

Rapid Publication

A Genome Screen of Maximum Number of Drinks as an Alcoholism Phenotype

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The Collaborative Study on the Genetics of Alcoholism (COGA) is a multicenter research program to detect and map susceptibility genes for alcohol dependence and related phenotypes. The measure M of “maximum number of drinks consumed in a 24-hour period” is closely related to alcoholism diagnosis in this dataset and provides a quantitative measure to grade nonalcoholic individuals. Twin studies have shown log(M) to have a heritability of approximately 50%. Genome screens for this trait were performed in two distinct genotyped samples (wave 1 and wave 2), and in the combined sample. MAPMAKER/SIBS was used to carry out Haseman-Elston based regression analyses. On chromosome 4, an unweighted all-pairs multipoint LOD of 2.2 was obtained between D4S2407 and D4S1628 in wave 1; in wave 2, the region flanked by D4S2404 and D4S2407 gave a LOD of 1.5. In the combined sample, the maximal LOD was 3.5 very close to D4S2407. This evidence for linkage is in the region of the alcohol dehydrogenase gene cluster on chromosome 4. These findings on chromosome 4 are consistent with a prior report from COGA in which strictly defined nonal-

coholic subjects in wave 1 were analyzed. The present analysis on log(M) allows more individuals to be included and thus is potentially more powerful. *Am. J. Med. Genet. (Neuropsychiatr. Genet.)* 96:632–637, 2000.

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INTRODUCTION

The study of qualitative phenotypes and diseases can be enhanced by investigating related quantitative traits. Complex diseases such as alcoholism may be influenced by several genes of small effect that are difficult to detect by direct analysis of the clinical phenotype. However, it may be possible to detect such genes if they have a major effect on related traits (or endophenotypes) under study. For example, the degree of coronary artery calcification has been shown to be an excellent predictor of coronary artery stenosis [Kaufmann et al., 1994; Rumberger et al., 1995]. It is a meaningful variable in the early pathogenesis of atherosclerosis and has been used to study the contributions of such candidate genes as apolipoprotein E (apoE) [Kardia et al., 1999]. Plasma cholesterol levels are also quantitative indicators of the risk for coronary heart disease and have been found to be significantly associated with apoE genotype [Boerwinkle et al., 1987; Kamboh et al., 1995; Kaprio et al., 1991].

The P3 component of human event-related potentials (ERPs) may provide a marker of genetic risk for alcoholism. It has evidenced reduced amplitude in alcoholics even after long-term abstinence [Porjesz et al., 1998; Porjesz and Begleiter, 1985]. Furthermore, the reduction in P3 amplitude appears to be highly correlated with the number of alcohol-dependent individuals in the family [Benegal et al., 1995; Pfefferbaum et al., 1991], and amplitudes in alcohol-naive preadolescent sons of alcoholics have been shown to be significantly lower than those of controls [Begleiter et al.,

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1984]. Quantitative linkage analysis has shown evidence of several loci linked to the P3 amplitude [Begleiter et al., 1998]. These loci may lead to further understanding of genetic factors underlying susceptibility to alcohol dependence.

Platelet monoamine oxidase (MAO) activity also has been suggested as an endophenotype for alcoholism. However, analyses of data from the Collaborative Study on the Genetics of Alcoholism (COGA) [Daw et al., submitted; Saccone et al., 1999] indicate that the association between MAO and alcoholism is most likely explained by the confounding effect of cigarette smoking. Thus, choosing an endophenotype requires careful work to establish its connection to the clinical disease of interest.

The present report describes analyses of the COGA data, using self-reported "maximum number of drinks consumed in a 24-hour period" (denoted by M) as a quantitative trait. This phenotype may be expected to be related to alcohol metabolism capacity as well as to individual social influences. Despite the fact that the occurrence of M may be a one-time event, adult twin studies have shown $\log(M)$ to have heritability on the order of 50% [A. Heath, unpublished data], while a related dichotomous trait defined by $M \geq 20$ has shown heritability of approximately 30% [Slutske et al., 1999]. Other studies have found that M is an indicator of alcoholism and differs significantly between alcohol dependent subjects with and without a physiological component [Schuckit et al., 1995, 1998]. These findings motivated this study of the maximum drinks phenotype.

