

# Alcoholism Susceptibility Loci: Confirmation Studies in a Replicate Sample and Further Mapping

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**Background:** There is substantial evidence for a significant genetic component to the risk for alcoholism. A previous study reported linkage to chromosomes 1, 2, and 7 in a large data set that consisted of 105 families, each with at least three alcoholic members.

**Methods:** Additional genotyping in the 105 families has been completed in the chromosomal regions identified in the initial analyses, and a replication sample of 157 alcoholic families ascertained under identical criteria has been genotyped. Two hierarchical definitions of alcoholism were employed in the linkage analyses: (1) Individuals who met both Feighner and DSM-III-R criteria for alcohol dependence represented a broad definition of disease; and (2) individuals who met ICD-10 criteria for alcoholism were considered affected under a more severe definition of disease.

**Results:** Genetic analyses of affected sibling pairs supported linkage to chromosome 1 (LOD = 1.6) in the replication data set as well as in a combined analysis of the two samples (LOD = 2.6). Evidence of linkage to chromosome 7 increased in the combined data (LOD = 2.9). The LOD score on chromosome 2 in the initial data set increased after genotyping of additional markers; however, combined analyses of the two data sets resulted in overall lower LOD scores (LOD = 1.8) on chromosome 2. A new finding of linkage to chromosome 3 was identified in the replication data set (LOD = 3.4).

**Conclusions:** Analyses of a second large sample of alcoholic families provided further evidence of genetic susceptibility loci on chromosomes 1 and 7. Genetic analyses also have identified susceptibility loci on chromosomes 2 and 3 that may act only in one of the two data sets.

**Key Words:** Alcoholism, Nonparametric Linkage Analysis, Sibling Pair, Susceptibility Genes.

**A**LCOHOL DEPENDENCE IS a common, familial disorder that is a leading cause of morbidity and premature death (Caces et al., 1995; Campbell et al., 1996; DeBakery et al., 1995). Family studies have documented a 3- to 5-fold increased risk for alcoholism among siblings and other first-degree relatives of affected individuals (Cotton, 1979; Reich et al., 1998). The risk for alcoholism is increased even if the offspring of an alcoholic is adopted away from the home (Cloninger et al., 1981; Goodwin et al., 1973). In addition, twin studies have identified a significant

genetic component to alcoholism risk, with estimates of heritability ranging from 50% to 60% (Heath et al., 1997). Based on these findings, it is likely that alcoholism is a complex genetic disorder that results from the action of multiple, possibly interacting, genes as well as environmental influences.

Initial efforts to identify the genetic loci that underlie alcoholism susceptibility in human subjects relied on the evaluation of candidate genes. Studies of several candidate genes, notably the dopamine D2 receptor (*DRD2*) gene on chromosome 11 (Amadeo et al., 1993; Arinami et al., 1993; Blum et al., 1990, 1991; Bolos et al., 1991; Chen et al., 1996a; Comings et al., 1991; Cook et al., 1992; Edenberg et al., 1998a; Gelertner et al., 1991; Goldman et al., 1992, 1993, 1997; Lu et al., 1996; Neiswanger et al., 1995; Noble et al., 1994; O'Hara et al., 1993; Parsian et al., 1991; Sander et al., 1995; Schwab et al., 1991; Suarez et al., 1994b; Turner et al., 1992) and the serotonin transporter gene (*HTT*) on chromosome 17 (Edenberg et al., 1998b; Sander et al., 1997) have resulted in inconsistent findings, which suggests it is unlikely that either plays a major role in alcoholism susceptibility. The only consistently replicated findings are those that involve the protective effects of certain functional polymorphisms of the alcohol metabolizing enzymes alcohol dehydrogenase (ADH) and the mitochondrial aldehyde dehydrogenase (Chen et al., 1996b; Higuchi et

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al., 1994; Maezawa et al., 1995; Nakamura et al., 1996; Shen et al., 1997; Tanaka et al., 1996; Thomasson et al., 1991).

