American Journal of Medical Genetics (Neuropsychiatric Genetics) 81:207–215 (1998)

Genome-Wide Search for Genes Affecting the Risk for Alcohol Dependence

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Alcohol dependence is a leading cause of morbidity and premature death. Several lines of evidence suggest a substantial genetic component to the risk for alcoholism: sibs of alcoholic probands have a 3–8 fold increased risk of also developing alcoholism, and twin heritability estimates of 50–60% are reported by contemporary studies of twins. We report on the results of a six-center collaborative study to identify susceptibility loci for alcohol dependence. A genome-wide screen examined 291 markers in 987 individuals from 105 families. Two-point and multipoint nonparametric linkage analyses were performed to detect susceptibility loci for alcohol dependence. Multipoint methods provided the strongest suggestions of linkage with susceptibility loci for alcohol dependence on chromosomes 1 and 7, and more modest evidence for a locus on chromosome 2. In addition, there was suggestive evidence for a protective locus on chromosome 4 near the alcohol dehydrogenase genes, for which protective effects have been reported in Asian populations. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 81:207–215, 1998. © 1998 Wiley-Liss, Inc.

KEY WORDS: linkage analysis; alcoholism; alcohol dependence; genome screening; genetic susceptibility

INTRODUCTION

Alcohol dependence (alcoholism) is a common, highly familial disorder that is a leading cause of morbidity and premature death [Campbell et al., 1995; Caces et al., 1995; DeBakery et al., 1995]. Modern definitions of the disorder include a pattern of compulsive, excessive use of alcohol, in spite of negative consequences. This is usually accompanied by tolerance to the intoxicating effects of alcohol so that increasing amounts are necessary to maintain the same effect. Debilitating withdrawal symptoms including tremors and confusion may also occur when consumption ceases or is abruptly decreased. The symptoms of withdrawal are rapidly abolished if sufficient alcohol is consumed. Physical, psychological, and social impairment is often progressive, or proceeds intermittently, resulting in deteriorating health, trouble at work, and destruction of family relationships. Symptoms tend to cluster at the onset, so that rapid deterioration may follow an apparent period of social or controlled drinking [American Psychiatric Association, 1994].

Alcohol dependence is strongly familial and has been reported more commonly in males than females. The relative risk to the brothers and sisters of affected individuals, however, has been similar (risk ratios from 3 to 8). Although the designs of studies to estimate the familial aggregation and heritability of alcoholism have been quite variable, a large genetic contribution to risk has been found [Cotton, 1979; Hall et al., 1983a; Reich et al., 1988; Merikangas et al., 1994]. The genetic contribution to the risk for alcoholism has been estimated by studies of adopted-away offspring of affected

Contract grant sponsor: National Institute on Alcohol Abuse and Alcoholism; Contract grant sponsor: USPHS; Contract grant numbers: U10AA08401, U10AA08402, U10AA08403; Contract grant sponsor: U.S. Public Health Service Resource Grant, Division of Research Resources; Contract grant number: 1 P41 RR03655.

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Received 20 October 1997; Revised 26 February 1998

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and unaffected parents [Goodwin et al., 1973; Cloninger et al., 1981; Sigvardsson et al., 1996] and by comparisons of concordance among monozygotic and dizygotic twins [Kendler et al., 1992; Pickens et al., 1991; Heath et al., 1997]. Studies of adopted-away Scandinavian offspring led to heritability estimates of 39%. Meta-analysis of twin data estimated the heritability to be approximately 60% in both men and women [Heath et al., 1996]. Segregation analyses have been carried out to test for Mendelian modes of inheritance and to detect the presence of loci of major effect [Yuan et al., 1996]. Although the underlying liability to develop alcoholism was, in part, controlled by a "major effect," Mendelian and major locus modes of transmission were not supported.

The Collaborative Study of the Genetics of Alcoholism (COGA) is a six-center program to detect and map susceptibility genes for alcohol dependence and related phenotypes [Begleiter et al., 1995]. Nuclear and multigenerational families were systematically ascertained through a proband in treatment for alcoholism. After determination of the familial distribution of alcoholism, a subset of densely affected families was chosen for genetic linkage and association studies. Because of the complexity of the alcoholism phenotype, comprehensive assessment was implemented in many different domains. Alcohol-dependent subjects were defined as those individuals who met *both* the DSM III-R (Diagnostic and Statistical Manual of the American Psychiatric Association-Revised) [American Psychiatric Association, 1987b] criteria for alcohol dependence *and* the Feighner [Feighner et al., 1972] criteria for alcoholism at the definite level. The requirement of a positive diagnosis in two diagnostic schemata was intended to minimize errors of diagnosis.

A subsample of 987 informative individuals from 105 multigenerational families, selected through 23 female and 82 male probands with 3 or more first-degree relatives diagnosed with alcohol dependence, was used in the linkage analyses reported here. A genome screen is reported with 291 markers at an average interval of 13.8 cM. Some preliminary results were presented as part of a symposium at the Research Society of Alcoholism in Washington, DC, in June 1996 [Reich et al., 1996].

