A sex-adjusted and age-adjusted genome screen for nested alcohol dependence diagnoses

J. Corbett[a], N. L. Saccone[a], T. Foroud[b], A. Goate[a], H. Edenberg[b], J. Nurnberger[b], B. Porjesz[c], H. Begleiter[c], T. Reich[a,†] and J. P. Rice[a]

Alcohol dependence is a complex disorder with a substantial genetic contribution to susceptibility. The Collaborative Study on the Genetics of Alcoholism is a multi-site study whose purpose is to detect, localize, and characterize genes contributing to this susceptibility. Previous linkage analyses of the trait of alcohol dependence in Collaborative Study on the Genetics of Alcoholism have used affected sib-pair methods with a dichotomous phenotype definition. In contrast, the analysis in this paper uses a sex-adjusted and age-adjusted multiple threshold liability model. The use of such a model, in that it includes unaffected as well as as affected subjects and in that it utilizes the differential severity of a diagnosis scale, should heuristically be more powerful than a straight affected sib-pair analysis. Three regions of interest are found on chromosome 1 (lod 5.17), chromosome 4 (lod 3.46), and chromosome 8 (lod 4.31). The region on chromosome 1 near the marker D1S532 is in the region previously reported as linked to alcohol dependence and correlated phenotypes in this dataset. The region on chromosome 4 near the alcohol dehydrogenase gene cluster has been reported to be linked to alcohol dependence in other studies, as well as to the alcohol consumption phenotype 'Maximum Number of Drinks in a 24-Hour Period' in this dataset. The region on chromosome 8 near the marker D8S1988 is homologous to a section of rat chromosome 5 to which an alcohol consumption phenotype has been linked. *Psychiatr Genet* 15:25–30

Psychiatric Genetics 2005, **15**:25–30

Keywords: alcoholism, variance components analysis, normal threshold liability model, polychotomous phenotype

[a]Washington University School of Medicine, St Louis, Missouri, USA, [b]Indiana University School of Medicine, Indianapolis, Indiana, USA and [c]SUNY Health Science Center, Brooklyn, New York, USA.

Sponsorship: The Collaborative Study on the Genetics of Alcoholism national collaborative study is supported by the NIH Grant U10AA08403 from the National Institute on Alcohol Abuse and Alcoholism.

Correspondence and requests for reprints to John Rice, Ph.D., Department of Psychiatry, Box 8134, Washington University School of Medicine, 660 South Euclid Avenue, St Louis, MO 63110, USA.
Tel: +1 314 286 2572; e-mail: john@zork.wustl.edu

Received 21 May 2004 **Accepted** 29 July 2004

Introduction

Previous affected sib-pair (ASP) linkage analyses of alcohol dependence in the Collaborative Study on the Genetics of Alcoholism (COGA) dataset have been performed using various diagnostic criteria of dependence. The diagnostic criteria used have included COGA DSM-III-R (American Psychiatric Association, 1987) plus Feighner Definite (Feighner *et al.*, 1972) criteria, DSM-IV criteria (American Psychiatric Association, 1994) and ICD-10 criteria (World Health Organization, 1993). Suggestive linkage to alcohol dependence has been found on regions of chromosomes 1, 2, and 7 (Reich *et al.*, 1998; Foroud *et al.*, 2000c). Additionally, a genome scan using individuals classified as pure unaffected, defined as individuals who had been exposed to alcohol but who showed no symptoms of alcohol dependence, yielded some evidence for a possible protective locus on chromosome 4 in the region around the alcohol dehydrogenase gene cluster (Reich *et al.*, 1998).

The ICD-10 diagnostic criteria are the most restrictive, the COGA criteria are the least restrictive, and DSM-IV criteria are intermediate (Grant, 1996; Foroud *et al.*, 2000c). Previous investigations of this dataset have used the strategy of parallel ASP analyses of 'broad' (COGA) and 'narrow' (ICD-10) alcohol dependence phenotypes to attempt to increase the power of linkage analysis. This is a common strategy employed in psychiatric genetics where multiple models of clinically defined affectation are employed (Reich *et al.*, 1998; Brzustowicz *et al.*, 2000; Foroud *et al.*, 2000b). However, this strategy does not take full advantage of the severity gradient underlying the aforementioned diagnoses of alcohol dependence, since the subjects considered affected under the broader diagnosis are excluded from analysis under the narrower diagnosis while there is no provision under the broader analysis for the differential severity within the sample. Furthermore, under neither the broad nor the narrow analyses are unaffected subjects included.

