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Linkage of an Alcoholism-Related Severity Phenotype to Chromosome 16

Tatiana Foroud, Kathleen K. Bucholz, Howard J. Edenberg, Alison Goate, Rosalind J. Neuman, Bernice Porjesz, Daniel L. Koller, John Rice, Theodore Reich, Laura J. Bierut, C. Robert Cloninger, John I. Nurnberger, Jr., T.-K. Li, P. Michael Conneally, Jay A. Tischfield, Raymond Crowe, Victor Hesselbrock, Marc Schuckit, and Henri Begleiter

There is substantial evidence for a significant genetic component to the risk for alcoholism. In searching for genes that contribute to this risk, the diagnostic criteria for alcohol dependence may not be the optimal phenotype; rather, creation of a more homogeneous phenotype will lead to a more homogeneous genetic etiology. Items from the Semi-Structured Assessment for the Genetics of Alcoholism collected from 830 individuals in 105 alcoholic families were used in a latent class analysis to identify a more homogeneous alcoholism-related phenotype. A four-class solution was chosen: class 1, unaffected group; class 2, mildly problematic group; class 3, moderately affected group; and class 4, severely affected group. Classes 3 and 4 had higher symptom endorsement probabilities than classes 1 and 2 for items reflecting severe alcohol dependence, and were combined to provide enough sibling pairs for genetic linkage analysis. A total of 291 markers distributed throughout the genome, with an average intermarker distance of 14 cM, were genotyped. Linkage analysis was performed to detect loci underlying classes 3 and 4, the moderately and severely affected alcoholics, of whom 88% met the Collaborative Study of the Genetics of Alcoholism, and >99% met ICD-10 criteria for alcohol dependence. Evidence for a locus on chromosome 16, near the marker D16S675, was found with a maximum multipoint lod score of 4.0. Analysis of additional markers on chromosome 16 yielded a lod score of 3.2, narrowed the critical region, and placed the gene between D16S475 and D16S675 in a 15 cM interval.

Key Words: Alcoholism, Linkage, Latent Class Analysis, Chromosome 16.

ALCOHOL DEPENDENCE is a common, familial disorder that is a leading cause of morbidity and premature death.[1–3] Family studies have documented a 3- to 5-fold increased risk for alcoholism among siblings and other first-degree relatives of affected individuals.[4] The risk for alcoholism is increased even if the offspring of an alcoholic is adopted away from the home.[5,6] Twin studies have identified a significant genetic component to alcoholism risk, with estimates of heritability ranging from 50 to 60%.[7]

Evidence from twin, adoption, and family studies suggests that, rather than being a disorder due to a single gene, alcoholism is more likely a complex genetic disorder resulting from the action of multiple, possibly interacting, genes. Efforts to identify the genetic loci underlying alcoholism susceptibility have primarily relied on the evaluation of candidate genes. Linkage was reported to the MNS blood group[8] and esterase D,[9] but neither finding has been replicated in subsequent studies.[10,11] The observation of low monoamine oxidase (MAO) activity in alcoholics has not led to significant linkage findings with the *MAO* genes.[12,13] The dopamine D2 receptor (*DRD2*) gene on chromosome 11, considered a candidate for involvement in alcoholism, as well as the personality trait of novelty seeking and central nervous system reward,[14,15] has been studied extensively by a number of research groups after the report of an association between the *Taq*I-A1 polymorphism in the *DRD2* gene and alcoholism,[16] with some positive,[17–22] but many negative results.[23–36] Most recently, a family-based association study from a large multisite family study of alcoholism [Collaborative Study of the Genetics of Alcoholism (COGA)] provided no evidence for linkage or association with the *DRD2* gene.[37] The only consistently replicated findings are those involving the protective effects of certain functional polymorphisms of the alcohol-metabolizing enzymes alcohol dehydrogenase and aldehyde dehydrogenase in Asian populations.[38–44]

One of the difficulties in working with a complex phenotype, such as alcohol dependence, is its definition. Several diagnostic systems, including DSM-III-R,[45] DSM-IV,[46] Feighner criteria,[47] and ICD-10,[48] have been used. Differences in the diagnostic criteria result in overlapping, but not identical, disease assignment.[49] As a result, it is possible that linkage analysis using different disease criteria may result in the identification of some similar and some unique genetic loci.

