Author's response to reviews

Title: Protective variant associated with alcohol dependence in a Mexican American cohort

Authors:

Trina M Norden-Krichmar (tnorden@scripps.edu)
Ian R Gizer (gizeri@missouri.edu)
Kirk C Wilhelmsen (kirk_wilhelmsen@med.unc.edu)
Nicholas J Schork (nschork@jcvr.org)
Cindy L Ehlers (cindye@scripps.edu)

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Author's response to reviews: see over
Dear Dr. Jin and Mr. Cenzon,

We are pleased that the reviewers agree that our manuscript presents an interesting and important study of the association of alcohol dependence in a Mexican American cohort. We also appreciate the reviewers’ suggestions and have added to and revised the manuscript accordingly. Our detailed responses to the comments are included below. We hope that you find this improved manuscript now suitable for publication in BMC Medical Genetics. Please feel free to contact me if you have any questions.

Sincerely,

Trina M. Norden-Krichmar

Reviewer #1: Victor Jin

Reviewer's report:

In this paper, the authors evaluated a cohort of 427 Mexican American men (n=171) and women (n=256). Information on alcohol dependence was obtained through interview using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA). They found a protective variant (rs991316) located downstream from the ADH7 gene showed suggestive significance in association with alcohol dependence symptom counts as well as to clustered alcohol dependence symptoms. Additional linkage analysis suggested that nearby variants in linkage disequilibrium with rs991316 were not responsible for the observed association with the alcohol dependence phenotypes in this study. The work is well designed. The manuscript is well written. I am wondering if the health conditions of people in the cohort would affect the results. Is this rs991316 found to be associated with any risk of other diseases?

Our response: As mentioned on page 12, line 287, rs991316 was deposited into the dbSNP database based on a study of hypertension in African Americans (Adeyemo et al., 2009, PLoS Genetics). In our laboratory, we focus mainly on alcohol dependence, substance dependence, and other psychiatric disorders. Therefore, we do not have phenotype information for many other health conditions, nor do we have blood pressure readings in order to determine if there is a link to hypertension. At this reviewers request we did investigate a possible association with Body Mass Index, but did not find rs991316 to be significantly associated. However, since this is a young cohort (age 18 – 30 years old), we might not expect to find a high incidence of hypertension or other chronic health conditions.
Reviewer #2: Junbai Wang

Reviewer’s report:

Manuscript by Norden-Krichmar et al investigated possible association between alcohol abuse and genetic variants in Mexican American population. They studied 427 people (171 men and 256 women), collected blood samples and Affy Exome array were used in this work. Authors found one variant (rs991316) is strongly linked with alcohol dependence symptom in Mexican American population, though the same marker was already known to play a role against alcoholism in other ethnic groups. Overall, authors presented a coherent study. However, the results are not novel and the data sets used in this work are not publicaly available.

I hope authors in their revised version can make their data sets and the analysis pipeline public available, then other researchers may reproduce the results.

Our response: We are planning to release the data through the newly formed NIAAA NIDA genetics consortium and will eventually place it in dbGaP.

Regarding the release of the analysis pipeline, most of the analyses were performed using freely available software written at other institutions, such as PLINK, Haploview, GEC, etc., so we do not have a general utility pipeline to provide externally. We would be more than happy to assist others that contact us about reproducing the results in this or other data sets.
Reviewer #3: Ulrich W. Preuss

Reviewer’s report:

The paper reports results from a GWAS in 427 Mexican Americans regarding their risk for alcohol dependence symptoms with focus on the ADH gene cluster on chromosome 4. The subjects were diagnosed using a semi-structured interview (SSAGA). GWAS was performed using an Affymetrix Axiom Exome 1A chip. Results indicate that an ADH7 variant (rs991316) has a protective influence on the phenotypes. Additional linkage analysis suggested that nearby variants in LD with this SNP are not responsible for the observed associations.

The authors conclude that ADH7 has been previously reported to have a protective role against alcohol dependence in other studies and may also play a protective role in the current sample of Mexican Americans.

The strengths of the paper certainly include the thorough assessment of the subjects and the elaborate genetic analyses as well as the elaborate discussion of results. The paper is well-structured and well-written. Selection of phenotypes and statistical methods are appropriate.

I have only few comments to make:

1. The authors should include a sample-size/power analysis to their study, since the sample is rather small for a GWAS/analysis of the ADH cluster and the authors also use “quantitative” phenotypes (DSM III R and IV symptom counts) and n = 125 of the subjects had an alcohol dependence diagnosis.

   Our response: We have performed a power analysis using GWAPower (Feng et al., 2011, BMC Genetics) on the quantitative phenotypes. We have added the following line to the Results section on Page 10, Line 227:

   “Power analyses using GWAPower (Feng et al., 2011, BMC Genetics) showed that the samples contained 80% power to detect an effect explaining 0.053% of the variance.”

2. Previous studies in other ethnicities also identified a number of other ADH gene variants which may influence the risk for alcohol dependence (e.g. ADH1B, C) which did not reach significance in the current study. This may also be due to low sample size and loss of power. Please comment.

   Our response: We have added some text to address this concern to the Discussion section, Page 14, Line 338:

   “Additionally, we may have missed some associations due to low sample size and loss of power. However, the rs991316 finding is supported given that this SNP showed an association with alcohol dependence in this cohort and in an independent sample of
Native Americans.”

3. Please use the term “alcohol dependence” throughout the paper (instead of “alcoholism”)

Our response: We have changed the term “alcoholism” to “alcohol dependence” throughout the paper, if possible. In some cases, we were not able to change the text, since “alcoholism” is a component of the names for the SSAGA, NIAAA, ACER journal, and in some reference article titles.

4. Please report ethnic background assessments in the results section.

Our response: We have changed and expanded the one-sentence description of the ethnic background assessments in the Results section on Page 8, Line 194:

“Ethnic background was self-reported by the subjects, by choosing the dominant heritage of each of their great grandparents from a list of possible ethnicities. For each subject, the numbers of great grandparents reported as Mexican, Mexican-American, Chicano, Mexican Indian, Caribbean, Cuban, Puerto Rican, South American, or other Spanish, were tallied, resulting in a percentage between 0 and 100%. From these tallies, 92% of the participants reported at least 50% Hispanic heritage.”

5. Methods: please explain the term “winsorization “

Our response: We have expanded the text in the Methods section to provide more information about winsorization on Page 7, Line 151:

“To reduce the effect of extreme outliers in the phenotypic values, we used a winsorization transformation, whereby the extreme values are replaced by certain percentiles rather than discarding the outliers. In particular, the lowest 5% of the numerically sorted phenotype values were replaced by the 5th percentile value, and the highest 5% of the values were set to the 95th percentile value. Custom R code was written to generate winsorized phenotype values at the 5% and 95% cutoffs, which were then used as the phenotype values in PLINK.”

6. Figure 2: please explain the phenotypes (“AlcDep3” and “AlcDep4”) in the legend, as with table 1.

Our response: As requested, the abbreviations for AlcDep3 and AlcDep4 have been added to the legend text of Figure 2.