[†]In memory of Theodore Reich, M.D., Co-Principal Investigator of the Collaborative Study on the Genetics of Alcoholism (COGA) since its inception, and one of the founders of modern psychiatric genetics. We acknowledge his immeasurable and fundamental scientific contributions to COGA and the field.

0955-8829

In order to simultaneously analyze the full spectrum of alcohol dependence states identifiable in this dataset, we propose the use of a multiple threshold liability model (Reich *et al.*, 1972). Under this model, we consider the trait of alcohol dependence to be derived from a standard normal liability variable encompassing all states from 'pure unaffected' to ICD-10 dependent. We thus create a polychotomous analysis variable. Simulation studies have shown that dichotomizing a polychotomous phenotype, as for example one would do in the case of alcoholism by simply selecting a single diagnosis to determine affectation, leads to a loss of power with no corresponding decrease in Type I Error (Corbett *et al.*, 2004). It is the associated liability score that is used to perform a linkage analysis. Since the liability score itself cannot be directly observed, it must be estimated from the prevalence of alcohol dependence states in base populations. Since there are strong sex or age-cohort effects for alcohol dependence (Rice *et al.*, 2003), the estimates for liability scores in this analysis were adjusted to reflect these effects. This adjustment cannot be made under standard ASP analyses. In effect, the method in this paper will overweight the occurrence of severe diagnoses in those cohorts where such diagnoses are rare and lower the impact of phenocopies on our analysis.

Materials and methods

Subjects

The dataset consists of 1228 families ascertained through an alcoholic proband in treatment, as well as 236 randomly ascertained control families. The control families were selected without regard to alcohol dependence to represent a random population sample. From these 1464 extended families, a subset of 250 multiplex families informative for linkage was selected for genotyping. The 250 extended families selected for inclusion in the genetic analysis sample comprised 327 nuclear families and 2263 non-independent sib pairs. Of these 250 families, 248 were selected from among the 1228 proband ascertained families, while two were selected from among the 236 control families. The protocols for ascertainment and the conditions required to be selected for genotyping have been previously described (Begleiter *et al.*, 1995). Informed consent was obtained from all subjects.

Overall, the sample is 77% Caucasian, 15% African-American, and 8% 'Other'. It is 44% male and 56% female.

Genotyping

This analysis uses a total of 351 markers spread across the genome with an average heterozygosity of 0.74. The average intermarker distance was 10.9 cM. Genotyping for COGA was performed at Washington University and at Indiana University. The majority of markers genotyped were trinucleotide or tetranucleotide repeat polymorphisms developed by the Cooperative Human Linkage Center, as well as markers from Genethon, the Marshfield Clinic, MIT, and the University of Utah (Murray *et al.*, 1994). Further details on genotyping procedures in COGA can be found in previous publications (Reich *et al.*, 1998; Foroud *et al.*, 2000c).

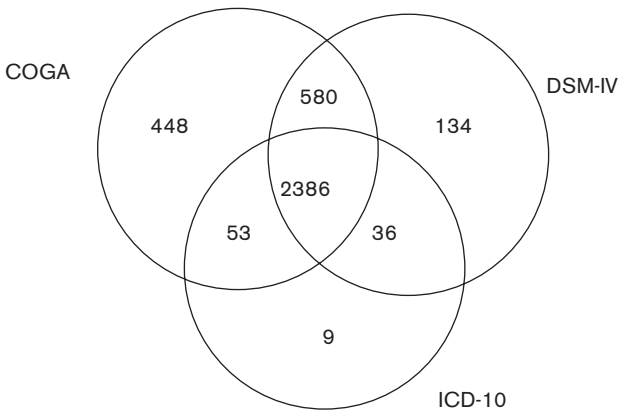
Markers were checked for Mendelian inheritance through use of the database manager GeneMaster as well as the program USERM13 (Boehnke, 1991). Marker frequency estimates were made from data on all genotyped individuals in COGA using USERM13. Marker order and intermarker distances were estimated in COGA through the use of the program CRI-MAP (Lander and Green, 1987).