SUBJECTS AND METHODS

Ascertainment and Assessment of Probands and Relatives

Probands were systematically recruited from inpatient and outpatient units if their families included a sibship of at least 3 individuals, and if parents were available for study. At least 2 first-degree relatives were required to live in the catchment area of one of the COGA centers. A sibship greater than 3 persons was required if parents were missing. The rationale for these requirements was provided by our intention to maximize the use of "identity-by-descent" methods of linkage analysis, in which the genotype of the parent is directly measured or inferred from multiple offspring. These methods are generally more powerful and less sensitive to bias than "identity-by-state" approaches, which are used if genotype information from parents is missing. Probands were excluded if they were intravenous drug users, had AIDS, were unable to participate, or had a terminal illness not related to alcoholism. The decision to exclude subjects who were intravenous drug users or who had AIDS was taken to reduce the proportion of HIV-infected samples in COGA laboratories. Fewer than 1% of potential probands were eliminated because of AIDS.

Systematic ascertainment criteria and criteria for extending families were standardized across all sites. Probands, spouses, and first-degree relatives were personally interviewed, using a semistructured lifetime psychiatric interview schedule developed for this study. Families that were not bilineal and that included 3 or more affected first-degree relatives were studied more intensively. They were extended using rules to optimize their informativeness for linkage. These pedigrees were extended through affected first-degree relatives, such as a parent or sib (diagnosed by history or interview) (Rice et al., 1995), into secondarily ascertained nuclear branches. Extension also occurred over an unaffected relative into a secondary branch, if at least 2 of the relatives in the branch were affected based on family history. Bilineal branches were not extended. Using these rules, 23.7% of nuclear families with 3 or more affected first-degree relatives were extended into second- and third-degree branches.

Adult lifetime psychiatric status was assessed by direct interview with the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA), designed for this study [Bucholz et al., 1994, 1995]. It was used to make diagnoses of alcohol dependence, alcoholism, or alcohol abuse by the Feighner [Feighner et al., 1972], DSM III [American Psychiatric Association, 1994], and DSM III-R [American Psychiatric Association, 1987b] criteria. A very close approximation to DSM IV [American Psychiatric Association, 1994] and ICD 10 [World Health Organization, 1993] criteria for the diagnosis of alcohol dependence was also available, allowing comparisons with studies that use these criteria. Alcohol consumption and symptoms of abuse and dependence were assessed to identify individuals who drank without adverse consequences and who may have been resistant to the development of alcohol dependence. Thus linkage to protective genes may be studied. Medical records were collected to assist with questionnaire diagnoses. These records were used to establish a diagnosis when the personal interview was deemed unsatisfactory or contradictory. A psychiatric history of all first- and second-degree relatives was also assessed, using a structured family history interview [Rice et al., 1995]. In the absence of personal interview data, family history assessments were used to determine which pedigrees should be extended.

In developing the SSAGA interview schedule, questions that operationalized each diagnostic criterion item for the various classification systems included in SSAGA were identified by a committee of senior diagnosticians. A computer program was written using these criterion items to produce psychiatric diagnoses consistent with each nosologic system, for all psychiat-

ric disorders covered in the SSAGA. These diagnoses were used in the linkage study reported here.

The total sample included 1,227 families of alcohol dependent probands. In these families we interviewed 7,847 adults, 18 years and older. A random sample of 234 control families that included 2 parents and at least 3 children over age 14 was similarly assessed. The familial distribution of the lifetime prevalence of alcohol dependence was estimated by personal interviews in the families of alcohol-dependent probands and control probands. The risk ratio of alcohol dependence was computed from these data as the lifetime prevalence in sibs of alcoholic probands divided by the lifetime prevalence in controls. The risk ratio was used in the estimation of the individual contribution of putatively linked loci to the risk for alcohol dependence.

A subset of cooperative families with 3 or more alcohol dependent first-degree relatives was used in the genetic linkage study. Blood was drawn from adults for the EBV transformation and cryopreservation of lymphoblastoid cell lines and for the extraction of DNA. One hundred and five families including 987 individuals were used in the initial genome screen. Eighty-six of the families were predominantly Caucasian, 13 were African-American, 5 were Hispanic, and 1 was Samoan.

Genotyping

Two hundred and ninety-one markers with an average heterozygosity of 0.72 and an average intermarker interval of 13.8 cM were genotyped. There were seven intermarker regions >30 cM and 28 which were >20 cM. 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]. Genotyping was conducted in two laboratories, with each responsible for approximately half the chromosomes. Therefore, a single laboratory was responsible for genotyping a particular marker in all individuals, avoiding interlaboratory differences in assigning allele sizes. Markers were amplified by the polymerase chain reaction (PCR). For fluorescently labeled markers, PCR reactions for each marker were performed separately and products combined into sets prior to electrophoresis. Data were collected using the 373A automated DNA sequencer (Applied Biosystems, Inc, Foster City, CA) and genotyped using the Genescan 672 and Genotyper software (Applied Biosystems, Inc.). For radioactively labeled markers, 3-μl aliquots of the denatured PCR products were electrophoresed on 6% denaturing polyacrylamide gels (Gel Mix-6, GIBCO/BRL, Gaithersberg, MD). Gels were exposed to Kodak XAR-5 film for 6–72 hr.

