Association between single nucleotide polymorphisms in the Mu Opioid receptor gene (OPRM1) and self-reported responses to alcohol in Southwest California Indians

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Abstract

Background: Variation in response to the hedonic and adverse effects of a substance is in part an inherited factor that may influence its use, abuse and dependence. The mu opioid receptor is the primary site of action for opiates and individuals with polymorphisms in this receptor appear to have variation in the CNS effects of opiates. Several studies have suggested that this receptor may also mediate some of the effects of non-opioid drugs, such as alcohol. The purpose of this study was to investigate associations between 14 single nucleotide polymorphisms in the mu opioid receptor gene (OPRM1) with self-reported responses to alcohol, an endophenotype associated with the development of alcohol dependence, in Southwest California Indians.

Methods: Each participant gave a blood sample and completed a structured diagnostic interview. Additionally, response to alcohol was indexed using the expectation version of the subjective high assessment scale (SHAS-E). SNPs were genotyped in 251 participants and data analyses were conducted using SOLAR.

Results: The estimated heritability ($h^2$) for the SHAS-E phenotypes ranged from 0.01 to 0.28. Endorsing the expectation of a more intense response on one or more of the following items from the SHAS-E: buzzed, clumsy, dizzy, drunk, effects, high, nausea, sleepy, talkative, terrible, and/or uncomfortable after imbibing 2-3 drinks was significantly associated with having at least one minor allele for at least one of 7 SNPs ($p<0.01$) in the OPRM1 receptor gene. The most commonly genotyped OPRM1 polymorphism in the literature, Asn40Asp (A118G) G allele was associated with reporting a less intense response to alcohol for the items: dizzy ($p<0.02$) and sleepy ($p<0.02$).
**Conclusion:** These studies provide data to suggest that the minor allele for most of the polymorphisms in the *OPRM1* receptor gene investigated were found to be associated with a more intense, and/or more adverse, response to alcohol, traits that are significantly correlated with lowered quantity of alcohol consumption and less susceptibility to dependence in this SWC Indian population. These data further suggest that making conclusions on the role of the mu opiod receptor gene in the development of alcohol dependence may be limited if only one polymorphism in the gene is evaluated in isolation.
Background

A number of studies have documented that the dosage requirements for targeted effects of CNS drugs can vary widely [1]. For example in a study of over 3,000 patients experiencing pain following postoperative hip replacement the therapeutic morphine dosage requirements varied almost 40-fold [2]. Wide inter-patient variability in response to, and therefore in the dosage requirement for morphine have been demonstrated in cancer patients receiving morphine for pain control [3].

It appears that a number of genetic and environmental factors can lead to significant variation in the doses of a drug necessary to produce therapeutic, hedonic and/or adverse effects. However, there is increasing evidence that gene polymorphisms may be an important factor in determining a person’s sensitivity and tolerance to a drug. The mu opioid receptor (OPRM1) is the primary site of action for opiates; about 20 variants in the mu opioid receptor gene (OPRM1) have been identified with amino acid substitutions that have polymorphic frequencies over 1% [4-11]. The most common single nucleotide polymorphism (SNP) reported on is A118G (rs1799971) which encoded the Asp40Asn codon change with most data suggesting that it is a functional variant [4, 12].

There have been a series of studies in both healthy volunteers and in clinical patients suggesting that, the A118G variant may alter response to opioid drugs (see [1] for review). Lotsch and colleagues reported the 118G allele conferred smaller analgesic effects and produced less pupillary constriction during morphine and morphine-6-glucuronide (MG6) infusion [13-15]. In an experiment using a measure of pain tolerance to electrical stimulation higher MG6 concentrations were associated with a 25% increase in current (C25) in participants with the 118G allele [16, 17]. Similar findings have been found for alfentanil [18] and levomethdone [19]. In clinical studies, data from patients with the 118G
polymorphism tend to confirm data from experimental pain studies where those patients with the variant required higher alfentanil doses for analgesia or more morphine during colorectal surgery [20] or for pain/toxicity associated with morphine use in renal failure [13, 14]. However, it appears that the effects may be drug or disease specific owing to presumed variation in environmental and/or other uncontrolled variables [1, 21, 22].