Phenotype and statistical methods

In previous analyses of this dataset, three different definitions of alcohol dependence were used. Regions of suggestive linkage for these definitions were often, but not always, overlapping. The three alcohol dependence diagnoses used in this dataset are almost completely nested and show evidence of a severity gradient running from COGA dependence (the least severe) to ICD-10 dependence (the most severe) (Grant, 1996). For example, of the 2484 subjects diagnosed with ICD-10 alcohol dependence in the entire dataset, only 45 were unaffected according to the COGA criteria and only 62 were unaffected according to DSM-IV criteria (see Fig. 1).

This nesting of diagnoses suggests the use of a multiple threshold liability model for alcohol dependence. We propose a model with five categories – pure unaffected, unaffected, COGA dependent, DSM-IV dependent, and ICD-10 dependent – where affected subjects are classified according to their most severe diagnosis. Pure

Fig. 1



Concordance of A1C. Dependence Diagnosis in COGA.

unaffected subjects are defined to be those subjects who have been exposed to alcohol but who show no symptoms of alcohol dependence according to any of the diagnostic systems (DSM-III-R, Feighner, DSM-IV, ICD-10) used in this dataset. Unaffected subjects are defined to be those subjects who have been exposed to alcohol and are not pure unaffected, but who do not meet dependence criteria under any of the the three alcohol dependence diagnostic systems. Subjects who had not been exposed to alcohol ($n = 238$) were not included in this analysis as their status was considered to be unknown.

Since alcohol dependence has well-known sex and cohort effects (Rice *et al.*, 2003), the thresholds for a multiple threshold liability model should depend upon the age and sex of the subject. We modeled this dependence through the partitioning of subjects into six classes based upon the sex of the subject as well as the age of the subject (≤ 25, 26–50, ≥ 51) at the time of interview. We used data from the 236 randomly ascertained families in the control sample to estimate population prevalences of the states of our multiple threshold liability model. We then estimated threshold values for each of these six age-sex cohorts separately.

Multipoint identity by descent estimates for all sib pairs in the combined genetic analysis dataset were obtained from MAPMAKER/SIBS (Kruglyak and Lander, 1995) at each marker locus. These identity by descent estimates were combined with the estimated liability values and were used as inputs to a variance components method implemented in the structural equation modeling package Mx (Eaves *et al.*, 1996) for linkage analysis. Linkage analysis was performed on all possible pairs of sibs without weighting for non-independence.

Lod scores were calculated for the results by converting *P* values. Twice the difference in $\log_e$-likelihood difference between a polygenic disease model and a polygenic model including a QTL serves as a test statistic that is distributed under the null hypothesis of no linkage at a given locus as a random variable with a 0.5:0.5 mixture of a χ^2 distribution with one degree of freedom and a point mass at zero (Self and Liang, 1987; Almasy and Blangero, 1998). Thus, one may calculate a traditional lod score at a locus by dividing the difference in $\log_e$-likelihoods of the polygenic and QTL models at that locus by $\log_e 10$.

Results

We observed a clear gradient among the age–sex cohorts, indicating that, for all age cohorts, alcohol dependence is both more common and more severe in males than in females. In particular, alcohol dependence in any form was found to be extremely rare among older female subjects in the control group.