A database manager, Genemaster (Rice, personal communication), was used to check for noninheritance by detecting alleles that were not present in either parent. Markers were further assessed for Mendelian inheritance using CRIMAP [Green, 1990], and the USERM13 [Boehnke, 1991] option of the Mendel package. Unresolved discrepancies were deleted. Approximately 4% of the genotypes that were assessed were deleted either because they were not successfully amplified by the PCR reaction or because of unresolved discrepancies. Marker allele frequencies were estimated from the data set, using maximum likelihood procedures implemented in the USERM13 program. Marker order and distances were calculated using CRIMAP. Three sets of monozygotic twins in the data were used to determine the rate of genotype disagreement after data cleaning. The rate of disagreement was approximately 0.8%.

Statistical Methods

The 105 multigenerational families were divided into 177 nuclear families. As a result, some individuals appeared in one nuclear family as a parent and in another as a sib; however, no sibships were redundant. Both parents were available for genotyping in 61% of these families, and an additional 29% had one available parent. Subjects who reported consuming alcohol but had no symptoms of alcohol abuse or dependence by any diagnostic system were considered unaffected. Subjects who reported one or more symptoms at some time during their lives but who did not meet the full set of diagnostic criteria were designated "unknown," as were individuals who had never used alcohol.

Nonparametric sib-pair methods were used. Two-point linkage analysis was performed with full sibs, using version 2.7.2 of SIBPAL from the SAGE package [SIBPAL:SAGE (Statistical Analysis for Genetic Epidemiology), 1994]. Half-sibs were omitted from the analyses. The proportion of alleles identical-by-descent (IBD) was estimated for each marker for all possible affected sib-pairs, i.e., n (n – 1)/2 affected sib-pairs for n affected sibs, and compared to the null hypothesis of 0.50 using a one-sided t-test [Blackwelder and Elston, 1985]. Evidence for increased allele sharing among unaffected sib-pairs was also evaluated. When parental genotypes were missing, allele frequency estimates were used to produce a weighted estimate of IBD. To simultaneously evaluate the evidence for linkage in affected, unaffected, and discordant sib-pairs, a regression analysis was performed [Haseman and Elston, 1972]. This method regresses the squared trait difference against the estimated proportion of alleles IBD for each sib-pair to detect a negative slope, which suggests linkage. In this method affected individuals are given the score of 1 and unaffected individuals the score of 0; unknowns are omitted.

The SIBPHASE option of the ASPEX program (D. Hines and N. Risch, personal communication) was used to conduct multipoint affected sib-pair linkage analyses. SIBPHASE estimated the proportion of alleles [P(IBD)] from all family data, incorporating estimates based on marker allele frequencies when necessary, if parents were missing or uninformative. Independent sib-pairs, calculated as (n – 1), were used. To reduce potential bias in linkage analyses due to heterogeneous marker allele frequencies in different ethnic groups [Rice et al., 1996], multipoint lod scores for alcohol dependence were computed separately for the subset of families (61%) for which both parents were genotyped. Due to the small number of unaffected sib-pairs, all families, regardless of parental genotype availability,

were included in linkage studies of the unaffected phenotype to maximize the number of observations.

The SIBIBD option of the ASPEX program was also employed, since it uses only unambiguous paternal and maternal IBD scores and it is therefore robust to differences in population allele frequencies. Neighboring loci were also used in the multipoint analyses to increase informativeness. Since this method depends on availability of genotype information in the parents as well as availability, fewer haplotypes can be unambiguously determined when compared with the SIBPHASE method, so it was used to complement other approaches. Independent sib-pairs, computed as (n – 1), were used.

The possibility of ethnic heterogeneity in susceptibility genes for alcohol dependence was tested by reestimating marker allele frequencies in Caucasian families and repeating the multipoint linkage analyses using the SIBPHASE program with only Caucasian families. The 86 Caucasian multigenerational families yielded 141 nuclear families with 779 members. Two-point as well as multipoint linkage analyses were conducted in these families.

RESULTS

The familial distribution of the lifetime prevalence of alcohol dependence, measured by interviewing the first-degree relatives of singly ascertained families, was compared to the randomly ascertained control sample to compute a risk ratio. The lifetime prevalence of alcohol dependence in controls was 17.5% in males and 4% in females. The risk ratio for sibs of alcoholic probands was approximately 2.8 for brothers of male probands and 3.1 for brothers of female probands. The risk ratio for females was 5.3 for sisters of male and 7.8 for sisters of female probands. The median age-at-onset, determined as the age of the cluster of symptoms that define DSM III-R alcohol dependence, was 19 years in males and 21 years in females. These findings are characteristic of family studies of alcohol dependence [Reich et al., 1988]. The relative risks for alcohol dependence in these data are sufficient to allow detection of genes of moderate effect size [Risch, 1990].