Several studies have suggested that the mu receptor may also mediate some of the hedonic and/or addictive effects of non-opioid drugs, such as alcohol [23, 24]. Indirect support for this hypothesis is provided by studies demonstrating the efficacy of naltrexone for the treatment of alcohol dependence [25-31]. Further support is provided by studies evaluating associations between response to naltrexone pharmacotherapy for alcohol dependence and the presence of the A118G variant. In a study that combined data from three different clinical trials, Oslin and colleagues [32] demonstrated that carriers of the 118G allele had a significantly lower rate of relapse and a longer time to a return to heavy drinking when compared to those individuals who were homozygous for the 118A allele. This finding was not supported in the Veterans Affairs (VA) Cooperative Study where no significant interactions were found between naltrexone treatment response and any polymorphic variants at each of the three opioid receptor genes [33]. The A118G polymorphism has also been associated with an individual’s response to a naloxone challenge with subjects with the 118G allele showing higher plasma cortisol concentrations [34, 35].

There has been a plethora of studies that have investigated the relationship between a diagnosis of drug and/or alcohol dependence and the A118G polymorphism. The results have been conflicting and inconsistent. In a recent meta-analysis of 28 different studies including over 8000 subjects, the
conclusion was that the \textit{OPRM1} A118G variant did not appear to affect risk for substance dependence. However, the authors further speculated that additional research would be needed to determine whether another polymorphism in the gene might influence receptor function and thus risk for substance dependence [36]. An additional feature of these studies that may have weakened the results is the use of a dichotomous phenotype, drug dependence, a diagnosis that is made based on both heritable and non-heritable factors [37]. Town and colleagues [38] suggested that genetic studies on the influence of mu opioid receptors polymorphisms be viewed within the broader context of alcoholism where the opioid receptor genes are taken to be partial, rather than complete, risk factors for the disorder. Thus, it may be that polymorphisms in \textit{OPRM1} encode for a variant that influences a more narrowly defined risk factor for alcoholism. This risk factor is envisioned to partially influence the development of the disorder but may or may not ultimately be associated with the diagnosis depending on the age of the participant, presence of other risk factors and environmental variables.

Individual sensitivity to alcohol represents such an inherited factor that affects the likelihood of drinking and mediates the disposition for developing alcoholism [39], and has a strong genetic basis [40]. In general, people at higher genetic risk for alcoholism are less sensitive to the effects of alcohol and people at lower genetic risk for alcoholism are more sensitive. Support for this theory is provided by many, but not all, studies examining the reaction to alcohol among children of alcoholics, who are at greatly elevated risk for developing alcoholism [41]. Results have indicated that at moderate doses of alcohol, subjects who are family history positive for alcoholism and subjects who are family history negative for alcoholism attain equivalent blood alcohol concentrations, but most studies have found that subjects with a positive family history rate themselves as significantly less intoxicated than control subjects with a negative family history [42-45]. Although not all studies agree [46], a meta-analysis
focusing on subjective level of intoxication confirmed a diminished response to alcohol as a characteristic more frequently seen in subjects with a positive family history than in those with a negative family history [47]. In addition, an 8-year follow-up of previously studied men with positive and negative family histories found that both a family history of alcoholism and a low response to alcohol were related to the development of alcohol-related problems [48].

Studies using similar methodologies among groups at lower risk for alcoholism have provided additional support for the idea that individual sensitivity to alcohol might also mediate protection from developing alcoholism. Individuals of Asian heritage, who have mutations in the aldehyde dehydrogenase gene (ALDH2) [49-52], and individuals of Jewish decent [53], two groups with low rates of alcoholism, were found to have more intense, although not necessarily more negative, responses to alcohol than matched control subjects of average alcoholism risk.

Genetic studies of complex phenotypes, such as sensitivity to alcohol, often have advantages when they are conducted in well-defined populations such as Native American tribes living on reservations [54]. A once popular notion, called the firewater myth, proposed that Native American Indians are constitutionally predisposed to an altered response to drinking alcohol [55]. In one empirical study, Native American Mission Indians, like Caucasian sons of alcoholics, were found to have less intense objective and subjective effects of alcohol in an alcohol challenge paradigm. Additionally, participants with at least 50% Native American heritage reported less intense effects of alcohol than did those with less than 50% Native American heritage, despite equivalent blood alcohol concentrations [56-58].
The present report is part of a larger study exploring risk factors for substance dependence among Native American Indians residing in southwest California [56-67]. The lifetime prevalence of substance dependence in this Indian population is high and evidence for heritability and linkage to specific chromosome locations has been demonstrated [64, 68-71]. The purpose of the present set of analyses was to determine if a significant association could be detected between 14 SNPs in the *OPRM1* receptor gene and self-report of subjective response to alcohol in this population.