Estimating liability thresholds separately for each age–sex cohort, we assigned to each subject the median value of a truncated standard normal random variable, where the truncation values were based upon the assigned class of the subject and the estimated lower and upper threshold values for that age–sex cohort. For example, if a subject were a 35-year-old female dependent under COGA criteria, but not under DSM-IV or ICD-10 criteria, the appropriate thresholds would be 1.53 and 1.76 (estimated from the population prevalences in Table 1), and the subject would be assigned an estimated liability score of 1.64. This score was computed by taking the median (instead of the mean, to reduce the influence of extreme values) of the probability distribution formed by starting with the standard normal distribution and limiting the allowed values of the distribution to be between the two threshold values. In the case of the oldest female cohort, where there were too few affected individuals to obtain threshold estimates for the affected classes, missing threshold values were estimated by taking medians in the intervals between estimated threshold values. Since alcohol dependence is rarer in the older cohorts as well as in the female cohorts, estimated liability scores for alcohol-dependent subjects in these cohorts will be more extreme than for similarly diagnosed subjects in the younger and male cohorts. Conversely, since pure unaffected subjects are less common in the younger and male cohorts than in the older and female cohorts, the estimated liability scores for pure unaffected individuals in these younger and male cohorts will be more extreme than pure unaffected subjects in the older and female cohorts. Table 2 presents estimated liability scores for each threshold state and age–sex cohort. We note that the resulting liability estimates do not form a true quantitative variable, but are rather limited to only five possible values for each age–sex cohort. However, the quantitative scale still gives a way to vary the impact a subject will have on this analysis based upon their age, sex and alcohol dependence state.

Table 1 Sex and cohort effects for alcohol dependence state in the control sample in Collaborative Study on the Genetics of Alcoholism (COGA)

	Male, under 26 years	Male, 26–50 years	Male, over 50 years	Female, under 26 years	Female, 26–50 years	Female, over 50 years
Pure unaffected	42 (24.4%)	37 (20.1%)	49 (38.9%)	52 (31.5%)	133 (52.2%)	79 (79.8%)
Unaffected	84 (48.8%)	99 (53.8%)	63 (50.0%)	91 (55.2%)	106 (41.6%)	18 (18.2%)
COGA	5 (2.9%)	15 (8.2%)	4 (3.2%)	4 (2.4%)	6 (2.4%)	0 (0.0%)
DSM-IV	24 (14.0%)	17 (9.2%)	5 (4.0%)	12 (7.3%)	7 (2.7%)	2 (2.0%)
ICD-10	17 (9.9%)	16 (8.7%)	5 (4.0%)	6 (3.6%)	3 (1.2%)	0 (0.0%)
Total	172	184	126	165	255	99

Affected subjects are classified by their most severe diagnosis.

Table 2 Estimated liability scores for each threshold by age–sex cohort

	Male, under 26 years	Male, 26–50 years	Male, over 50 years	Female, under 26 years	Female, 26–50 years	Female, over 50 years
Pure unaffected	−1.16	−1.27	−0.86	−1.01	−0.64	−0.26
Unaffected	−0.03	−0.07	0.36	0.23	0.61	1.22
COGA	0.67	0.77	1.31	1.17	1.64	1.55
DSM-IV	0.96	1.11	1.56	1.46	1.95	2.32
ICD-10	1.65	1.71	2.06	2.09	2.52	2.57

Table 3 All markers with lod score > 2.0

Chromosome	Marker	Location (cM)	Lod score
1	D1S1665	112.2	2.61
	D1S532	124.2	5.17
	D1S2614	128.2	3.42
4	D4S1628	127.1	3.45
	ADH3	131.1	3.19
	D4S1651	146.1	3.11
	FABP2	152.2	2.25
8	D8S549	10.0	2.21
	D8S1119	103.7	2.43
	D8S1988	109.7	4.31
18	D18S844	107.0	2.27

The most significant result for alcohol dependence was found on chromosome 1 at the marker D1S532, at which there was estimated to be a quantitative trait locus (QTL) explaining 13.2% of the heritable variance (11.1% of overall variance) in liability, with a lod score equivalent of 5.17. Another significant result was found on chromosome 4 near the ADH3 locus, at which there was estimated to be a QTL effect explaining an estimated 10.7% of the heritable variance (9.1% of overall variance) in liability, with a lod score equivalent of 3.46. The final significant result was found on chromosome 8 at the marker D8S1988, where there was estimated to be a QTL explaining 12.6% of the heritable variance (10.6% of the overall variance) in liability. This peak had a lod score equivalent of 4.31.

Table 3 presents all markers that had a lod score equivalent greater than 2.0. As one can see, adjacent markers also showed evidence of linkage to liability for all three of these peaks.