The 105 probands in the sample who were used in the initial genomic screen were typical of probands in the entire sample. Twenty-two percent were female, 13% were African-American, and 5% were Hispanic. Comorbidity with other psychiatric diagnoses was common. In addition to alcohol dependence, the lifetime prevalence of other disorders in probands was: substance dependence (excluding marijuana dependence), 49.5%; antisocial personality, 16.2%; and affective disorder (unrelated to alcohol dependence), 21.9%. The number of families that were included in the linkage study was approximately equally distributed across COGA centers, and apart from one site that oversampled female probands (33% of female probands were recruited from one center), there were no prominent center effects on demographic or diagnostic variables.

The two-point nonparametric linkage analyses, carried out by the SIBPAL option of the SAGE statistical package, used 382 affected-affected, 182 affected-unaffected, and 47 unaffected-unaffected sib-pairs. The unaffected-unaffected sib-pairs included only those who had no symptoms of alcohol abuse or dependence by any criterion. Twenty-four loci for which SIBPAL provided evidence for linkage at the $P < 0.01$ level are presented in Table I (detailed results for all loci will be posted on a web page). This includes 9 loci in 8 chromosomal regions for the affected-affected sib-pairs, and 13 loci in 8 regions for the unaffected-unaffected sib-pairs. The multipoint linkage analyses used the SIBPHASE and SIBIBD options of the ASPEX suite of programs (D. Hinds and N. Risch, personal communication). The number of haplotypes that can be unambiguously scored with this method at each locus is variable because of variation in marker heterozygosity (Table II). The number of affected sib-pairs in the analyses that used SIBPHASE was 140. Seven loci with evidence for linkage at the $P < 0.01$ level of significance, using the SIBIBD program, are displayed in Table II.

Evidence in favor of linkage was observed with two-point and multipoint methods on chromosome 1. The adjacent markers D1S532 and D1S1588, which are 20 cM apart, both had significantly increased allele sharing by two-point analysis of affected sib-pairs (P(IBD) = 0.56 and 0.55, respectively; Table I). Linkage analysis using the regression method to include affected, unaffected, and discordant pairs also supported linkage to this region ($P = 0.0003$; Table I). Multipoint analyses of unambiguous haplotypes (SIBIBD) in affected sib-pairs showed even greater allele sharing for these markers (61% and 65%, respectively; Table II). Multipoint linkage analysis limited to those affected sib-pairs with genotypic data on both parents (SIBPHASE) supported a locus near D1S1588 at 168.9 cM, with a peak lod score of 2.93 (Fig.1a). The consistency of these results, using all types of sib-pairs and methods of analysis, supports the finding of elevated haplotype sharing among affected sib-pairs and suggests that a locus contributing to the rise for alcoholism lies in this region of chromosome 1p21–22. A second region on chromosome 1, near D1S224, approximately 60 cM proximal to D1S1588, had a multipoint lod score of 1.65 (Fig. 1a). This locus also had increased sharing with [P(IBD)] of over 60% among affected sib-pairs (Table II). Although clarification of these results awaits replication, it seems likely that the two regions on chromosome 1 are unrelated.

Chromosome 7 also provided evidence of linkage using affected sib-pairs. The marker D7S1793 had increased sharing of alleles among affected sib-pairs with two-point analysis (P(IBD) = 0.54; Table I). The proportion of alleles shared by affected sib-pairs, estimated by multipoint analysis (SIBIBD, SIBPHASE), was 64% (Table II). The maximum multipoint lod score, estimated using nuclear families with genotyped parents, was 3.49, near D7S1793 at 93.9 cM (Fig. 1c).

Only one other chromosomal region gave a peak lod score >1.5, using multipoint linkage analysis methods with affected sib-pairs. A region on chromosome 2 near D2S1790 had a multipoint lod score of 1.81 (Fig. 1b) and P(IBD) of 60% among affected sib-pairs (Table II). There was no evidence of increased allele sharing at