**Methods**

Participants, known collectively as Southwest California (SWC) Indians (also known as “Mission Indians”), who were of mixed heritage but at least one-sixteenth Native American, were recruited from eight geographically contiguous reservations with a total population of about 3,000 individuals. They were recruited using a combination of a venue-based method [72, 73], and a respondent-driven procedure [74], as described previously [63, 75]. To be included in the study a participant had to be a SWC Indian between the age of 18 and 70 without major medical problems that would preclude mobility.

Potential participants gave written informed consent using a protocol approved for the study by The Institutional Review Board (IRB) of The Scripps Research Institute, the Scientific Advisory Committee of the GCRC, and the Indian Health Council, a tribal review group overseeing health issues for the reservations where recruitment was undertaken. They also responded to a screening questionnaire that was used to gather information on demographics, personal medical history, ethnicity and detailed measures of substance abuse history [76] and weight & height. Each participant also completed an interview with the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) [77] which
was used to make diagnoses [78]. Response to alcohol was assessed using the subjective high assessment scale-expectations version (SHAS-E). This scale consists of 14 items rated on Likert scales ranging from 0 (normal) to 36 (extreme effect). The participants indicated how intoxicated they felt after drinking 2-3 drinks for the following items: buzzed, clumsy, dizzy, drunk, effects of alcohol, energy, good, high, nausea, sleepy, talkative, uncomfortable, terrible overall and great overall. A total score was all calculated for the first 12 SHAS-E items.

Two hundred (251) individuals have both genotype and phenotype data for this analyses. DNA was isolated from whole blood using an automated DNA extraction procedure. Since genotype data was not available from the International HapMap Project public database ([http://www.hapmap.org](http://www.hapmap.org)[79] at the time this study was conceived (October 2004), seventeen single nucleotide polymorphisms (SNPs) in or near the \( OPRM1 \) locus were selected from the Applied Biosystems SNP database ([http://www.appliedbiosystems.com](http://www.appliedbiosystems.com)[80]). SNPs were initially chosen to be evenly distributed across \( OPRM1 \) with an average intermarker spacing of 5,133bp. Assays for three SNPs (rs561720, hCV32237184, rs3798687) failed and were excluded from analyses. The locations of the fourteen remaining SNPs typed in the study are shown in Figure 1 and SNP information, including the observed minor allele frequency (MAF), is described in Table 2.

All primers, probes and reagents were purchased from ABI (Applied Biosystems, Foster City, CA). SNPs were genotyped using TaqMan™ fluorescence 5' exonuclease technology. Each 5 µL reaction contained 25 ng genomic DNA, 1.6X TaqMan assay primer/probe mix, 1X PCR Buffer A, 2.5 mM MgCl\(_2\), 250 µM dNTPs, and 0.5 U AmpliTaq Gold polymerase. Thermocycling was performed as recommended by ABI. Genotypes were determined on an ABI 7900HT Fast Real-Time PCR System.
using the allelic discrimination mode. Hardy-Weinberg equilibrium analyses were completed in Haploview (version 4.0) [81].

The total additive genetic variance (heritability, \( h^2 \)) and its standard error were estimated for the SHAS-E phenotypes using SOLAR (http://www.sfbr.org/solar/) [82]. A genetic association analyses was conducted where the number of copies of the minor allele of each individual was used as a covariate in a variance component analysis as implemented in SOLAR v2.0.4 [83], and the statistical significance of the ability of the covariate to explain phenotypic variance was determined. In these exploratory analyses nominal significance was set at the \( p<0.05 \) level.

**Results**

The demographic characteristics of the sample are virtually equivalent to the U.S. census data for these tribes and have been presented previously [64, 84]. The mean age of the sample was 30.2 (± 0.7) yrs, there were 110 males and 141 females with a mean of 11.5(0.1) yrs of education, 60% of the sample was over 50% Native American heritage as estimated by their federal Indian blood quantum and 55% reported income of less than $20,000 per annum.