Discussion

This analysis yielded evidence for linkage of the liability trait to three regions of the genome. The first region is on chromosome 1, around the marker D1S532. This region was also highlighted as the strongest signal in the original ASP genome screen for this dataset under both the COGA and ICD-10 models. Since the liability trait draws much of its information from these diagnoses, and since both diagnoses yield signals in this area, it is not surprising that a quantitative linkage analysis of the estimated liability should also yield a signal of suggestive linkage in the same area. The region on chromosome 1 is currently being investigated due to its earlier implication as a region of suggestive linkage under both the COGA and ICD-10 alcohol dependence models as well as other correlated phenotypes including COGA alcohol dependence and depression (Nurnberger *et al.*, 2001) and subjective response to ethanol (Schuckit *et al.*, 2001).

While the signal on chromosome 4 near the ADH3 locus was not selected as a region of suggestive linkage under ASP analysis using any of the three dependence models, analyses of this dataset with other phenotypes have pointed to this region. This region was implicated to contain a possible protective locus based upon an analysis (Reich *et al.*, 1998) of the relatively small number of genotyped pure unaffected sib pairs ($n = 30$), as well as in an analysis of a slightly broadened version of the pure unaffected phenotype with a larger number ($n = 126$) of sib pairs. Additionally, quantitative linkage analysis of the trait 'Maximum Number of Drinks Consumed in a 24 Hour Period' gives evidence of a locus influencing this trait in this same region (Saccone *et al.*, 2000).

The region of interest on chromosome 4 is near the alcohol dehydrogenase gene cluster, containing *ADH1*, *ADH2*, *ADH3*, *ADH4*, *ADH5*, *ADH6*, and *ADH7*. Previous studies have indicated that alleles at *ADH2* and *ADH3*, which affect enzyme kinetic properties, reduce the risk for alcoholism in Chinese and Japanese populations (Thomasson *et al.*, 1991; Crabb *et al.*, 1995; Muramatsu *et al.*, 1995; Higuchi *et al.*, 1996; Edenberg and Bosron, 1997; Shen *et al.*, 1997). In addition, evidence of linkage of alcohol dependence to a region of chromosome 4 near the ADH gene cluster has been reported in a Native American population (Long *et al.*, 1998), using two-point Haseman–Elston regression. This was also seen in an initial analysis of this dataset by the same method (Reich *et al.*, 1996). The two-allele system at *ADH3* has been genotyped in this dataset and does not appear to account for the QTL observed in this analysis, as the distribution of diagnoses does not vary significantly by *ADH3* genotype for the individuals in the combined genetic analysis dataset. Further exploration of the *ADH* gene cluster and other candidate genes in this chromosomal region is underway.

The final region of interest suggested by this analysis is on chromosome 8, near the marker D8S1988. The relative strength of this signal along with the homology of this

region with a region of suggestive linkage to an alcohol-related trait in rat (Foroud *et al.*, 2000a) combines to give some confidence that this signal is not simply due to noise, but is generated by the aggregate information gained by using all available diagnoses, including unaffected subjects, as well as appropriate corrections for age and cohort effects.

The liability trait did not give a strong indication of linkage to other regions at which suggestive linkage to alcohol dependence was found using ASP methods. While there was a lod score of 1.73 seen about 20 cm distal to the main signal found on chromosome 3 in the combined genetic analysis sample of this dataset (Foroud *et al.*, 2000c), no regions on chromosome 2 or chromosome 7 showed lod scores above 0.80 under the model used in this paper. The estimated liability in this model seems to capture some, but not all, of the linkage information of an ASP analysis. This model gains additional information through the mechanism of the assumed severity gradient in addition to the benefits derived from taking sex and birth cohort effects into account. This method of passing to an estimate of an underlying normal liability in order to perform linkage analysis could be performed on other polychotomous traits that have a well-defined, although not necessarily completely ordered, severity gradient. This analysis of the COGA dataset suggests that such an approach may find information for linkage that is not discovered through analysis of dichotomous diagnoses that are at different points on the severity spectrum for the trait under inspection.