TABLE I. Nominally Significant Loci: Two-Point Linkage Analyses†

Marker	P(IBD)UU	P(IBD)AU	P(IBD)AA	P(reg)
D1S548	0.60 (39)*	0.50 (168)	0.53 (361)	0.0701
D1S1592	0.62 (38)**	0.51 (155)	0.50 (354)	0.4891
D1S1598	0.62 (36)**	0.49 (159)	0.51 (356)	0.0677
D1S532	0.52 (40)	0.45 (131)	0.56 (301)**	0.0003
D1S1588	0.54 (41)	0.45 (146)	0.55 (310)**	0.0003
D2S426	0.58 (45)	0.51 (167)	0.54 (347)*	0.1172
D4S244	0.62 (42)**	0.50 (164)	0.50 (317)	0.3122
D4S2393	0.69 (45)**	0.49 (177)	0.50 (370)	0.1489
ADH3	0.50 (45)	0.46 (172)**	0.51 (361)	0.0032
D4S2457	0.52 (32)	0.45 (165)	0.53 (355)	0.0010
D6S1018	0.55 (39)	0.55 (155)	0.53 (351)*	0.8198
D7S1793	0.47 (41)	0.49 (161)	0.54 (353)*	0.0761
D7S1809	0.60 (28)*	0.55 (131)	0.52 (292)	0.7502
D8S549	0.67 (45)**	0.47 (163)	0.53 (333)	0.0021
AFM333TH1	0.63 (42)**	0.52 (160)	0.51 (349)	0.4609
D8S280	0.64 (41)**	0.50 (164)	0.49 (350)	0.4656
D11S2359	0.49 (40)	0.52 (145)	0.54 (280)**	0.3265
D12S393	0.66 (33)**	0.48 (151)	0.49 (336)	0.2039
D12S1045	0.66 (31)*	0.56 (136)	0.53 (303)	0.6658
D13S321	0.59 (46)**	0.49 (168)	0.53 (354)	0.0252
D13S762	0.61 (42)**	0.49 (170)	0.53 (332)	0.0392
D15S642	0.48 (43)	0.48 (160)	0.55 (319)*	0.0234
D16S675	0.49 (33)	0.50 (140)	0.55 (259)**	0.0893
D19S49	0.59 (43)	0.51 (166)	0.55 (334)**	0.0549

†Twenty-four of the 291 markers on 12 chromosomes, displayed here, gave nominal evidence of linkage ($P < 0.01$) by two-point linkage analysis (SIBPAL). P(IBD) is the estimate of the average proportion of alleles shared IBD over all sib-pairs for the locus. The identity-by-descent [P(IBD)] scores are shown for affected-affected (AA), unaffected-unaffected (UU), and discordant (AU) sib-pairs. The statistical significance of the regression of phenotypic sib-pair differences on IBD, i.e., P(reg), is given to include UU and AU sib-pairs. In these analyses, "unaffecteds" are individuals who drink but have no symptoms of alcohol dependence or abuse by any criterion. Repeating these analyses using only Caucasian sib-pairs (309 AA, 121 AU, and 32 UU) reduced the estimate of IBD for AA sib-pairs at D11S2359 and D6S1018 and the significance of P(reg) at D8S549 to levels that are no longer significant. With the reduced number of UU sib-pairs, D1S548, D1S1598, D7S1809, D8S549, AFM333TH1, D8S280, D13S321, and D13S762 were no longer significant.
*$P < 0.01$.
**$P < 0.005$.

this locus using two-point analysis (Table I). Other chromosomal regions had evidence for increased allele sharing using two-point methods (Table I); however, none of these had a multipoint lod score >1.5.

TABLE II. Nominally Significant Loci in Multipoint Analyses†

	COGA			
	SIBIBD		SIBPHASE	
Markers	N	%Shr	P(IBD)	MLOD
D1S224	150	61*	60.4	1.62
D1S532	163	61*	56.2	0.67
D1S1588	129	65**	64.9	2.91
D2S1790	193	60*	60.2	1.81
D4S2457	230	57*	51.4	0.04
D7S1793	216	64*	64.0	3.29
D8S549	125	62*	55.0	0.46

†Allele sharing and multipoint lod scores calculated by ASPEX for seven loci that gave nominal evidence for linkage ($P < 0.01$) using SIBIBD, which analyzes only unambiguous informative haplotypes. N, number of haplotypes analyzed, with % sharing. SIBPHASE calculates P(IBD), a maximum likelihood estimate of allele sharing for informative sib-pairs, and MLOD, the multipoint lod score from these data. Both analyses used independent sib-pairs, of whom 140 have DNA on both parents. Repeating SIBPHASE analyses using only Caucasian sib-pair, of whom 124 have DNA on both parents, resulted in minor changes in P(IBD). The MLOD of D7S1793 was reduced to 2.89, and of D2S1790 to 1.70. The MLOD of D1S1588 was unchanged.
*$P < 0.01$.
**$P < 0.005$.

Several markers in the region of chromosome 4 near the alcohol dehydrogenase (ADH) gene cluster showed nominally significant two-point results. There was increased allele sharing among unaffected sib-pairs near ADH3, P(IBD) = 0.69 at D4S2393, and reduced allele sharing among discordant affected-unaffected sib-pairs (Table I). Regression analyses showed evidence for linkage at the ADH3 and D4S2457 loci (P(reg) = 0.003, 0.001; Table I). Multipoint analysis of the unaffected phenotype suggested linkage to this region of chromosome 4, with a maximum lod score of 1.72 near D4S2393 at 77.8 cM (Fig. 1d). Since the number of strictly defined unaffected individuals who drank without adverse consequences was low, there were few sib-pairs (N = 30 independent pairs) for analysis. To increase the number of pairs, we broadened the definition of the unaffected phenotype to include individuals who drank and who had up to 8 out of 37 sporadic symptoms but who did not meet the criteria for a diagnosis of alcohol dependence by any system. This definition increased the number of unaffected sib-pairs to 126 and increased the maximum multipoint lod score to 2.50. The peak lod score using the broader definition was near D4S2361 at 86.6 cM. These data suggest a protective factor in this region of chromosome 4.

