Reviewer's report

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

**Version:** 2  **Date:** 8 January 2008

**Reviewer:** Jonathan Covault

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This study sought to examine whether significant associations existed between genetic variation in an important candidate gene for the effects of addictive substances, OPRM1, and self-reported subjective effects of alcohol in a sample of 251 Southwest California Mission Indians. The strengths of the study include examination of a defined population of subjects with a higher risk of developing alcohol use disorders and the use of multiple markers spread across the OPRM1 gene. The authors report that the minor allele for several markers was associated with greater self-reported alcohol effects for several items.

The report could be improved by revisions to address the following major concerns:

1) No reference is given for the SHAS-E scale. What is the correlation between SHAS-E response items? Do responses in this population cluster into effects groups? What is the average response and range for each item for the sample? How do responses compare with those using this instrument in other samples? Abbreviating the term alcohol effects to effects may be necessary for formatting the table 3 but should be avoided in the text and table 1 (also in table 1 do not abbreviate talkative and uncomfortable).

2) What variables were included together with genotype in the statistical analysis? Age? Gender? Drinking involvement?

3) The sample description in the methods or results should include more information about the alcohol involvement of the study sample. This should include the proportion of subjects with and alcohol use disorder, current alcohol use, years of use, average drinks per use, frequency of drinking etc.

4) The basis for the first sentence on page 15: “Within our SWC Indian population, the rs524731 A-allele and rs648893 T-allele were generally associated with a more intense response to alcohol” is obscure. Table 3 lists an enhanced response for only 2 of the 14 SHAS-E items as being associated with the rs524731 A-allele. None of the 14 items was associated to genotype for the rs648893 snp.

5) There was no comment on the potential Type I error introduced by multiple testing - 14 items plus total score x 14 SNPs. While the SNPs likely represent
fewer than 14 genetic elements, even if only the number of phenotype measures were considered a Bonferroni corrected p-value of 0.003 would be indicated. With this in mind, perhaps the comment that the Asn40Asp minor allele was associated with less intense response for items dizzy and sleepy p<0.02 in the abstract places excess emphasis on strength of this observation (note the statistic for these items is shown as p=0.02 in table 3). Similarly, the second sentence of the conclusion regarding the study â##corroborated the possible importance of the relationship of the A118G polymorphism â#ï and substance abuse related phenotypesâ## is not well supported by this dataset.

6) The authors should examine and report on the haplotype structure for this sample using the 14 markers examined. The result would not only inform the current study results, but also allow comparison of haplotype structure for this sample of American Indians with that published previously using overlapping markers in European American samples. With respect to OPRM1 haplotype structure, line 19 p 14 does not make sense.

More minor issues which should be addressed:

1) In the second paragraph of the results it is stated that subjects were from 124 families with an average of 7 members per familyâ#ï. This description would suggest a sample size of 124 x 7 =868 rather than the stated 251.

2) Paragraph 3 of results states â##a more intense response â#ïâ#ï was significantly associated with having at least one minor allele for 8 SNPs (p<0.01).â## Table 3 only lists 7 SNPs with p<0.01 which is also the number of SNPs noted in the abstract.

3) The March 2006 genome assembly (www.genome.ucsc.edu) places the SNP rs2075572 in intron 2 not intron 1 as shown in the figure and table 2.

4) Table 2 is described in the text prior to Table 1. Recommend renumber these Tables.

5) Paragraph 3 of results â## use consistent style to note the SHAS-E item (ie near to zero (energy) to .28 for â##terribleâ##).

6) Table 2. The terms chromosomal and functional should be switched for column 4 and 5 headings. A column with the data indicated in the table footnote c (percentage of subjects genotyped for each SNP) should be added to the table.

What next?: Unable to decide on acceptance or rejection until the authors have responded to the major compulsory revisions

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a
statistician.

Declaration of competing interests:

I declare that I have no competing interests