To more efficiently identify genetic loci that contribute to alcoholism susceptibility, recent studies have focused on a genome-wide approach, which would allow novel genetic loci to be identified. After extended pedigrees with multiple members diagnosed with alcoholism are collected, genetic analysis techniques can be employed to evaluate the evidence for linkage throughout the genome. Such a strategy was employed by the Collaborative Study of the Genetics of Alcoholism (COGA), which ascertained, evaluated, and genotyped 105 pedigrees as part of an initial genome screen (Reich et al., 1998). The initial linkage analyses of the COGA data set used only one definition of alcoholism, with individuals defined as affected if they fulfilled criteria for alcoholism based on DSM-III-R (American Psychiatric Association, 1987) and Feighner et al. (1972) criteria (termed *COGA criteria*). In a study of 382 affected sibling pairs, regions on chromosomes 1, 2, and 7 were identified as harboring genes that predispose an individual to alcoholism (Reich et al., 1998). The most significant finding of the study was on chromosome 7, with a LOD score of 3.5 near the marker D7S1793. On chromosome 1, a peak LOD score of 2.9 was found near the marker D1S1588. A second locus on chromosome 1, about 60 cM from the initial linkage finding, had a LOD score of 1.6. A LOD score of 1.8 was found on chromosome 2, near the marker D2S1790.

Additional analyses that used individuals without a diagnosis of alcoholism supported a protective locus on chromosome 4 near the *ADH* genes (Reich et al., 1998). Linkage to chromosome 4 was particularly interesting, because the COGA sample has few Asian families but rather consists primarily of non-Hispanic White and African American families. This linkage result on chromosome 4 suggests that the protective effects of *ADH* are not limited to the Asian population.

We report the results of additional genotyping performed in the initial sample of 105 pedigrees to further confirm and localize these initial linkage findings. In addition, linkage analyses have been completed in a replication data set of 157 pedigrees ascertained and evaluated by using criteria identical to those used in the initial sample. These analyses further support the initial linkage finding on chromosome 1 and provide additional evidence for the loci on chromosomes 2 and 7. The replication sample also identified additional chromosomal regions that may harbor susceptibility loci underlying 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 (American Psychiatric Association, 1987) alcohol dependence and Feighner et al. (1972) definite alcoholism; (2) had two first-degree relatives living within the catchment area of one of the COGA centers; (3) were free of non-alcohol-related, life-threatening illnesses; and (4) neither injected illicit substances within 6 months of admission nor reported more than 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) (Bucholz et al., 1994, 1995; Hesselbrock et al., 1999), as described previously (Foroud et al., 1998; Reich et al., 1998). Symptoms that reflect criteria from multiple diagnostic systems for alcohol dependence, including DSM-III-R (American Psychiatric Association, 1987), Feighner et al. (1972), DSM-IV (American Psychiatric Association, 1994), and ICD-10 (World Health Organization, 1993), are covered by the SSAGA.

Probands were considered affected if they met criteria for DSM-III-R alcohol dependence and Feighner definite alcoholism (termed *COGA alcoholism*) during their lifetime, based on the SSAGA 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 (Rice et al., 1995) was used, which asked each respondent about all other relatives both interviewed and noninterviewed (Foroud et al., 1998; Reich et al., 1998). Individuals were 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, which allowed noninterviewed individuals to be assigned a diagnosis. Present diagnoses were made by algorithm for those relatives interviewed with SSAGA. COGA alcoholic subjects were defined as previously described. ICD-10 alcoholic subjects were defined by using a modified ICD-10 criteria, as noted by Reich et al. (1998).

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 previously. 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 were obtained were reviewed by a 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 initial sample, which was reported previously (Reich et al., 1998), includes 105 families that consist of 987 individuals with genotypic data. On average, 9.5 individuals were genotyped per family. A replication sample was collected by using the same ascertainment and genotyping criteria and consisted of 157 families with genotypic data for 1295 individuals. An average of 8.3 individuals were genotyped in each replication family. In the initial sample 395 and 243 individuals met COGA and ICD-10 criteria for alcoholism, respectively, whereas in the replication data set 500 and 335 individuals met COGA and ICD-10 criteria, respectively. The number of affected sibling pairs included in each analysis is shown in Table 1.