Although there was evidence for linkage with the unaffected phenotype (0 symptoms) using two-point

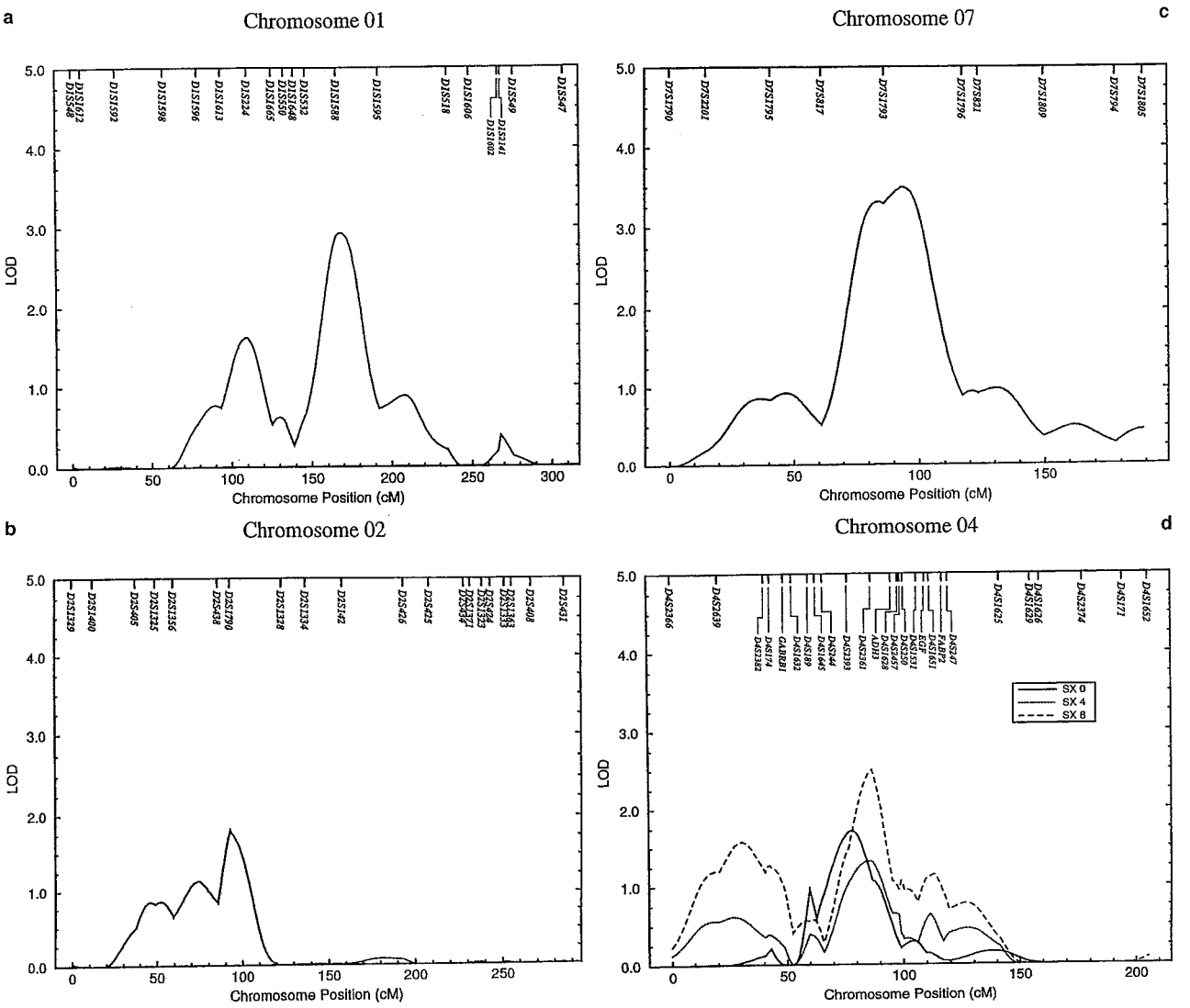

Fig. 1. Maximum likelihood estimates of multipoint scores for linkage with the alcohol dependence and the unaffected phenotypes. Lod scores were calculated and plotted at 1-cM intervals by the SIBPHASE option of ASPEX. The alcohol-dependence analyses (a–c) used data from families in which genotyping has been carried out on both parents. a–c: Analysis of affected sib-pairs. **a:** Chromosome 1. **b:** Chromosome 2. **c:** Chromosome 7. **d:** Chromosome 4 shows multipoint lod scores for linkage with unaffected sib-pairs. In d, all families were used due to the low number of unaffected sib-pairs (30 independent pairs). To increase the number of sib-pairs, analyses were also done with unaffected pairs of individuals with up to four sporadic symptoms (98 pairs) and up to eight sporadic symptoms (126 pairs). These individuals did not meet any criteria for alcohol dependence. Repeating all analyses in affected (n = 124) and unaffected (n = 26) Caucasian sib-pairs reduced some of the maximum lod scores. The maximum lod score on chromosome 7 was reduced to 2.9 and on chromosome 2 to 1.6. There was little change on chromosome 1. The maximum lod score on chromosome 4 for the unaffected group with up to eight sporadic symptoms (n = 92) was reduced to 2.1.

methods on chromosomes 1, 8, 12, 13, and 19 (Table I), multipoint analyses did not support these findings. The relatively small number of unaffected-unaffected sib-pairs may in part be responsible for the scarcity of findings with this phenotype.

DISCUSSION

The strongest evidence for loci linked to alcohol dependence was on chromosomes 1 and 7. The maximum lod score on chromosome 1 was 2.93 near D1S1588, using multipoint analysis (Fig. 1a). Two-point affected sib-pair analysis in this region provided evidence of significant allele sharing, and regression analysis also supported linkage. The consistency of these findings supports the conclusion that a gene in this region is important in the etiology of alcohol dependence. The maximum multipoint lod score on chromosome 7 was 3.49 near D7S1793 (Fig. 1c), the highest lod score in our genomic survey. There was some support (Table I) for this region in the two-point analyses of affected sib-pairs; however, the regression analyses using all three types of sib-pairs added little support for linkage.

There was evidence for linkage on chromosome 4 from two-point analyses of affected sib-pairs, regression analyses, and most strikingly from multipoint analyses of the unaffected phenotype (Tables I and II;

Fig. 1d). The evidence extended over a 38-cM region near D4S2393 and D4S2361 (Fig. 1d), with a maximum lod score of 2.50. Allele sharing of up to 0.69 among unaffected sib-pairs using two-point analysis at D4S2393 added strong support, although the number of unaffected pairs was small (Table I). The evidence is intriguing because it suggests the presence of a locus with protective alleles near the region that includes the human ADH genes, for which a protective role has been found in Asian populations. Alleles at the adjacent loci ADH2 and ADH3 on chromosome 4 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]. These alleles encode isozymes that metabolize ethanol to acetaldehyde at a high rate. This may discourage further alcohol intake in a manner analogous to that caused by the accumulation of acetaldehyde in people with the aldehyde dehydrogenase allele ALDH2*2. The accumulated acetaldehyde causes an aversive flushing reaction, thereby reducing the risk for alcoholism [Edenberg and Bosron, 1997; Crabb et al., 1995].