Recognizing this difficulty, the assessment protocol used

From the Indiana University School of Medicine (T.F., H.J.E., D.L.K., J.I.N., T.-K.L., P.M.C., J.A.T.), Indianapolis, Indiana; Washington University School of Medicine (K.K.B., A.G., R.J.N., J.R., T.R., L.J.B., C.R.C.), St. Louis, Missouri; SUNY Health Science Center at Brooklyn (B.P., H.B.), Brooklyn, New York; University of Iowa School of Medicine (R.C.), Iowa City, Iowa; University of Connecticut School of Medicine (V.H.), Farmington, Connecticut; and University of California–San Diego School of Medicine (M.S.), La Jolla, California.

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Reprint requests: Tatiana Foroud, Ph.D., Department of Medical and Molecular Genetics, Indiana University School of Medicine, 975 West Walnut Street IB-155, Indianapolis, IN 46202.

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in the multicenter COGA study is multidimensional and permits classification according to several diagnostic systems. Previous genome-wide genetic analyses of individuals considered alcoholic by both DSM-III-R and Feighner criteria found suggestive lod scores on chromosomes 1, 2, and 7.[50] A maximum lod score of 2.93 was observed near the marker D1S1588 with a secondary peak near D1S224 having a lod score of 1.65. On chromosome 2, near the marker D2S1790, a multipoint lod score of 1.81 was identified. The highest lod score for the alcoholism phenotype (lod = 3.49) was observed on chromosome 7 near the marker D7S1793.

Because the ultimate goal of the COGA study is to use linkage analysis to localize genes contributing to alcohol susceptibility, defining phenotypes or subtypes of alcohol dependence that are likely to be genetically homogeneous with regard to the underlying etiology of alcoholism is critical. In this article, we present results from an application of latent class analysis to alcoholism-related symptom data to identify homogeneous classes of alcohol-dependent individuals. Comparison of results from the linkage analyses based on the latent class phenotype with the more conventional alcohol dependence phenotype based on formal diagnostic criteria (e.g., DSM-III-R or ICD-10) may provide additional insights into the genetics of alcoholism.

METHODS

Sample and Assessment

COGA is a six-site study conducted at centers in Farmington, CT, Brooklyn, NY, Indianapolis, IN, St. Louis, MO, Iowa City, IA, and San Diego, CA. Individuals were systematically recruited from both inpatient and outpatient units if they met the following ascertainment criteria: (1) fulfilled requirements for both lifetime DSM-III-R[45] alcohol dependence and Feighner[47] definite alcoholism; (2) had two first-degree relatives living within 100 miles of a COGA center; (3) were free of nonalcohol-related, life-threatening illnesses; and (4) neither injected illicit substances within 6 months of admission nor reported >30 injections in their lifetime.

Adult lifetime psychiatric status was assessed by direct interview with the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA[51,52]). The SSAGA is a comprehensive, reliable interview that elicits lifetime and current information for axis I psychiatric disorders, including alcohol dependence and abuse, dependence and abuse for five drug classes, major depression, dysthymia, mania, as well as antisocial personality disorder and anxiety disorders. Symptoms reflecting criteria from multiple diagnostic systems for alcohol dependence—including DSM-III-R, Feighner, DSM-IV, and ICD-10—are covered by the SSAGA.

Probands were considered affected if they met criteria for lifetime DSM-III-R alcohol dependence and Feighner definite alcoholism (COGA alcoholism definition) by subject report at interview. Families were eligible for further intensive study, including biological and neurophysiological assessments, if two first-degree relatives, in addition to the proband, met the criteria for COGA alcoholism. In cases where family members were unavailable for interview, a reliable, structured Family History Assessment Module,[53] which asked each respondent about all other relatives, both interviewed and noninterviewed, was used. In contrast to other family history instruments, the Family History Assessment Module contains structured questions that directly map onto diagnostic criteria for alcoholism based on DSM-III-R and Feighner. Individuals are considered to have an "implicated diagnosis" of alcoholism by virtue of an informant report of three DSM-III-R symptoms and three Feighner symptoms. Three such "implicated diagnoses" were required to classify a study subject as affected by family history report, thus allowing noninterviewed individuals to be assigned a diagnosis.

Pedigrees were extended through either affected first-degree relatives or by extension over an unaffected relative into a secondary branch of the pedigree if at least two of the relatives in the new branch were affected by family history report, as described herein. All available members of the secondarily ascertained nuclear families were sought for study. Bilineal branches where both parents were defined as alcohol-dependent by either interview or family history report were not included. Families in which recruitment of all eligible members was completed, and blood samples obtained, were reviewed by an internal COGA committee composed of the Principal Investigators and other key investigators from all six sites. The genetically most informative families, which typically included multiple affected sibling pairs, were selected for genotyping. The sample used in the latent class linkage study came from 105 families, and included 830 individuals with latent class assignment and 987 individuals with genotypic data. On average, 9.5 individuals were genotyped in each family for this study.