### Genotyping and Error Detection

An initial genome screen was reported previously (Reich et al., 1998) that uses 292 markers with an average intermarker distance of 13.8 cM. Regions with linkage findings as well as intermarker gaps of greater than 20 cM have been genotyped more extensively. Data in this report are based on a total of 351 markers. Most markers were tri- or tetranucleotide repeat polymorphisms.

**Table 1.** Analysis Sample: Number of Affected Sibling Pairs ( $n-1$ )

| Diagnosis              | Initial sample | Replication sample | Combined sample |
|------------------------|----------------|--------------------|-----------------|
| COGA                   |                |                    |                 |
| All                    | 225            | 266                | 491             |
| Both parents genotyped | 137            | 122                | 259             |
| ICD-10                 |                |                    |                 |
| All                    | 101            | 143                | 244             |
| Both parents genotyped | 59             | 60                 | 119             |

Note: COGA criteria were DSM-III-R alcohol dependence and Feighner definite alcoholism.

Genotyping was completed in two laboratories (H.J.E. and A.G.) by using radioactive and fluorescence-based detection systems, as described previously (Foroud et al., 1998; Reich et al., 1998). The vast majority of markers were genotyped in both the initial data set and the replication data set. Genotypic data for the initial and replication data sets were stored separately by using the GeneMaster database management system. Each data set was checked separately for Mendelian inheritance of marker alleles with the program CRIMAP (Green et al., 1990) and the USERM13 option of the MENDEL suite of linkage programs (Boehnke, 1991). Maximum likelihood estimates of marker allele frequencies were obtained for each data set separately by using the USERM13 program. Marker order and distance were estimated independently from the two data sets by using CRIMAP. By comparing the maps developed from each data set independently, it was possible to identify the small number of markers whose map position was poorly estimated and to eliminate these markers from the chromosomal maps and further genetic analyses. Subsequently, data from the two data sets were combined, and marker allele frequencies, marker order, and distance were reestimated to obtain the most accurate maps and data for the linkage analyses. These maps represent a significant improvement over the marker maps developed in the initial data set (Reich et al., 1998) and are used in all figures and analyses presented herein.

#### Linkage Analysis

We used linkage analysis to identify loci that underlie susceptibility to alcoholism. Two models of alcoholism were used in the analyses. In the broader definition, termed COGA alcoholism, individuals were considered affected if they met criteria for both lifetime DSM-III-R alcohol dependence and Feighner definite alcoholism. A narrower model of alcoholism was also used, in which only individuals who met ICD-10 criteria for alcohol dependence were considered affected. Linkage analyses were performed separately in the initial and replication samples. The two data sets were also combined for a joint analysis to detect genes of smaller effect size that might not be identified in the analysis of the individual data sets.

We employed nonparametric methods of linkage analysis, which do not rely on the specification of a model of inheritance of susceptibility for the alcoholism diagnosis. Rather, the linkage tests are based on the sharing of marker alleles identical by descent among affected relative pairs. A marker allele is considered to be identical by descent (IBD) if both affected individuals have inherited the allele from a common ancestor. If the marker being tested is in close physical proximity to a gene that influences alcohol dependence, then alcoholic relatives would be more likely to share the chromosomal region near the susceptibility locus than to share a random chromosomal region.

Initial analyses reported by Reich and colleagues (1998) used affected sibling pairs. We have confirmed and expanded that initial report by performing linkage analyses that used affected sibling pairs in the initial and replication data sets as well as the combined data set, which includes both groups of families. Multipoint affected sibling pair analyses were performed by using the program ASPEX (Hinds and Risch, 1999). The subroutine SibIBD was used in the initial analyses presented herein,

because this is the most conservative analytic technique and uses only those sibling pairs with genotyped parents. As a result, inferences regarding allele sharing based on population-derived allele frequencies are not necessary. Complementary analyses were performed with the ASPEX subroutine SibPhase by using all affected sibling pairs, regardless of the status of parental genotyping. When sibships with more than two affected individuals are encountered, the test statistic may be biased by the formation of large numbers of sibling pairs from some families. Therefore, analyses were performed by using the more conservative analytic technique that employed independent ( $n-1$ ;  $n$  = number of siblings in a nuclear family) sibling pairs.