MATERIALS AND METHODS

Subjects

The COGA dataset contains case families ascertained from an alcoholic proband in a treatment program ($N = 1228$) and control families ($N = 236$) selected randomly to reflect population-based rates of alcoholism. A subset of 250 multiplex families has been genotyped. The ascertainment protocols for COGA are described in Begleiter et al. [1995]. Informed consent from subjects was obtained.

For genetic analysis, two distinct sets of phenotyped and genotyped families (and the combined dataset) were examined. The first dataset, designated wave 1, is composed of 105 extended families, providing 170 nuclear families and 1158 nonindependent phenotyped and genotyped sib-pairs. Wave 2 is a distinct sample of 157 extended families. Of these, 145 families had interview data and genotypes for sib-pair analyses of maximum drinks, providing 200 nuclear families and 1105 nonindependent sib-pairs. Wave 1 and wave 2 together form the combined sample.

Genotyping

A total of 351 markers was genotyped; the average intermarker distance was 10.9 cM and the average heterozygosity was 0.74. Genotyping was performed in the labs of Alison Goate at Washington University and Howard Edenberg at Indiana University. Most markers were tri- and tetranucleotide repeat polymorphisms

developed by the Cooperative Human Linkage Center, with additional markers from Genethon, the Marshfield Clinic, M.I.T., and the University of Utah [Murray et al., 1994]. Details of the genotyping protocols are in Reich et al. [1998].

Mendelian inheritance of markers was confirmed using the database manager Genemaster [Rice, personal communication] as well as the programs CRI-MAP [Lander and Green, 1987] and USERM13 [Boehnke, 1991]. Maximum likelihood estimates of marker allele frequencies were obtained from data on all genotyped individuals in the COGA combined dataset using USERM13. CRI-MAP was used to calculate marker order and distances using the combined genotype data in COGA.

Phenotype Definitions and Statistical Methods

The measure M was recorded as part of the SSAGA interview [Bucholz et al., 1994, 1995; Hesselbrock et al., 1999], via the question "What is the largest number of drinks you have ever had in a 24-hour period." This phenotype was specifically selected for study, and no other diagnostic, grouped, or individual items from the SSAGA were analyzed. Selection of this phenotype was motivated by reports of the heritability of $\log(M)$ and related phenotypes [A. Heath, personal communication; Slutske et al., 1999]. The sample analyzed by Heath consists of adult Australian twins, both male and female [Heath et al., 1997], whereas Slutske et al. analyzed male twins from the Vietnam Era Twin Registry. Since heritability findings may not generalize from those samples to the COGA sample, sibling correlations for the COGA control families were examined for the present study and also indicate $\log(M)$ to be familial. The correlations were 0.41 for female-female pairs, 0.46 for male-male pairs, and 0.22 for male-female pairs.

The full sample of case and control families was analyzed for descriptive information on M . The relationship between M and alcoholism diagnosis was also examined. Individuals were considered affected if they met both the DSM III-R (*Diagnostic and Statistical Manual of Mental Disorders—Revised*) [American Psychiatric Association, 1987] criteria for alcohol dependence and the Feighner criteria for definite alcoholism [Feighner et al., 1972]. Individuals were defined as strictly unaffected if they reported consuming alcohol but had no symptoms of alcohol abuse or dependence by any diagnostic system. Remaining individuals who reported some symptoms but did not meet the above criteria for dependence were defined as unaffected.

Prior to linkage analyses, M was transformed to $\log(M)$ (\log denotes logarithm base e). Individuals reporting $M = 0$ have unknown response to alcohol exposure and were excluded from analysis. The log transformation reduces skewness due to individuals who report extremely high consumption.

Linear regression was used to correct $\log(M)$ for the significant covariate of gender. After correction, the quantitative trait $(\log M)_{\text{corr}}$ was recorded as a residual from the predicted value. Body mass index was not a significant covariate in these data.

A discrete phenotype was defined using eight threshold classes corresponding to a \log_2 scale ($0 < M \leq 2$, $2 < M \leq 4$, $4 < M \leq 8$, etc.), with a linear correction for gender. This grouping allowed examination of the rates of diagnoses at different consumption levels. This threshold phenotype also was used for linkage analyses to compare with results for $(\log M)_{\text{corr}}$.