Latent Class Analysis

Latent class analysis is a statistical methodology based on the assumption that the association among a set of observed variables can be explained by the existence of a discrete latent (unobserved) variable with multiple levels or classes. A critical implication of this assumption is that within each class, the observed variables are statistically independent (i.e., the observed relationships among the variables are conditional upon a third, unobserved or latent variable, such that once this variable is taken into account, the other measures are unrelated). This is the assumption of local independence. Latent class analysis may be understood as a categorical form of factor analysis applied to discrete variables, because in factor analysis the latent variable is assumed to be continuously distributed.[54] The latent class parameters that are estimated are: (1) the class membership probabilities, which are estimates of the prevalence of each class; and (2) symptom endorsement probabilities (SEPs). SEPs reflect the probability that an individual will have a given response to an item, conditional on being a member of that particular class. In the case of psychiatric data, where symptoms are typically dichotomous, these parameters reflect the probability that a symptom will be endorsed by individuals within that class. Classes are thus characterized by their estimated prevalence and their SEPs. Several statistics are available to evaluate whether a latent class solution explains an observed association.[54] An m-class solution can be compared with an $(m + 1)$ class solution by a likelihood ratio χ^2 test with degrees of freedom equal to $p + 1$, where p is the number of symptom variables.

We applied latent class analysis to the alcohol symptom variables listed in Appendix A. The analyses used a program written by one of us (R.J.N.) in which maximum likelihood methods via the EM algorithm[55] were used to fit the data to 2-class through 5-class solutions. In the analyses reported herein, individuals were assigned to class membership based on the "most likely" class for their symptom profile (i.e. the class for which their probability of membership was greatest, conditional upon their symptom response profile). Although it has been observed[56] that statistics computed using this approach will not be unbiased, because they ignore the fact that conditional probabilities for membership in other classes are >0, our experience working with the very large sample sizes available with the COGA data has indicated such problems are minimal.

Latent Class Phenotype

In an earlier series of latent class analyses using the full COGA dataset designed to investigate whether subtypes of alcoholism could be found, Bucholz et al.[57] reported that, whereas alcoholism appeared to be distributed on a severity spectrum overall, there was only one class that was distinguished by the presence of the alcohol withdrawal syndrome. In the DSM-IV classification system, the presence of withdrawal or tolerance is sufficient for the specifier of "physiological dependence." Bucholz et al. observed evidence to support the inclusion of withdrawal in that specifier,

Table 1. Results of Latent Class Model Fitting

Model	Log-likelihood (−2ln L)	Likelihood ratio χ^2 test (df = 14)	p-value
2 class	7998.05	—	—
3 class	7578.61	419.44	<0.001
4 class	7510.64	67.97	<0.001
5 class	7470.19	40.45	<0.001

but not tolerance. For both men and women, only one class was marked by very high (>0.60) SEP for withdrawal. In contrast, SEPs for tolerance were quite high for 3 of 4 classes for men and 2 of 4 for women. The authors interpreted this as suggesting that withdrawal denoted a distinct subtype of alcohol dependence. Elaborating on this finding, Schuckit et al.,[58] in further analyses of the full COGA dataset, divided individuals who met DSM-III-R criteria for alcohol dependence into two groups based on the presence or absence of withdrawal and tolerance. The group with tolerance or withdrawal met significantly more lifetime DSM-III-R alcohol dependence criteria, and had a larger maximum number of drinks on a single occasion, more binge drinking, and more alcohol-related emotional problems. The authors concluded that tolerance and withdrawal, but primarily the latter, meaningfully distinguished a clinically more severe group of alcohol-dependent individuals.