## RESULTS

### Initial Sample

Based on the linkage findings reported by Reich et al. (1998), 10 additional markers were genotyped on chromosome 1. Multipoint sibling pair linkage analysis resulted in a LOD score of 2.5 near the marker D1S1588, which corresponded to 63.5% allele sharing among affected sibling pairs both of whom had alcoholism based on the COGA criteria (Table 2, Fig. 1a). When all COGA affected sibling pairs were used, regardless of parental genotyping (SibPhase), the maximum LOD score was 1.9 near the markers D1S2614 and D1S1588 with 59.8% allele sharing. Consistent with the previous report by Reich et al. (1998), there was a second linkage finding with a maximum LOD score of 1.7 near the markers D1S224 and D1S1665.

The evidence for linkage to chromosome 2 increased with the genotyping of eight additional markers. Initial linkage analyses (SibIBD) that used the smaller set of COGA affected sibling pairs who had both parents genotyped resulted in a LOD score of 3.0 with 64.5% allele sharing near the marker D2S379 (Table 2, Fig. 2a). Expanding the sibling pair analyses to include all COGA affected sibling pairs, regardless of parental genotyping, also supported linkage to this same region with a maximum LOD score of 2.2, which corresponded to lower allele sharing (60.3%) among affected sibling pairs.

Genotyping was performed on an additional 10 markers on chromosome 7. Multipoint sibling pair linkage analyses resulted in a LOD score of 2.0 near the marker D7S1793 for sibling pairs with both parents genotyped. Marker allele sharing among these sibling pairs affected with alcoholism based on the COGA criteria was 58.4%. Nearly 40 cM distal to this region is a second chromosomal region with a LOD score of 1.6 and 58.3% allele sharing among COGA affected sibling pairs with both parents genotyped. This finding is near the markers D7S821 and D7S1796 (Table 2, Fig. 3a).

Analysis of the ICD-10 affected sibling pairs identified two chromosomal regions with LOD scores greater than 1.5 in the initial sample. On chromosome 8, near the marker GA12B06, a maximum LOD score of 1.6 with 61.4% allele sharing was detected when the reduced data set of sibling pairs with genotyped parents was analyzed (Table 2). On chromosome 16, a maximum LOD score of 2.7 was identi-

**Table 2.** Summary of Multipoint Sibling Pair Analysis Using COGA and ICD-10 Alcoholism Criteria

| Chromosome | Diagnosis     | Initial sample |             |              | Replication sample |             |              | Combined sample |             |              |
|------------|---------------|----------------|-------------|--------------|--------------------|-------------|--------------|-----------------|-------------|--------------|
|            |               | Lod score      | % IBD       | cM           | Lod score          | % IBD       | cM           | Lod score       | % IBD       | cM           |
| 1          | <b>COGA</b>   | <b>2.5</b>     | <b>63.5</b> | <b>145.7</b> | 0.7                | 55.2        | [127.4]      | <b>2.6</b>      | <b>59.2</b> | <b>143.8</b> |
| 1          | ICD-10        | 1.0            | 63.2        | [145.7]      | <b>1.6</b>         | <b>61.2</b> | <b>127.4</b> | <b>1.9</b>      | <b>60.1</b> | <b>139.0</b> |
| 2          | <b>COGA</b>   | <b>3.0</b>     | <b>64.5</b> | <b>98.8</b>  | 0.0                | 50.0        | [98.8]       | <b>1.8</b>      | <b>56.6</b> | <b>120.6</b> |
| 3          | COGA          | 0.11           | 54.0        | [97.7]       | <b>3.4</b>         | <b>72.5</b> | <b>97.7</b>  | <b>2.4</b>      | <b>64.0</b> | <b>100.5</b> |
| 3          | ICD-10        | 0.0            | 50.0        | [97.7]       | <b>1.6</b>         | <b>71.6</b> | <b>97.7</b>  | 0.8             | 62.1        | [100.5]      |
| 7          | <b>COGA</b>   | <b>2.0</b>     | <b>58.4</b> | <b>91.4</b>  | 0.2                | 53.0        | [91.4]       | <b>2.1</b>      | <b>58.1</b> | <b>105.2</b> |
|            | <b>COGA</b>   | <b>1.6</b>     | <b>58.3</b> | <b>128.0</b> | 1.3                | 57.5        | [129.1]      | <b>2.9</b>      | <b>57.9</b> | <b>128.5</b> |
| 8          | <b>ICD-10</b> | <b>1.6</b>     | <b>61.4</b> | <b>88.2</b>  | 0.4                | 55.7        | [88.2]       | <b>1.8</b>      | <b>58.5</b> | <b>88.2</b>  |
| 22         | <b>ICD-10</b> | 0.0            | 50.0        | [58.3]       | <b>1.9</b>         | <b>64.4</b> | <b>58.3</b>  | 1.1             | 58.1        | [58.3]       |