To increase robustness, we used several methods to test for linkage in addition to the two-point affected sib-pair method. The regression method combines data from affected-affected, affected-unaffected, and unaffected-unaffected sib-pairs and is, therefore, insensitive to a uniform shift in allele sharing among all pairs. The multipoint methods depend on data at consecutive loci, reducing the effect of a genotype error at a single locus. Our findings on chromosomes 1 and 4 were consistent across methods, increasing confidence in these results. The strong result on chromosome 7, however, was mainly supported by multipoint methods.

Lander and Kruglyak [1995] put forth stringent criteria for the significance of genome-wide surveys, under which our findings on chromosomes 1, 4, and 7 are considered suggestive of linkage, rather than definitive. Simulating multipoint linkage analysis of unlinked affected sib-pairs, they proposed criteria for genome-wide significance. As a result of their simulations they noted that a lod score of 1.9 is expected to occur approximately once per genome scan for unlinked loci, and this value was proposed as being "suggestive" of linkage. A lod score of 3.3 with an unlinked genomic region is expected to occur approximately once per 20 genome scans and was proposed to represent "significant" linkage.

The recommendations of Lander and Kruglyak [1995] were based on simulation studies that assumed only one method of analysis was used to analyze a single phenotype. We analyzed several phenotypes with multiple methods, increasing the risk of false-positive results. The nominal P values in this study, therefore, provide a working assessment of the relative strength of the evidence for linkage and suggest areas of interest for high-resolution mapping. Definitive conclusions for any genomic survey require confirmation in another sample.

This report is based on the initial genome scan of the alcohol dependence phenotype, rigorously defined as alcoholic at the definite level by the Feighner criteria and alcohol dependent by DSM III-R criteria. The "unaffected" phenotype was narrowly defined as the absence of any symptoms of alcohol dependence or abuse by any criterion in individuals that consumed alcohol and were "exposed" to the risk for alcohol dependence. The problem of loss of power as a consequence of multiple phenotypes and multiple analyses is a constant feature of linkage analysis of psychiatric phenotypes. We approached this problem in two ways. First, the initial sample was large, the diagnosis was stringent, and an attempt was made to ascertain both parents so that identity-by-descent approaches could be used. Second, we ascertained a large replication sample so that precisely defined linkage hypotheses could be formally tested on new data collected using the same methods.