Analyses reported herein build on the observations from these two studies. A series of latent class analyses using items identified from the interview data thought to reflect more severe alcohol symptoms—including components of physiological dependence (see Appendix A for a listing of these items) and nondiagnostic items, such as maximum alcohol consumed—were included. Whenever available, the number of times a behavior had occurred was used in the initial analysis and recoded into six categories that were determined based on the distribution of the number of occurrences reported for each item. Results from these initial analyses identified items that differentiated groups of alcohol-dependent individuals, as judged by the estimates of SEPs. Items that did not differentiate classes, as indicated by nearly equal SEPs, were discarded. Most of the items eliminated at this step reflected adverse social consequences of drinking, which are no longer considered part of the diagnosis of alcohol dependence in DSM-IV. Eleven items were retained for a second latent class analysis. These were: persistent desire/being unable to quit drinking, a three-level variable, with individuals coded as 0, 1 (for either behavior), and 2 if they had both; morning drinking; craving; one or more episodes of binge drinking, with a binge defined as drinking for 2 or more days without sobering up; spending a great deal of time drinking or recovering from the effects of drinking, a three-level variable with individuals coded as 0, 1 if they had the behavior, and 2 if the behavior lasted for 1 month or more; narrowing of the drinking repertoire; giving up activities in order to drink; 12 or more blackouts; five or more co-occurring withdrawal symptoms; any health problems due to drinking; and any psychological problems due to drinking.

Using the 11 items, 2-class through 5-class latent class solutions were computed. Although the 5-class solution did theoretically improve the model fit over the 4-class solution, the interpretation of the classes in the two solutions was the same except the 5-class solution yielded a small class of binge drinkers. We selected the 4-class solution because it was our assessment that the more complex 5-class solution did not contribute substantially to the interpretability of the classes. Likelihood-ratio statistics are presented in Table 1 for all solutions, and a graph of the SEPs by class is displayed in Fig. 1. The binary items are graphed as 1 point, which reflects the probability of endorsing the behavior. For the two items that are trichotomous, 2 points are graphed: the probability of being at level 1, and at level 2. For example, for the behavior "great deal of time spent drinking," the points reflect those who had the behavior for <1 month (level 1) and those who had the behavior that lasted 1 month or more.

The classes may be characterized as follows: an unaffected group (class 1) with very low SEPs for most items and that contained 47% of the individuals (n = 419); a mildly problematic group, with most SEPs under 0.2 (class 2) and that contained 23% of the sample (n = 172); a moderately affected group, with most SEPs between 0.2 and 0.6 (class 3) and included 17% of the individuals (n = 137); and a severely affected group, with 13% of the sample (n = 104), whose SEPs exceeding 0.6 and in many cases were >0.8 (class 4). Although there are differences in the severity between classes 3 and 4, individuals in both classes had a greater number of symptoms and higher SEPs for items reflecting severe alcohol dependence, such as withdrawal, craving, spending a lot of time drinking, narrowing of the repertoire, giving up activities to drink, and having psychological problems from drinking, compared with individuals in class 2.

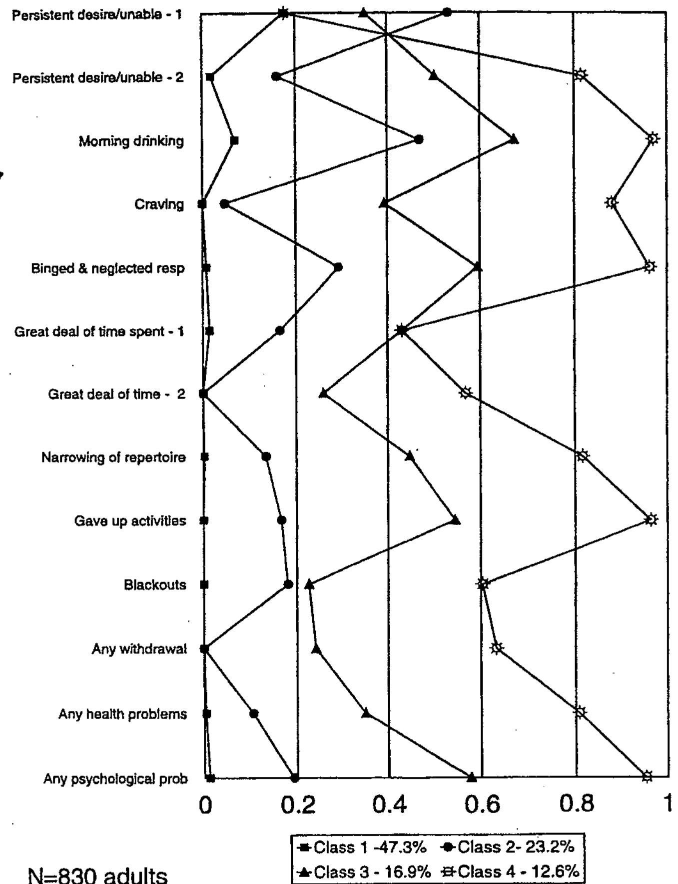

Fig. 1. SEPs by class membership. Four-class SSAGA linkage sample only; resp, responsibility; prob, problem.