Results from initial linkage analyses performed using only those sibling pairs with both parents genotyped (SibIBD). Analyses were performed in the initial, replication, and combined samples using the same marker map. Linkage results with lod scores greater than 1.5 are shown in bold, with the location of the maximum lod score indicated under the column marked cM (centimorgans). For comparison purposes, results are also shown in the other data sets at the same marker position (cM), although those results which are not greater than 1.5 are denoted by brackets around the map position.

fied when all sibling pairs regardless of parental genotyping were analyzed. This LOD score corresponded to 64.5% allele sharing and was between the markers D16S475 and D16S509. Analyses of the COGA affected sibling pairs identified a region on chromosome 15 near the marker D15S217 with a LOD of 1.6 and 57.5% allele sharing.

#### Replication Sample

In the replication sample, there was evidence of linkage to chromosome 1 when ICD-10 alcoholism criteria were used. Near the marker D1S224, the maximum multipoint LOD score was 1.6, with 61.2% IBD marker allele sharing among affected sibling pairs with both parents genotyped (Table 2; Fig. 1b). Linkage to chromosome 3, not identified in the initial sample, was also observed. The region near the marker D3S1766 gave the highest LOD score in the replication sample, a LOD score of 3.4 with 72.5% allele sharing among COGA diagnosed sibling pairs with genotyped parents (Table 2, Fig. 4a). Evidence of linkage to this region with a LOD score of 1.6 and 71.6% allele sharing also was observed among sibling pairs who met the more stringent ICD-10 diagnostic criteria for alcoholism and had both parents genotyped (Table 2, Fig. 4b). On chromosome 22, near markers D22S684 and D22S1267, there was evidence of linkage with a LOD score of 1.9 and 64.4% allele sharing when ICD-10 affected sibling pairs with both parents genotyped were analyzed (Table 2). This region also was supported (LOD = 2.0, allele sharing 61.7%) by the larger data set that consisted of all ICD-10 affected sibling pairs regardless of parental genotyping.

#### Combined Sample

To begin fine mapping the chromosomal regions identified in the initial and replication samples, we performed a combined analysis of both the initial and replication data sets. On chromosome 1, the combined data set of 257 pedigrees produced a LOD score of 2.6 near the markers D1S2614 and D1S1588 for the COGA diagnosis when sibling pairs with both parents genotyped were analyzed. This

corresponds to 59.2% allele sharing among affected sibling pairs (Table 2, Fig. 1a). When only sibling pairs with ICD-10 defined alcoholism who had genotyped parents were analyzed, linkage also was observed to the same chromosomal region with a LOD score of 1.9 and 60.1% allele sharing (Table 2, Fig. 1b). Linkage also was observed in the enlarged data set of all ICD-10 affected sibling pairs regardless of parental genotyping. In this full sample, the LOD score was 1.5, with 57% allele sharing with the maximum LOD score near the same markers.