In total, 14 \( OPRM1 \) SNPs spanning a region of 81.9 Kb were genotyped in 251 individuals selected from 124 families comprising of 1 to 4 generations with an average number of 7 members per family (range, 1-30). Marker information including genetic map position, location within \( OPRM1 \), and minor allele frequencies within our SWC Indian population (as well as four reference populations for comparison) are listed in Table 2. Mendelian inconsistencies were identified using PEDSTATS [85] and made up 0.03% of the data. One SNP, rs12333298 in intron 1, showed significant (\( p=0.018 \)) deviations
from Hardy-Weinberg equilibrium (HWE) at a p<0.05 level. The physical locations of and pattern of linkage disequilibrium (LD) between the 14 SNPs typed across the **OPRM1** gene are schematically presented in Figure 1.

The estimated heritability ($h^2$) for the SHAS-E phenotypes ranged from near to zero (energy) to .28 for “terrible” (see Table 1). The only three phenotypes with significant heritability were sleepy talkative and terrible. As seen in Table 3, endorsing a more intense response on one or more of the following items: buzzed, clumsy, dizzy, drunk, effects, high, nausea, sleepy, talkative, terrible, and/or uncomfortable after imbibing 2-3 drinks, as indexed by the SHAS-E, was significantly associated with having at least one minor allele for 8 SNPs (p<0.01) in or near the OPRM1 receptor gene. Whereas, the 118G allele of the most commonly genotyped Asn40Asp polymorphism, was nominally associated with reporting a less intense response to alcohol for the items: dizzy (p<0.02) and sleepy (p<0.02).

**Discussion**

The CNS effects of alcohol range from mild euphoria (high), to impaired coordination, to ataxia, decreased mentation, labile mood, to poor judgment, slurred speech, nausea and vomiting, and finally to respiratory failure, coma and death, depending on the dose imbibed [86]. The final level of impairment appears to depend on a number of factors including a persons’ gender, age, weight, prior experience with alcohol and level of tolerance [39]. Another source of variation in response to alcohol is individual variation in alcohol metabolism. Some individuals, particularly East Asians who are homozygous for the **ALDH2*2** allele, are intolerant of alcohol and report intense facial flushing, tachycardia, hypotension, headache, nausea and vomiting following drinking more than one drink [66]. African Americans with at least one **ADH1B*3** also report expecting to have a more intense response to a standard dose of alcohol.
when compared to African Americans who are homozygous for the \textit{ADH1B*1} allele \cite{87}. Other sources of the genetic variation in sensitivity and tolerance to alcohol not attributed to differences in alcohol metabolism are less well understood. However, a genome-wide segregation analysis evaluating subjective response to alcohol challenge found nine chromosome regions with LOD scores between 2.2 and 3.2 suggesting other potential regions of interest in the genome that may contribute to the variance in alcohol responsivity \cite{40}.

In the present study, evidence was obtained for an association between expectations of the effects of a standard dose of alcohol and polymorphisms in the \textit{OPRM1} receptor gene. Participants with at least one 118G allele for the Asp40Asn polymorphism reported that they expected to feel a less intense response to alcohol for the items: dizzy (p<0.02) and sleepy (p<0.02) when compared to individuals without any 118G alleles. These data are consistent with data from Kim and colleagues \cite{88}, who found that alcoholics with two copies of the 118G allele spent more days drinking than those who were heterozygous or homozygous for the 118A allele, perhaps suggesting a less intense response to alcohol. Assuming that alcohol may act as a partial agonist at the mu opioid receptor, the findings in the present study of a reduced effect of alcohol in participants with the 118G allele, are also consistent with studies that evaluated response to opioid agonists where a reduced response to drug challenge (pupillary diameter, pain, respiratory depression) and/or increased dosage requirements are seen in those individuals with the 118G allele (see [1] for review).