The majority of individuals in class 3 (83%) and class 4 (93%) fulfilled the ICD-10 criteria for alcohol dependence (Fig. 2). A COGA definition of alcoholism had been developed for this study, with alcohol-dependent individuals defined as those who met both the DSM-III-R criteria for alcohol dependence and the Feighner criteria for alcoholism at the definite level. All but one individual in class 3 and 100% of those in class 4 fulfilled the COGA definition of alcoholism (Fig. 2). Over half (54%) of the individuals with a COGA diagnosis of alcohol dependence were in latent classes 3 or 4 and 77% of individuals with ICD-10 alcohol dependence were in classes 3 or 4. In addition, rates of ICD-10 and COGA defined alcohol dependence were substantially higher in these two classes, compared with class 2 (post-hoc test significance $p < 0.001$). Therefore, based on post-hoc comparison of the classes, an exploratory analysis was performed combining classes 3 and 4 to define an affected group of individuals with more severe dependence. There are 241 individuals in classes 3 or 4, among which there are 101 affected sibling pairs for analysis.

Genotyping and Error Detection

A total of 291 markers, with an average intermarker distance of 13.8 cM, were used in a genome screen. Most markers were tri- or tetranucle-

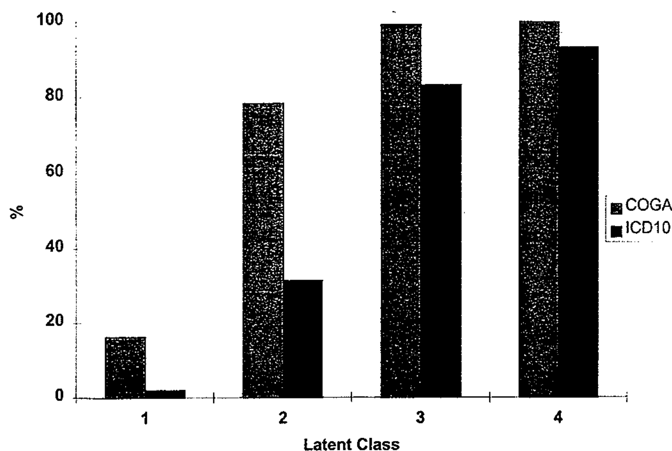

Fig. 2. Percentage meeting criteria for the COGA definition of alcoholism (both DSM-III-R criteria for alcohol dependence and Feighner criteria for alcoholism at the definite level) and ICD-10. n for classes 1 to 4: 417, 172, 137, and 104.

otide repeat polymorphisms. These markers were highly informative, with an average heterozygosity of 0.72. Markers were developed by the Cooperative Human Linkage Center, Genethon, Marshfield Clinic, M.I.T., and the University of Utah. After the genome screen, additional markers were genotyped on chromosome 16 to further delineate the critical region of linkage.

Genotyping was completed in two laboratories (H.J.E. and A.G.) using radioactive and fluorescence-based detection systems. Labeled markers were amplified by the polymerase chain reaction (PCR) using a 96-well format and standard conditions. For fluorescence-labeled markers, PCR reactions for each marker were performed separately and products combined into single chromosome sets before gel electrophoresis. Data were collected using the 373A automated DNA sequencer (ABI) and genotyped using the Genscan 672 and Genotyper software (ABI). For radioactively labeled markers, 3 μl aliquots of the denatured PCR products were electrophoresed on 6% denaturing polyacrylamide gels (Gel Mix-6, GIBCO/BRL). Gels were exposed to Kodak XAR-5 film for 6 to 72 hr.

Genotypic data were stored in the GeneMaster database management system and checked for Mendelian inheritance of marker alleles with the programs CRIMAP[59] and the USERM13 option of the MENDEL suite of linkage programs.[60] Maximum-likelihood estimates of marker allele frequencies were obtained using the USERM13 program. Marker order and distance were estimated from these data using CRIMAP and then used in all subsequent analyses.

Linkage Analysis

We defined post-hoc the individuals in latent class 3 or 4 to be affected in these analyses, so as to focus on a more homogeneous phenotype related to severe alcohol dependence. Genotypic data from individuals in classes 1 and 2 were used to maximize the marker information and improve the power of the linkage analyses to identify genetic loci; however, the latent class assignment for individuals in classes 1 and 2 was not used. Therefore, all statistical tests were confined to the identification of genes underlying latent classes 3 and 4.