In addition to the gender and birth cohort, ethnic ethnicity is another variable that impacts the risk to alcohol dependence (Rice *et al.*, 2003), with African-Americans having a lower risk. However, there are too few non-Caucasians in the linkage sample to permit separate analyses by group. The prior analyses of the linkage sample (Reich *et al.*, 1996; Foroud *et al.*, 2000c) used the entire set of families, and performed separate analyses on the Caucasians-only subset and on sibships that had both parents genotyped. They found consistent results in these analyses. Accordingly, we analyzed the entire set of families to provide comparability to the prior analyses. For studies with a significant ethnic admixture, separate liability classes for different ethnic groups would be advisable.

Acknowledgements

The Collaborative Study on the Genetics of Alcoholism (COGA) (Principal Investigator, H. Begleiter; Co-Principal Investigators, L. Bierut, H. Edenberg, V. Hesselbrock, Bernice Porjesz) includes nine different centers where data collection, analysis, and storage take place. The nine sites and Principal Investigators and Co-Investigators are: University of Connecticut (V. Hesselbrock); Indiana University (H. Edenberg, J. Nurnberger, Jr., P.M. Conneally, T. Foroud); University of Iowa (R. Crowe, S. Kuperman); SUNY HSCB (B. Porjesz, H. Begleiter); Washington University in St Louis (L. Bierut, J. Rice, A. Goate); University of California at San Diego (M. Schuckit); Howard University (R. Taylor); Rutgers University (J. Tischfield); and Southwest Foundation (L. Almasy). Lisa Neuhold serves as the NIAAA Staff Collaborator.

The authors would also like to thank Dr Erik Rasmussen (Washington University), Dr Jennifer Kwon (Washington University), and Dr Kathleen Bucholz (Washington University) for many helpful discussions.

References

Almasy L, Blangero J (1998). Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet* **62**:1198–1211.

American Psychiatric Association (1987). Diagnostic and Statistical Manual of Mental Disorders, Third Edition, Revised. Washington, DC: American Psychiatric Association Press; pp. 166–175.

American Psychiatric Association (1994). Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition. Washington, DC: American Psychiatric Association Press; pp. 194–196.

Begleiter H, Reich T, Hesselbrock V, Porjesz B, Li T-K, Schuckit MA, *et al.* (1995). The collaborative study on the genetics of alcoholism. *Alcohol Health Res World* **19**:228–236.

Boehnke M (1991). Allele frequency estimation from pedigree data. *Am J Hum Genet* **48**:22–25.

Brzustowicz LM, Hodgkinson KA, Chow EWC, Honer WG, Bassett AS (2000). Location of a major susceptibility locus for familial schizophrenia on chromosome 1q21-q22. *Science* **288**:678–682.

Corbett J, Gu CC, Rice JP, Reich T, Province MA, Rao DC (2004). Power loss for linkage due to the dichotomization of trichotomous phenotypes. *Hum Hered* **57**:21–27.

Crabb DW, Edenberg HJ, Thomasson HR, Li T-K (1995). Genetic factors that reduce the risk for developing alcoholism in animals and human. In: Begleiter H, Kissin B (editors): *The Genetics of Alcoholism*. New York: Oxford University Press; pp. 202–220.

Eaves LJ, Neale MC, Maes H (1996). Multipoint multivariate linkage analysis of quantitative trait loci. *Behav Genet* **26**:519–525.

Edenberg HJ, Bosron WF (1997). Alcohol dehydrogenases. In: Guengerich FP (editor): *Comprehensive Toxicology*, Vol 3: Biotransformation, chapter 3.08. Oxford: Elsevier Science.

Feighner JP, Robins E, Guze SB, Woodruff RA, Jr, Winokur G, Munoz R (1972). Diagnostic criteria for use in psychiatric research. *Arch Gen Psychiatry* **26**:57–63.

Foroud T, Bice P, Castellucio P, Bo R, Miller L, Ritchotte A, *et al.* (2000a). Identification of quantitative trait loci influencing alcohol consumption in the high alcohol drinking and low alcohol drinking rat lines. *Behav Genet* **30**:131–140.

Foroud T, Castelluccio PF, Koller DL, Edenberg HJ, Miller M, Bowman E, *et al.* (2000b). Suggestive evidence of a locus on chromosome 10p using the NIMH genetics initiative bipolar affective disorder pedigrees. *Am J Med Genet (Neuropsychiatr Genet)* **96**:18–23.