Few studies have evaluated whether an association exists between response to alcohol and polymorphisms in the \textit{OPRM1} gene. In one study, the ability of naltrexone to blunt an alcohol-induced high was found to be greater in those participants with the 118G allele \cite{89}. The finding of a more
intense response to a mu opioid receptor antagonist found by Ray and Hutchinson [89] is consistent with previous studies that have demonstrated that subjects with the 118G allele that were given naloxone had higher cortisol concentrations [35]. It is also consistent with the finding that naltrexone may be more efficacious for the treatment of alcoholism in those with at least one 118G allele [32]. However, Ray and Hutchinson [89, 90] have also reported that young participants in an IV alcohol challenge, with one 118G allele, reported feeling more subjective feelings of “high” across rising breath alcohol concentrations, as compared to those participants homozygous for the 118A allele. These findings are not consistent with the findings in the present study for the 118G allele, nor are they particularly consistent with studies that have found a less intense response to opioid agonists. However, the findings of Ray and Hutchinson [89, 90] are consistent with the findings in the present study of an association between expecting to experience a more intense response to alcohol and carrying at least one minor allele for eight other SNPS in the opioid receptor gene. Since, in the study of Ray and Hutchinson [89, 90], only one SNP was genotyped and the ethnic characteristics of the sample were not specified, it is possible that the findings reflected stratification of the sample or that the A118G variant was in linkage disequilibrium with several other alleles that may encode for a more intense response to alcohol. These data further suggest that making conclusions on the role of the mu opioid receptor gene in the development of alcohol-related behaviors may be limited if only one polymorphism in the gene is evaluated in isolation.

Several alcohol- or drug-related association studies [91-94] have expanded their investigations to include up to 20 SNPs in or near **OPRM1**, although all include the A118G variant. Ide and colleagues [91] genotyped 20 SNPs including 10 SNPs in the 3’UTR region among Japanese subjects meeting ICD-10 criteria for methamphetamine (MAP) dependence/psychosis and controls. Four SNPs (including the
A118G and rs2075572 variants that were genotyped in the present study) representing the major haplotypes observed in the study sample were tested for association with four features of MAP dependence/psychosis. While A118G and two other SNPs were not associated with MAP dependence/psychosis, the rs2075572 G-allele was significantly associated with increased risk for a diagnosis of MAP dependence/psychosis (p=0.011), as well as four aspects/symptoms of the disorder (p<0.01). Interestingly, within our SWC Indian population, the rs2075572 G-allele was related to expecting to feel a more intense response to alcohol in four of the 14 items of the SHAS-E, an indication that carriers of this allele may be protected from developing alcohol dependence. Zhang and colleagues [93] investigated the relationship between heroin-induced subjective responses in a Chinese population and ten SNPs selected throughout OPRM1. They found three SNPs in intron 1 were associated with an increased risk of positive responses on first use of heroin and were likely contributing to further heroin consumption. However, A118G and rs2075572 were not associated with any differences in heroin-induced subjective responses. In another study, Luo and colleagues [92] typed eight variants in alcohol, cocaine and opioid and poly-substance dependent European Americans (EA) and African Americans (AA). They found that the A-allele of the -2044C/A polymorphism was a susceptibility allele for the combination of alcohol and opioid dependence in the EA sample, but not the AA sample. Once again, A118G was not associated with any of the substance dependent phenotypes. Finally, Zhang and colleagues [94] studied the role of OPRM1 genetic variation in a large case-control sample of alcohol dependent and/or drug (cocaine and/or opioid) dependent European Americans. Thirteen SNPs, five of which were typed in the present study, were genotyped representing the major haplotypes observed in HapMap and this study were found. Seven SNPs (but not A118) were associated with alcohol, cocaine, opioid plus opioid/cocaine dependency. Zhang and colleagues [94] found that the frequencies of the rs524731 A-allele and rs648893 T-allele were significantly higher among the dependent EA subjects.
Within our SWC Indian population, the rs524731 A-allele and rs648893 T-allele were generally associated with a more intense response to alcohol.

**Conclusion**

In conclusion, these data represent the first association analysis of a level of response to alcohol phenotype with multiple SNPs in the *OPMR1* receptor gene in Native Americans. The results corroborated the possible importance of the relationship between the A118G polymorphism that is thought to have functional significance, and substance abuse related phenotypes. SNPs highlighted in prior studies of substance dependence phenotypes were also identified as well as new SNPs of potential importance to substance dependence research. The results of this study should, however, be interpreted in the context of several limitations. First, the findings may not generalize to other Native Americans or represent all SWC Indians. Second, comparisons of association findings to non-Indian populations may be limited by differences in a host of potential genetic and environmental variables. Finally, because this population has significant admixture estimates of allele frequencies may produce biased results. Despite these limitations, this report represents an important step in an ongoing investigation to understand the genetic determinants associated with the development of substance use disorders in this high risk and understudied ethnic group.
List of Abbreviations

CNS, Central Nervous System; DNA, Deoxyribonucleic Acid; \textit{ADH1B}*3, Alcohol Dehydrogenase 1B*3; \textit{ADH1B}*1, Alcohol Dehydrogenase 1B*1

Competing interests

The author(s) declare that they have no competing interests.