We have chosen to employ nonparametric methods of linkage analysis, which do not rely on the specification of a model of susceptibility for the latent class phenotype. Instead, all statistical tests are based on the sharing of marker alleles identical by descent. An allele is considered to be identical by descent if both members of a sibling pair have inherited the marker allele from the same parent. If the marker being tested is physically close to a gene influencing alcohol dependence, then alcoholic siblings would be more likely to share the chromosomal region near the susceptibility locus than a random chromosomal region and, consequently, would be more likely to share alleles at markers in this region. Nonparametric linkage methods test whether the observed marker allele sharing for affected pairs is >50%, the expected proportion of alleles shared identical by descent when there is no linkage. Significantly increased marker allele sharing would support linkage of a susceptibility locus to the marker being tested.

The 105 multigenerational pedigrees were divided into 177 nuclear families for the purposes of linkage analysis. An individual could appear as a parent in one nuclear family and as a sibling in another; however, no individual appeared in the same role in more than one nuclear family and no sibship was redundant. The program SIBPAL, version 2.7.2, part of the S.A.G.E. (Statistical Analysis for Genetic Epidemiology) suite of programs was used for all 2-point linkage analyses, which evaluated each marker individually for evidence of linkage to latent classes 3 and 4. In addition, the program ASPEX[61] was used to conduct multipoint linkage analysis. This program evaluates multiple markers simultaneously for evidence of linkage and, as a result, is a more powerful tool for linkage detection. To utilize all the information in the dataset, the ASPEX subroutine SibPhase was used for multipoint analyses. When sibships with >2 affected individuals are encountered, it is possible that the test statistic may be biased by the formation of large numbers of sibling pairs from these large families. The program SIBPAL analyzes all possible pairs of siblings $[n(n-1)/2]$ as though they were independent, whereas SibPhase was run with the option to contribute only $n-1$ sibling pairs to the analysis.

RESULTS

Two-point sibling pair analysis using the program SIBPAL identified 18 markers with nominal evidence in favor of linkage at the $p < 0.05$ level. These markers were located on 13 chromosomes. Of these 18 markers, 7 were significant at the $p < 0.01$ level, and 4 were significant at the $p < 0.001$ level (Table 2). Three markers on chromosomes 16 (D16S475, D16S2622, and D16S675) are within a 15 cM region, and all were below the $p < 0.01$ significance level. The estimated sharing of marker alleles for these markers D16S475, D16S2622 and D16S675 was 62%, 56% and 66%, respectively.

Multipoint linkage analysis, using the program ASPEX, provided significant evidence of linkage only to a group of markers on chromosome 16. A maximum lod score of 4.0 was obtained near the marker D16S675 (Fig. 3). Four markers spanning a region of ~15 cM on chromosome 16 had lod scores >3.0. The estimated marker allele sharing in this region exceeded 65%. No other chromosomal region had multipoint lod scores >2.0.

To further evaluate the chromosome 16 linkage finding, six additional markers were genotyped on chromosome 16 (Fig. 4). Five markers were between D16S2618 and D16S298, and the sixth was the most telomeric 16p marker. Two-point analysis was nominally significant with the marker D16S768 ($p = 0.03$) (Table 2) and suggestive with D16S2616 and D16S687 ($p \leq 0.10$). Allele-sharing with these markers exceeded 54%. Multipoint analysis of the chromosome 16 data with the additional markers continued to support linkage with a maximum lod score of 3.2 at the marker D16S475 and a secondary maximum of 3.0 between D16S2618 and D16S2616 (Fig. 4). Estimated marker allele-sharing in this region exceeded 65%.

Table 2. Nominally Significant 2-Point Linkage Results (SIBPAL) Using the COGA Alcoholism or Latent Class Phenotypes