The deviation from null expectations of IBD scores at a locus can be used to estimate the risk ratio in sibs or offspring due to that locus, assuming a single or multilocus (multiplicative) model of disease transmission [Risch, 1990]. Assuming no recombination between marker and disease loci, we computed the risk ratio due to loci on chromosome 1 and 7 in two ways. First, the null distribution of IBD scores of 0, 1, and 2 alleles was assumed to be 0.25, 0.50, and 0.25, respectively. Under this assumption, the risk ratio for the D1S1588 locus was 1.8 and the risk ratio for the D7S1793 locus was 2.0. We noted that the average observed chromosomal IBD score for all types of sib-pairs irrespective of diagnosis (N = 1,322) was slightly in excess of 0.50, with an average IBD of 0.511 and a range of 0.491–0.532 on individual chromosomes. Using this observed IBD distribution, the risk ratio for the D1S1588 locus was reduced to 1.5, and that for the D7S1793 locus was 1.6. Assuming a multiplicative oligogenic model, these relative risks represent the fraction of the total sib risk ratio for alcohol dependence (from 3 to 8 in the COGA study) due to activity at each of these single loci. Similar gene effects for individual loci have been reported for other common familial disorders such as asthma [Collaborative Study on the Genetics of Asthma, 1997].

Our genome survey included markers in or near several candidate genes. No evidence for linkage with the dopamine D2 receptor gene (DRD2) was found: two-point affected sib-pair analyses were not significant (P(IBD) = 0.52). More detailed analyses, including family-based association tests, were also negative [Edenberg et al., 1998]. Markers within several clusters of GABA receptor genes were analyzed as part of this survey. The GABRB1 marker, clustered with GABRA2, GABRA4, and GABRB2 on 4p13–p12 [Dean et al., 1991], is near the region on chromosome 4 with evidence for a protective effect; there is modest allele sharing at this locus when all types of sib-pairs are considered (P(reg) < 0.05). The region of chromosome 5 (5q34–35) containing the GABRA1, GABRA6, and GABRB2 loci [Russek and Farb, 1994] did not show increased allele sharing. The GABRB3–GABRA5 cluster on 15q11–q13 [Sinnett et al., 1993] showed a modest increase (P(IBD) = 0.53, $P < 0.05$). No evidence for linkage was found on the X chromosome in the region of the monoamine oxidase (MAO) loci, i.e., P(IBD) = 0.54, $P = 0.35$, at MAOB [Lan et al., 1989].

This is the first genome-wide survey of linkage with alcohol dependence in a largely Caucasian population. Long et al. [1998] studied a Southwest Amerindian population and presented evidence for linkage with alcohol dependence on chromosomes 4 and 11. Our data provided no evidence for linkage on chromosome 11, but the region of interest we identified on chromosome 4 with the unaffected phenotype overlaps the region they delineated (Fig. 1d). An alcohol preference locus on mouse chromosome 3 has been mapped to a region that is homologous with the region on human chromosome 4 close to the ADH gene cluster (Fig. 1d), adding further interest to the findings on this chromosome [Phillips et al., 1994; Markel et al., 1996].

These results provide the rationale for high-resolution mapping of the regions of interest we have identified on chromosomes 1, 2, 4, and 7. We have assembled a large replication sample to confirm and extend these findings. The replication sample was ascertained in the same manner as the sample in this report but is larger (157 families with 1,313 informative members), which should increase the likelihood of replicating linkage [Suarez et al., 1994]. We are also conducting a 5-year prospective follow-up interview study of the families that we genotyped. The juvenile members of these families are at high risk for alcohol dependence and provide an excellent opportunity to directly measure the effect of susceptibility loci. Gene-environment hypotheses will also be testable in the prospectively studied cohorts.

ACKNOWLEDGMENTS

The Collaborative Study on the Genetics of Alcoholism (COGA) (H. Begleiter, State University of New York, Health Sciences Center at Brooklyn, Principal Investigator, and T. Reich, Washington University in St. Louis, Co-Principal Investigator) includes six different centers where data collection takes place. The six sites and Principal Investigators and Co-Investigators are: Indiana University (J. Nurnberger, Jr., T.K. Li, P.M. Conneally, and H.J. Edenberg); University of Iowa (R. Crowe and S. Kuperman); University of California at San Diego and Scripps Institute (M. Schuckit and F. Bloom); University of Connecticut (V. Hesselbrock); State University of New York, Health Sciences Center at Brooklyn (H. Begleiter, B. Porjesz); and Washington University in St. Louis (T. Reich, C.R. Cloninger, A. Goate, and J. Rice). Some of the results of this paper were obtained by using the program package S.A.G.E., which is supported by a U.S. Public Health Service Resource Grant (1 P41 RR03655) from the Division of Research Resources.

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