Authors' contributions

Cindy L Ehlers contributed to the recruitment, collection and analysis of the clinical and genetic data on the subjects. Kirk C. Wilhelmsen contributed to the genetic and heritability analyses. Penelope A Lind did the genotyping and its analysis. All authors contributed to writing the paper.

Acknowledgements

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References


80. Applied Biosystems SNP Database. [http://www.appliedbiosystems.com](http://www.appliedbiosystems.com)


**Figure Legend**

**Figure 1. A schematic representation of OPRM1 Gene Structure, Linkage Disequilibrium and Genotyped SNPs.**

The gene structure of *OPRM1* is shown with exons numbered from 1 to 4 and relative exon size denoted by the width of the vertical bars. Fourteen SNPs analyzed in this study are shown in relation to their location across *OPRM1*. 
**Table 1.** Estimated heritability ($h^2$) for the Subjective High Assessment Scale-Expectations (SHAS-E) phenotypes. Significant values (p<0.05) are highlighted in bold.

<table>
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<th>Item</th>
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<th>heritability</th>
<th>Std. Err.</th>
<th>p</th>
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<td>0.10</td>
<td>0.18</td>
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<td>2</td>
<td>CLUMSY</td>
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<td>0.09</td>
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<td>3</td>
<td>DIZZY</td>
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<td>0.10</td>
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<td>4</td>
<td>DRUNK</td>
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<td>0.10</td>
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<td>5</td>
<td>EFFECTS</td>
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<td>0.11</td>
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Table 2. OPRM1 marker information, including genetic map position, location within OPRM1 and minor allele frequency.

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<th>Marker Name</th>
<th>Gene Chromosomal Location</th>
<th>Chromosomal Location Location&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Functional Location (bp)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Alleles</th>
<th>MAF&lt;sup&gt;f&lt;/sup&gt;</th>
<th>References&lt;sup&gt;g&lt;/sup&gt;</th>
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<td>154,402,490</td>
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<td>Intron 3</td>
<td>+57,468</td>
<td>154,459,840</td>
<td>T C</td>
<td>0.18</td>
<td>0.333</td>
</tr>
<tr>
<td>rs648007 hCV1691794</td>
<td>Intron 3</td>
<td>+61,932</td>
<td>154,464,304</td>
<td>T C</td>
<td>0.18</td>
<td>0.333</td>
</tr>
<tr>
<td>rs681243 hCV3073596</td>
<td>Intron 3</td>
<td>+67,062</td>
<td>154,469,434</td>
<td>A G</td>
<td>0.18</td>
<td>0.239</td>
</tr>
<tr>
<td>rs648893 hCV3073587</td>
<td>Intron 3</td>
<td>+77,949</td>
<td>154,480,321</td>
<td>C T</td>
<td>0.11</td>
<td>0.203</td>
</tr>
<tr>
<td>rs642489 hCV3073582</td>
<td>3' UTR</td>
<td>+81,996</td>
<td>154,484,368</td>
<td>A C</td>
<td>0.11</td>
<td>0.208</td>
</tr>
</tbody>
</table>

<sup>a</sup>bp = base-pair position on Genome Build 127.

<sup>b</sup>Position relative to transcription start site at 154,402,372 on Chromosome 6 (NCBI Build 35 version 1, June 2004).

<sup>c</sup>Percentage of subjects genotyped for each SNP.

<sup>d</sup>pHWE = p-value for the Hardy-Weinberg Equilibrium test run in Haploview (Barrett et al 2005)[81]

<sup>e</sup>The allele with the lowest frequency.

<sup>f</sup>MAF = Minor Allele Frequency for Southwest California Indians (SWC), and the reference populations CEPH (European), China, Japan, Yoruba (African). nd = no data.

Table 3. Association of OPRM1 SNPs with response to alcohol, as measured by the Subjective High Assessment Scale-Expectations (SHAS-E) questionnaire. Significant values (p<0.05) are highlighted in bold with values <0.01 also italicized.