Multipoint linkage analyses were carried out on all sib-pairs ($n(n-1)/2$ pairs for a sibship of size n) using version 2.0 of MAPMAKER/SIBS. As described by Kruglyak and Lander [1995], this program analyzes quantitative trait data using Haseman-Elston type regression [Haseman and Elston, 1972], regressing the squared trait difference on a true maximum likelihood estimate for alleles shared identical by descent.

This study was designed to identify chromosomal regions that provide evidence for linkage in both wave 1 and wave 2. Results for the combined data also are reported for such regions. Although wave 1 and wave 2 are distinct samples, the same ascertainment protocol was used for both, so that wave 2 may be viewed as a replication sample for wave 1.

RESULTS

In the full sample of case and control families, M ranged from 0 to 336. Nineteen individuals were in the highest threshold class ($M > 128$ drinks). The highest report of $M = 336$ was confirmed to be the subject's response and not an entry error. It is likely that the extremely high values, given by self-report, are inflated; however, use of the log transform minimizes this inflation's impact on the regression analysis. Reliability of this measure was examined using available responses at a five-year follow-up interview. For men ($N = 884$), correlation in responses was 0.59 for M and 0.79 for $\log(M)$. For women ($N = 1076$), correlation in responses was 0.74 for M and 0.80 for $\log(M)$. Based on these findings, the use of $\log(M)$ appears appropriate.

In the case families, the mean M for probands was 36.6 and the mean for nonprobands was 13.8, whereas in control families the mean was 10.6. In the full sample, the mean was 10.2 for females and 24.2 for males. Subsetting by alcoholism diagnosis, affecteds had a mean of 29.5; strictly defined unaffecteds had a mean of 4.4.

The rates of alcoholism diagnosis in the threshold classes indicated a close relationship between diagnosis and M . Table I shows the percentage of affecteds, unaffecteds with some symptoms, and strict unaffecteds in each of the eight classes, stratified by gender. Strictly defined unaffected individuals reported relatively low levels of maximum consumption. Among the high maximum consumption classes, affected individuals predominate.

Multipoint linkage analyses of $(\log M)_{\text{corr}}$ found evidence for linkage to chromosome 4 in each sample analyzed (wave 1, wave 2, and combined). Figure 1 shows LOD score graphs from MAPMAKER/SIBS multipoint Haseman-Elston analyses (all pairs unweighted) of chromosome 4 for the wave 1, wave 2, and combined samples. In wave 1, a maximal LOD of 2.2 was obtained at 124 cM between D4S2407 and D4S1628; in

TABLE I. Alcohol Diagnosis Percentages for Threshold Classes

Maximum drinks	% Affected	% Unaffected ^A	% Strict unaffected ^B	N
Men				
1-2 drinks	0.00	0.00	100.00	108
3-4	0.61	15.34	84.05	163
5-8	5.62	56.43	37.95	498
9-16	30.03	60.46	9.51	999
17-32	71.76	26.81	1.42	1544
33-64	92.49	6.84	0.67	746
65-128	94.51	5.49	0.00	164
>128	100.00	0.00	0.00	18
Women				
1-2 drinks	0.00	0.00	100.00	593
3-4	0.34	15.87	83.79	846
5-8	8.11	59.75	32.15	1431
9-16	34.84	56.74	8.42	1128
17-32	76.24	21.99	1.77	623
33-64	90.54	9.46	0.00	148
65-128	93.33	6.67	0.00	30
>128	100.00	0.00	0.00	1

^AUnaffected with some symptoms.

^BUnaffected with no symptoms of alcohol abuse or dependence.

wave 2 a LOD of 1.5 was attained at 119 cM between D4S2404 and D4S2407. In the combined sample, the LOD increased to 3.5 on chromosome 4 at 121 cM, 1.5 cM from D4S2407.

Table II shows all regions with LOD scores above 1 in either wave 1 or wave 2, using all pairs unweighted. Only the chromosome 4 region near D4S2407 gave LOD scores above 1 in both waves.

Linkage analyses of the threshold class phenotype gave results very similar to those for $(\log M)_{\text{corr}}$, and are not displayed. The signalled regions gave slightly lower LOD scores; this reflects the information lost by imposing discrete classes.