Chromosome	Location	Marker	Latent class *p*-value*	COGA *p*-value†
1	78.6	D1S1596	0.05	0.09
1	146.3	D1S532	0.0006	0.0002
1	167.4	D1S1588	0.17	0.002
1	176.3	D1S534	0.0006	0.0002
2	193.3	D2S426	0.41	0.01
2	220.8	D2S408	0.02	0.31
4	65.5	D4S2457	0.03	0.02
6	61.8	D6S1018	0.49	0.01
6	76.9	D6S493	0.05	0.08
6	85.6	GCT16B	0.05	0.46
6	179.4	D6S1009	0.05	0.13
7	0.0	D7S1790	0.01	0.09
7	86.2	D7S1793	0.02	0.01
8	13.8	D8S1109	0.01	0.11
8	17.1	D8S1106	0.02	0.13
8	31.2	D8S549	0.03	0.03
8	144.8	D8S1119	0.03	0.54
10	42.6	D10S1426	0.01	0.08
10	111.7	D10S610	0.01	0.30
11	147.3	D11S901	0.01	0.04
14	95.7	D14S302	0.01	0.08
15	65.5	D15S644	0.04	0.12
15	130.6	D15S642	0.49	0.01
16	0.0	D16S475	0.0002	0.01
16	1.5	D16S2622	0.01	0.01
16	14.4	D16S675	0.0000	0.01
16‡	28.3	D16S768	0.03	0.32
16	117.6	D16S539	0.02	0.27
17	63.6	D17S250	0.05	0.13
18	90.6	D18S541	0.04	0.02
19	38.4	D19S49	0.49	0.003

* *p*-value obtained for the test of linkage using the latent class phenotype.
† *p*-value obtained for the test of linkage using the COGA phenotype.
‡ Flanking marker not included in the initial genome screen.

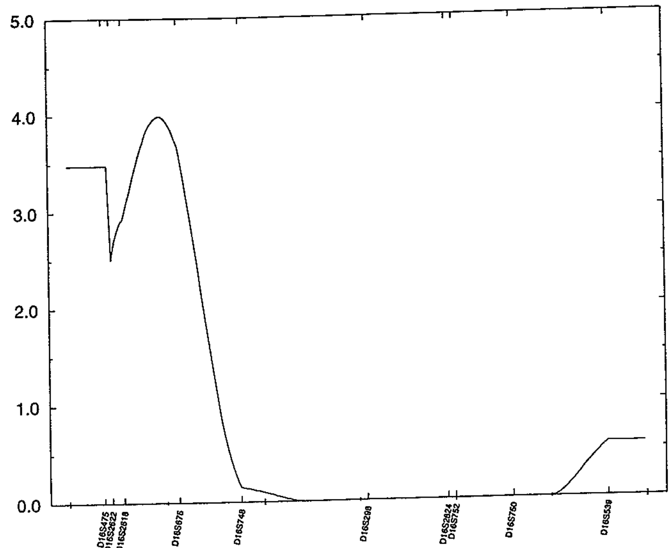

Fig. 3. Initial multipoint linkage analysis of chromosome 16 using the screening set of marker. Marker distances were estimated from the COGA dataset.

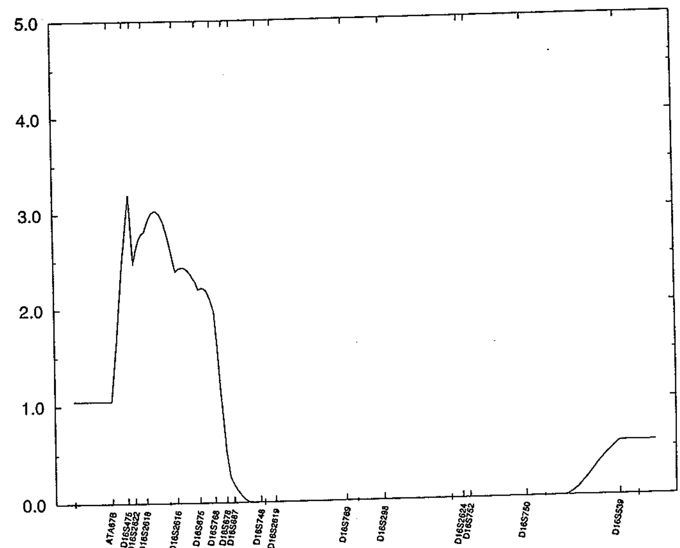

Fig. 4. Multipoint linkage analysis following the genotyping of 6 additional chromosome 16 markers. Marker distances were estimated from the COGA dataset.

DISCUSSION

Failure to consistently replicate linkage or association findings for alcoholism may be due to the potentially large number of genes, each with small individual effects, that underlie alcoholism susceptibility. Such genes would require a very large sample of families to be detected, and a larger sample for confirmation. The COGA collaboration represents the largest family sample collected for the purpose of identifying genes underlying alcohol dependence. The extensive interview administered to each participant not only made it possible to perform linkage analyses of the core alcoholism diagnostic phenotype, but also allowed for refinement of a phenotype using a smaller set of symptoms in a latent class analysis. The goal of this phenotypic narrowing is to provide a more homogeneous phenotype underlying particular aspects of alcoholism that are influenced by a smaller number of genes.

