and environmental influences on stages of alcohol use across adolescence and into young adulthood. *Behav Genet* 36:483–497.
- Papineau KL, Roehrs TA, Petrucci N, Rosenthal LD, Roth T (1998) Electro-physiological assessment (The Multiple Sleep Latency Test) of the biphasic effects of ethanol in humans. *Alcohol Clin Exp Res* 22:231–235.
- Prescott CA, Cross RJ, Kuhn JW, Horn JL, Kendler KS (2004) Is risk for alcoholism mediated by individual differences in drinking motives? *Alcohol Clin Exp Res* 28:29–39.
- Ray LA, Hutchison KE (2004) A polymorphism of the mu opioid receptor gene and sensitivity to the effects of alcohol in humans. *Alcohol Clin Exp Res* 28:1789–1795.
- Ray LA, Hutchison KE (2007) Effects of naltrexone on alcohol sensitivity and genetic moderators of medication response. *Arch Gen Psychiatry* 64:1069–1077.
- Ray LA, MacKillop J, Monti PM (2009) Subjective responses to alcohol as endophenotypes: advancing genetics into etiological and treatment models of alcoholism. *Subst Use Misuse*. In press.
- Rommelspacher H, Smolka M, Schmidt LG, Samachowiec J, Hoehe MR (2001) Genetic analysis of the μ -opioid receptor in alcohol-dependent individuals. *Alcohol* 24:129–135.
- Rosenthal R (2001) Effect sizes in behavioral and biomedical research, in *Validity and Social Experimentation* (Bickman I ed.), pp. 121–139. Sage, Thousand Oaks, CA.
- Schuckit MA (1994) Low level of response to alcohol as a predictor of future alcoholism. *Am J Psychiatry* 151:184–189.
- Schuckit MA, Smith TL (1996) An 8-year follow-up of 450 sons of alcoholic and control subjects. *Arch Gen Psychiatry* 53:202–210.
- Schuckit MA, Smith TL, Danko GP, Pierson J, Hesselbrock V, Bucholz KK, Kramer J, Kuperman S, Dietiker C, Brandon R, Chan G (2007) The ability of the self-rating on the effects of alcohol (SRE) Scale to predict alcohol-related outcomes five years later. *J Stud Alcohol* 68:371–378.
- Schuckit MA, Smith TL, Trim RS, Heron J, Horwood J, Davis J, Hibbeln J, the ALSPAC Study Team (2008) The self-rating of the effects of alcohol questionnaire as a predictor of alcohol-related outcomes in 12-year-old subjects. *Alcohol Alcohol* 43:641–646.
- Sinha M, Larkin EK, Elston RC, Redline S (2006) Self-reported race and genetic admixture. *N Engl J Med* 354:421–422.
- Sobel ME (1982) Asymptomatic confidence intervals for indirect effects of structural equation models, in *Sociological Methodology* (Leinhard S ed.), pp. 290–312. Jossey-Bass, San Francisco.
- Somogyi AA, Barratt DT, Collier JK (2007) Pharmacogenetics of opioids. *Clin Pharmacol Ther* 81:429–444.
- Substance Abuse and Mental Health Services Administration (SAMHSA), Office of Applied Studies (2008) The NSDUH Report: trends in Substance Use, Dependence, or Abuse, and Treatment among Adolescents: 2002 to 2007. SAMHSA, Rockville, MD.
- Substance Abuse and Mental Health Services Administration (SAMHSA), Office of Applied Studies (2007) The DASIS Report: Adolescent Treatment Admissions by Gender: 2005. SAMHSA, Rockville, MD.
- Tang H, Quertermous T, Rodriguez B, Kardia SL, Zhu X, Brown A, Pankow JS, Province MA, Hunt SC, Boerwinkle E, Schork NJ, Risch NJ (2005) Genetic structure, self-identified race/ethnicity, and confounding in case-control association studies. *Am J Hum Genet* 76:268–275.
- Tidey JW, Monti PM, Rohsenow DJ, Gwaltney CJ, Miranda R Jr, McGeary JE, MacKillop J, Swift RM, Abrams DB, Shiffman S, Paty JA (2008) Moderators of naltrexone's effects on drinking, urge, and alcohol effects in non-treatment-seeking heavy drinkers in the natural environment. *Alcohol Clin Exp Res* 32:58–66.
- Town T, Abdullah L, Crawford F, Schinka J, Ordonica PI, Francis E, Hughes P, Duara R, Mullan M (1999) Association of a functional opioid receptor allele (+118A) with alcohol dependence. *Am J Med Genet B* 88:458–461.

- Varlinskaya EI, Spear LP (2000) Acute effects of ethanol on social behavior of adolescent and adult rats: role of familiarity of the test situation. *Alcohol Clin Exp Res* 26:1502–1511.
- Varlinskaya EI, Spear LP (2006) Differences in the social consequences of ethanol emerge during the course of adolescence in rats: social facilitation, social inhibition, and anxiolysis. *Dev Psychobiol* 48:146–161.
- Walker AH, Najarian D, White DL, Jaffe JF, Kanetsky PA (1999) Collection of genomic DNA by buccal swabs for polymerase chain reaction-based biomarker assays. *Environ Health Perspect* 107:517–520.
- Weiss F, Porrino LJ (2002) Behavioral neurobiology of alcohol addiction: recent advances and challenges. *J Neurosci* 22:3332–3337.
- White HR, Labouvie EW (1989) Towards the assessment of adolescent problem drinking. *J Stud Alcohol* 50:30–37.
- van den Wildenberg E, Wiers RW, Dessers J, Janssen RG, Lambrichs EH, Smeets HJ, van Breukelen GJ (2007) A functional polymorphism of the mu-opioid receptor gene (OPRM1) influences cue-induced craving for alcohol in male heavy drinkers. *Alcohol Clin Exp Res* 31:1–10.
- Zhang H, Lou X, Kranzler HR, Lappalainen J, Yang BZ, Krupitsky E, Zvaartau E, Gelernter J (2006) Association between two μ -opioid receptor gene (OPRM1) haplotype blocks and drug or alcohol dependence. *Hum Mol Genet* 15:807–810.
- Zhang Y, Wang D, Johnson AD, Papp AC, Sadée S (2005) Allelic expression imbalance of human mu opioid receptor (OPRM1) caused by variant A118G. *J Biol Chem* 280:32618–32624.
- van der Zwaluw CS, van den Wildenberg E, Wiers RW, Franke B, Buitelaar J, Scholte RHJ, Engles R (2007) Polymorphisms in the μ -opioid receptor gene (OPRM1) and the implications for alcohol dependence in humans. *Pharmacogenomics* 8:1427–1436.