DISCUSSION

In this study, the only consistent, and strongest, evidence for linkage to the maximum drinks phenotype was on chromosome 4. Both wave 1 and wave 2, as well as the combined data, support linkage near D4S2407, in the 4q21.3 region (University of Southampton map,

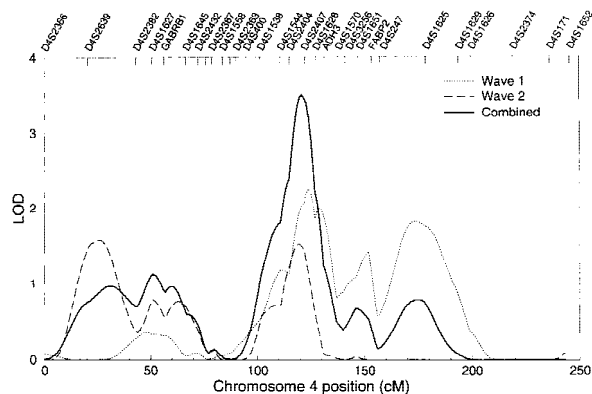


Fig. 1. Multipoint LOD scores for chromosome 4, generated by MAPMAKER/SIBS (all pairs unweighted) using traditional Haseman-Elston regression of $(\log M)_{\text{corr}}$. Analysis of wave 1 is indicated by the dotted line, wave 2 is the dashed line, and the combined is the solid line.

TABLE II. Regions With LOD > 1 in Either Wave 1 or Wave 2*

Chromosome and location (flanking markers)	LOD score
Wave 1	
Chr 4, D4S2407-D4S1628	2.25
Chr 4, D4S1625-D4S1629	1.82
Chr 3, GATA128C02-D3S3045	1.64
Chr 4, D4S1651-FABP2	1.42
Chr 8, D8S1107-D8S549	1.49
Chr 17, D17S1308	1.27
Wave 2	
Chr 4, D4S2639-D4S2382	1.57
Chr 4, D4S2404-D4S2407	1.52
Chr 1, D1S1592	1.15

*MAPMAKER/SIBS Haseman-Elston regression, all pairs unweighted, trait ($\log M$)_{corr}.

<http://cedar.genetics.soton.ac.uk/pub/chrom4/gmap>). Further investigation is warranted, especially in view of the presence of the alcohol dehydrogenase (ADH) gene cluster in this region. *ADH1*, *ADH2*, and *ADH3*, which encode subunits of the class I alcohol dehydrogenases, are tandemly organized in an 88-kb region of 4q21-q23 [Tsukahara and Yoshida, 1989; Yasunami et al., 1990]. Other ADH genes cluster in or near this region as well. Alleles at *ADH2* and *ADH3* on chromosome 4 affect enzyme kinetic properties and have been reported to reduce the risk for alcoholism in Chinese and Japanese populations [Edenberg and Bosron, 1997; Crabb et al., 1995; Thomasson et al., 1991; Shen et al., 1997; Muramatsu et al., 1995; Higuchi et al., 1996]. Evidence linking alcohol dependence to the region of the ADH genes also has been reported in an American Indian population [Long et al., 1998], using two-point Haseman-Elston regression on the dichotomous trait.

We have initiated analyses of ADH genes using *ADH3*, a locus already genotyped in COGA. On the genetic map constructed, *ADH3* is on chromosome 4 at 131.6 cM. However, the signals observed on chromosome 4 for maximum drinks do not appear to be due to variation at the two-allele system at *ADH3*, as the maximum drink phenotype does not significantly vary by *ADH3* genotype for individuals in our dataset. Additional genotyping is underway to more fully evaluate the role of the ADH cluster.

The issue of appropriate choice of sib-pair use ($n - 1$ independent pairs versus all possible $n(n - 1)/2$ nonindependent pairs) can complicate interpretation of the results reported. These genome screens used all possible sib-pairs, which gives a larger sample but opens concerns due to the nonindependence of the pairs. To evaluate the appropriateness of using all pairs, two point MAPMAKER/SIBS results from all pairs were compared with results from the SIBPAL routine from the Statistical Analysis for Genetic Epidemiology package [S.A.G.E., 1994], for the combined sample. SIBPAL allows use of all possible pairs for two-point linkage analysis with a correction for nonindependence.