Our genome screen, using this latent class phenotype, identified only one chromosomal region that provided significant evidence for linkage using both 2-point and multipoint-affected sibling pair methods. Three markers on chromosome 16p were among the most significant using 2-point methods ($p < 0.01$). Multipoint methods provided even greater evidence for linkage with a maximum lod score of 4.0. This multipoint result meets the stringent criteria for linkage (lod > 3.6) proposed by Lander et al.[62] The multipoint lod score dropped somewhat (to 3.2), but this might, in part, reflect variable marker heterozygosity and some ambiguity in marker order and distance. Nevertheless, evidence for linkage remained in the 15 cM interval between D16S475 and D16S2616, with estimated allele-sharing exceeding 65%.

Reich et al.[50] obtained a suggestive 2-point result ($p < 0.01$) at marker D16S675 using the broad COGA definition of alcoholism; however, the multipoint lod score in this region of chromosome 16 was <1.0.[50] Evidence for linkage with the chromosome 16p markers has also been found in the COGA sample using the ICD-10 diagnoses for alcoholism.[63]

Most other significant 2-point linkage results were primarily with single markers. Only one other region had

significant 2-point results with adjacent markers. However, these markers on chromosome 8 were only nominally significant ($0.01 < p < 0.05$). Multipoint methods in this region were not significant, with the maximum lod score only 1.2. This region had also previously provided suggestive evidence for linkage (lod ~ 2.0) using the ICD-10 definition of alcoholism.[63]

The consistent linkage findings on chromosome 16 using the latent class phenotype and the two definitions of alcoholism used in the COGA sample may be attributed to the overlap in the three definitions. Nearly all individuals with the ICD-10 diagnosis of alcoholism also fulfilled the criteria for the COGA definition. However, all but one individual in latent classes 3 and 4 had COGA-defined alcoholism, and <50% of the individuals who met COGA criteria for alcoholism were in latent class 3 or 4. In addition, not all individuals in classes 3 and 4 met ICD-10 criteria for alcoholism. In comparing the affected sibling pairs in the various analyses, there was substantial overlap among the samples. All 101 sibling pairs used in these analyses were also included in the 382 affected sibling pairs used in the COGA-defined alcoholism linkage study. Therefore, the sample used in this genome screen is a distinct sample from that used in the alcoholism studies, because it is not solely defined in terms of conventional diagnostic definitions of alcohol dependence. The development of the latent class phenotype required detailed data from the SSAGA, which have not been collected in other alcoholism linkage studies. As a result, it is critical that this phenotype be analyzed in the replication sample that has been constructed by COGA.

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APPENDIX A: ITEMS USED IN LATENT CLASS ANALYSIS

Item No.	Description
1	Total no. of drinks in the last week
2	Total no. of drinks in a typical drinking week in the last 6 months
3	Maximum no. of drinks in a 24-hr period, lifetime
4	Largest no. of drinks consumed every day for at least 1 week
5	Largest period of abstention (in months)
6*	Persistent desire/being unable to stop drinking (coded 2 if both endorsed)
7*	Morning drinking
8*	Craving
9*	Total no. of binge drinking episodes
10	Drank longer/more than expected (3 or more times, coded 2)
11	Became drunk when didn't want to be (3+ times coded as 2)
12*	Spent so much time drinking that had little time for anything else (coded 2 if it lasted 1 month or longer)
13*	Narrowing of drinking repertoire
14	Drank nonbeverage alcohol
15	Tolerance (50% increase to get an effect or intoxicated)
16	Made rules to control drinking
17*	Gave up activities in order to drink
18	Drinking interfered with carrying out of responsibilities
19	Drinking caused marital problems (coded 2 if R continued to drink)
20	No. of DWI's or driving accidents when drinking
21	No. of drinking-related arrests
22	No. of nondriving accidents when drinking
23	No. of times used alcohol in situations when could have hurt self
24*	No. of blackouts (12 or more)
25*	No. of co-occurring withdrawal symptoms (5 or more)
26	No. of episodes of withdrawal
27	No. of alcohol-related seizures
28	No. of times experienced DT's
29*	Any health problems caused by drinking
30	Continued to drink in the presence of a health condition that alcohol exacerbated
31	Drank when using medication's that were hazardous with alcohol
32*	Any psychological problems caused by drinking

* Selected for final analysis.

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