<table>
<thead>
<tr>
<th>Marker</th>
<th>Minor Allele</th>
<th>Increaser Allele</th>
<th>Subjective High Assessment Scale-Expectations Item</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>G</td>
<td>A</td>
<td>BUZZED    CLUMSY    DIZZY    DRUNK      EFFECTS     ENERGY    GOOD    GREAT    HIGH    NAUSEA    SLEEPY    TALK    TERRIBLE    UNCOM    TOTAL</td>
</tr>
<tr>
<td>rs1799971</td>
<td>G</td>
<td>A</td>
<td>0.26      0.84      0.02      0.23      0.67      0.64      0.98      0.49      0.20      0.32      0.02      0.67      0.71      0.07      0.24</td>
</tr>
<tr>
<td>rs553202</td>
<td>A</td>
<td>A</td>
<td>0.03      0.10      0.01      0.001     0.10      0.48      0.32      0.64      0.001     0.01      0.15      0.21      0.008     0.008     0.02</td>
</tr>
<tr>
<td>rs524731</td>
<td>A</td>
<td>A</td>
<td>0.03      0.10      0.01      0.001     0.09      0.56      0.26      0.94      0.002     0.06      0.13      0.16      0.01      0.07      0.02</td>
</tr>
<tr>
<td>rs3778148</td>
<td>T</td>
<td>T</td>
<td>0.02      0.02      0.01      0.01      0.02      0.92      0.39      0.52      0.000     0.003     0.09      0.001     0.11      0.000     0.003</td>
</tr>
<tr>
<td>rs12333298</td>
<td>C</td>
<td>C</td>
<td>0.45      0.18      0.23      0.30      0.25      0.42      0.97      0.59      0.25      0.22      0.46      0.16      0.19      0.09      0.2</td>
</tr>
<tr>
<td>rs1461773</td>
<td>T</td>
<td>T</td>
<td>0.02      0.03      0.01      0.03      0.03      0.94      0.67      0.60      0.003     0.01      0.07      0.005     0.08      0.009     0.01</td>
</tr>
<tr>
<td>rs506247</td>
<td>G</td>
<td>G</td>
<td>0.40      0.95      0.51      0.06      0.91      0.58      0.63      0.55      0.35      0.44      0.47      0.48      0.07      0.83      0.64</td>
</tr>
<tr>
<td>rs563649</td>
<td>A</td>
<td>A</td>
<td>0.73      0.77      0.56      0.12      0.70      0.91      0.73      0.39      0.84      0.62      0.58      0.38      0.06      0.57      0.91</td>
</tr>
<tr>
<td>rs2075572</td>
<td>G</td>
<td>G</td>
<td>0.12      0.04      0.01      0.003     0.31      0.69      0.76      0.21      0.002     0.05      0.74      0.07      0.17      0.06</td>
</tr>
<tr>
<td>rs548646</td>
<td>T</td>
<td>T</td>
<td>0.01      0.01      0.001     0.02      0.02      0.12      0.77      0.91      0.06      0.02      0.04      0.03      0.33      0.02      0.005</td>
</tr>
<tr>
<td>rs648007</td>
<td>T</td>
<td>T</td>
<td>0.09      0.02      0.01      0.08      0.10      0.21      0.85      0.95      0.12      0.01      0.23      0.09      0.82      0.05      0.03</td>
</tr>
<tr>
<td>rs681243</td>
<td>A</td>
<td>A</td>
<td>0.02      0.01      0.001     0.02      0.03      0.15      0.90      0.91      0.05      0.006     0.06      0.06      0.37      0.02      0.007</td>
</tr>
<tr>
<td>rs648893</td>
<td>C</td>
<td>C</td>
<td>0.51      0.34      0.37      0.62      0.45      0.09      0.85      0.82      0.78      0.82      0.60      0.28      0.42      0.50      0.41</td>
</tr>
<tr>
<td>rs642489</td>
<td>A</td>
<td>A</td>
<td>0.43      0.25      0.31      0.55      0.40      0.09      0.83      0.80      0.79      0.71      0.45      0.28      0.50      0.55      0.36</td>
</tr>
</tbody>
</table>

The increaser allele for the TOTAL phenotype (i.e., the allele associated with a higher TOTAL score) TOTAL = sum of scores reported for the first 12 SHAS-E items.
Figure 1