Under SIBPAL, there are three markers in the signalled chromosome 4 region that are significant at the 0.05 level. The neighboring markers of D4S2407 and D4S1628 have p -values of 0.016 and 0.026, respectively. The nearby D4S1538 gives a p -value of

0.028. The LOD score from all-pairs analysis with MAPMAKER/SIBS is not a true LOD due to nonindependence. However, treating it as though it were, the corresponding p -values may be compared with those of SIBPAL. From two-point MAPMAKER/SIBS using all pairs unweighted, D4S2407 gives a LOD of 1.08 (corresponding to a p -value of 0.013) and D4S1628 gives a LOD of 1.08 (p -value of 0.013). D4S1538 gives a LOD of 0.80 (p -value 0.027). These p -values are more significant than, but comparable with, those from SIBPAL. In contrast, two-point MAPMAKER/SIBS results using the recommended weighting for all pairs are markedly conservative (p -values of 0.110, 0.053, and 0.076 for D4S2407, D4S1628, and D4S1538, respectively.) Independent pairs analysis is even more conservative. Hence, it appears appropriate to report the multipoint interpolation for MAPMAKER/SIBS analysis of all pairs, unweighted, although the underlying nonindependence should be noted as a limitation. The corresponding two point p -values are significant at only the 0.05 level and do not meet criteria for genomewide significance; however, multipoint interpolation extracts more information from the region, leading to the evidence reported on chromosome 4.

The sibling correlations in the COGA controls for $\log(M)$ are high, especially in like-sex pairs. These correlations reflect familiarity of the trait, and possibly also the age similarity of sibs together with a cohort effect. In the COGA data, age is negatively correlated both with $\log(M)$ and also with alcoholism diagnosis; this effect is under further study and may indicate a secular trend. Such an age effect would tend to elevate the correlation between siblings, and may limit interpretation of this correlation as an indication of familiarity of M . However, an increased sibling correlation for the trait due to an age effect would not tend to infer linkage where none exists, and thus does not detract from the significance of these linkage results. Rather, loss of power may be the expected consequence, due to overall increased similarity across all sib-pairs.

Of the 105 extended families in wave 1, 86 were predominantly Caucasian, 13 were African-American, 5 were Hispanic, and 1 was Pacific Islander. In wave 2, 127 extended families were predominantly Caucasian, 25 were African-American, 4 were Hispanic, and 1 was Pacific Islander. These groupings are based on the majority of responses among interviewed family members. Genetic heterogeneity can be a concern when studying ethnically diverse samples. However, although affected-affected sib-pair analyses can be greatly affected by allele frequency misspecification [Hauser et al., 1996], analyses of quantitative traits are more robust in this regard. Analyses on a subset of 154 extended families for which all interviewed members reported themselves as Caucasian gave signals on chromosome 4 supporting results from the full sample (maximal LOD of 2 at 126 cM). Hence, these linkage findings do not appear to be artifacts of genetic heterogeneity.

The linkage to chromosome 4 reported here is consistent with a previous report on the wave 1 data of COGA [Reich et al., 1998], which detected significantly increased allele sharing among strictly defined unaffected-unaffected sib-pairs, and decreased sharing in

TABLE III. Sib-Pair Counts by Diagnosis and by Wave*

Diagnoses	Wave 1	Wave 2
Affected-affected	381 (226)	425 (266)
Affected-unaffected	182	150
Unaffected-unaffected	46 (31)	21 (17)

*First number is for all possible pairs; number in parentheses is for $n - 1$ independent pairs per sibship. Unaffecteds are strictly defined as having no alcohol symptoms.

discordant pairs, in a nearby region of chromosome 4. However, these findings have not been confirmed in wave 2, as the sample of strict unaffecteds in wave 2 is very small.

Table III gives the sib-pair counts for genotyped sib-pairs available in each wave, by diagnosis, where unaffecteds are strictly defined. Numbers are further reduced when only pairs with genotyped parents are considered. In comparison, the maximum drinks phenotype is available for many more people than those diagnosed under this schema, and yet is closely related to diagnosis. This quantitative phenotype of maximum drinks has proved to be a useful proxy for alcoholism diagnosis in these analyses, with the results suggesting a possible role for alcohol dehydrogenase in this phenotype.

It is perhaps unexpected, but instructive, to find that a simple measure based on a single self-reported interview item appears to capture important information relating to diagnosis. These findings reveal that it will be important to study further the basic characteristics of the maximum drinks phenotype to better understand the information it contains, and to understand any bias to which it may be liable, such as in subjects' reporting or remembering. Analogous measures of substance exposure also may be useful in future studies of this and other substance abuse disorders.

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