Foroud T, Edenberg H, Goate A, Rice JP, Flury L, Koller DL, *et al.* (2000c). Alcohol susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcohol Clin Exp Res* **24**:933–945.

Grant BF (1996). DSM-IV, DSM-III-R, and ICD-10 alcohol and drug abuse/harmful use and dependence, United States, 1992: a nosological comparison. *Alcohol Clin Exp Res* **20**:1481–1488.

Higuchi S, Matsushita S, Muramatsu T, Murayama M, Hayashida M (1996). Alcohol and aldehyde dehydrogenase genotypes and drinking behavior in Japanese. *Alcohol Clin Exp Res* **20**:493–497.

Kruglyak L, Lander ES (1995). Complete multipoint sib-pair analysis of quantitative and qualitative traits. *Am J Hum Genet* **57**:439–454.

Lander ES, Green P (1987). Construction of multilocus genetic linkage maps in humans. *Proc Natl Acad Sci USA* **84**:2363–2367.

Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E, *et al.* (1998). Evidence for genetic linkage to alcohol dependence on

chromosomes 4 and 11 from an autosome-wide scan in an American Indian population. *Am J Med Genet (Neuropsychiatr Genet)* **81**:216–221.

Muramatsu T, Wang Z-C, Fang Y-R, Hu K-B, Yan H, Yamada K, *et al.* (1995). Alcohol and aldehyde dehydrogenase genotypes and drinking behavior of Chinese living in Shanghai. *Hum Genet* **96**:151–154.

Murray JC, Buetow KH, Weber JL, Ludwigsen S, Scherpbier-Heddema T, Manion F, *et al.* (1994). A comprehensive human linkage map with centimorgan density. *Science* **265**:2049–2054.

Nurnberger J, Foroud T, Flury L, Meyer E, Crowe R, Edenberg H, *et al.* (2001). A Locus on chromosome 1 contributes to vulnerability to alcoholism and affective disorder. *Am J Psychiatry* **158**:632–637.

Reich T, James JW, Morris CA (1972). The use of multiple thresholds in determining the mode of transmission of semi-continuous traits. *Ann Hum Genet* **36**:163–184.

Reich T, Edenberg HJ, Begleiter H, Cloninger CR (1996). A genomic survey of alcohol dependence and related phenotypes: results from the Collaborative Study on the Genetics of Alcoholism (COGA). *Alcohol Clin Exp Res* **20**:133–137.

Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, *et al.* (1998). Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Genet (Neuropsychiatr Genet)* **81**:207–215.

Rice JP, Neuman RJ, Saccone NL, Corbett J, Rochberg N, Hesselbrock V, *et al.* (2003). Age and birth cohort effects on rates of alcohol dependence. *Alcohol Clin Exp Res* **27**:93–99.

Saccone NL, Kwon JM, Corbett J, Goate A, Rochberg N, Edenberg HJ, *et al.* (2000). A genome screen of maximum number of drinks as an alcoholism phenotype. *Am J Med Genet (Neuropsychiatr Genet)* **96**:632–637.

Schuckit MA, Edenberg HJ, Kalmijn J, Flury L, Smith TL, Reich T, *et al.* (2001). A genome-wide search for genes that relate to a low level of response to alcohol. *Alcohol Clin Exp Res* **25**:323–329.

Self SG, Liang K-Y (1987). Asymptotic properties of maximum likelihood estimators and likelihood ratio tests under non-standard conditions. *J Am Stat Assoc* **82**:605–610.

Shen Y-C, Fan J-H, Edenberg HJ, Li T-K, Cui Y-H, Wang Y-F, *et al.* (1997). Polymorphism of ADH and ALDH genes among four ethnic groups in China and affects upon the risk for alcoholism. *Alcohol Clin Exp Res* **21**:1272–1277.

Thomasson HR, Edenberg HJ, Crabb DW, Mai XL, Jerome RE, Li T-K, *et al.* (1991). Alcohol and aldehyde dehydrogenase genotypes and alcoholism in Chinese men. *Am J Hum Genet* **48**:677–681.

World Health Organization (1993). International Classification of Disease, 10th edition. Geneva: World Health Organization; pp. 56–59.