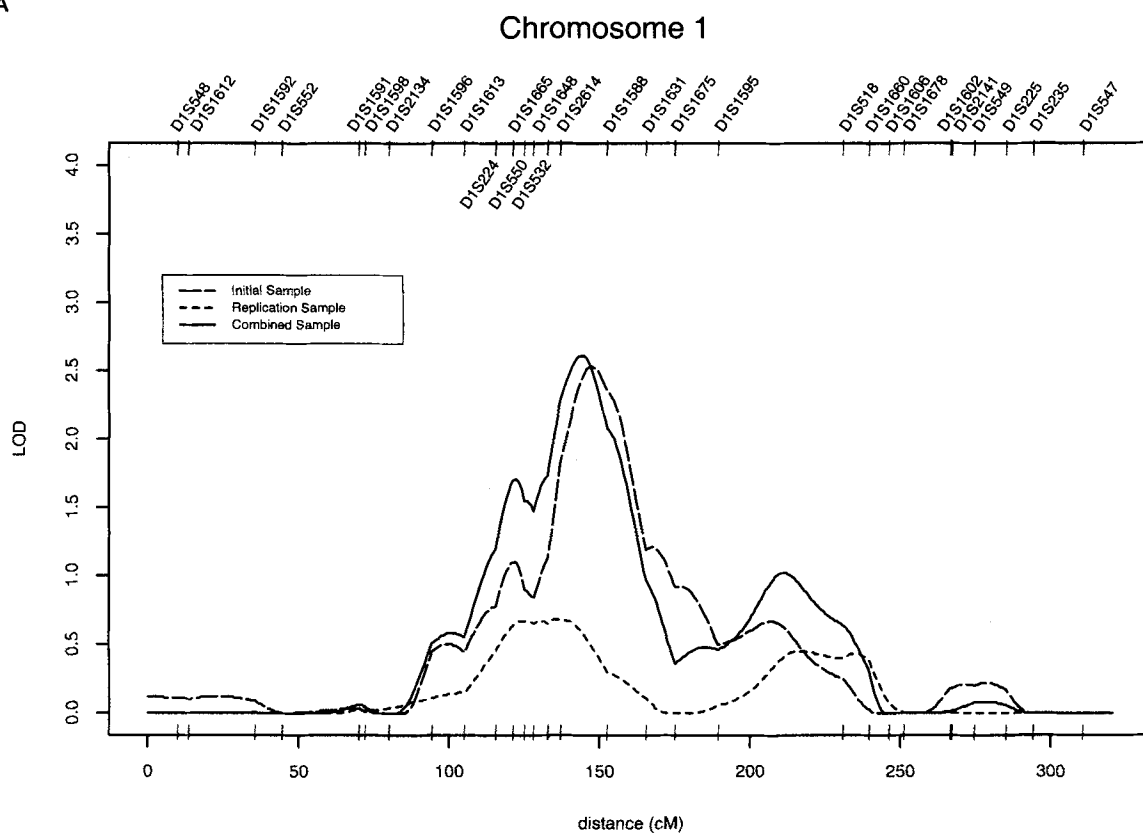
On chromosome 2, analyses of COGA diagnosed affected sibling pairs produced a LOD score of 1.8 with 56.6% allele sharing among affected siblings with both parents genotyped. The peak LOD score occurred near the markers D2S438 and D2S1790 (Table 2, Fig. 2a). Evidence of linkage to chromosome 3 remained in the combined analysis, with a maximum LOD score of 2.4 between the markers D3S1766 and GATA128. This linkage corresponds to 64.0% allele sharing among sibling pairs diagnosed with COGA-defined alcoholism that had genotyped parents (Table 2; Fig. 4a).

Two regions on chromosome 7 had evidence of linkage in the combined sample of COGA affected sibling pairs. The highest LOD score (LOD = 2.9) was near the marker D7S821 with 57.9% allele sharing among COGA diagnosed affected sibling pairs with genotyped parents (Table 2, Fig. 3a). A second region, more proximally located at about 105 cM, also provided evidence of linkage, near the markers D7S1830 and D7S1797. In this region, the maximum LOD score was 2.1 with 58.1% allele sharing among COGA affected sibling pairs with genotyped parents. On chromosome 8, ICD-10 affected sibling pairs with both parents genotyped produced a LOD score of 1.8 with 58.5% allele sharing at the marker GA12B06.

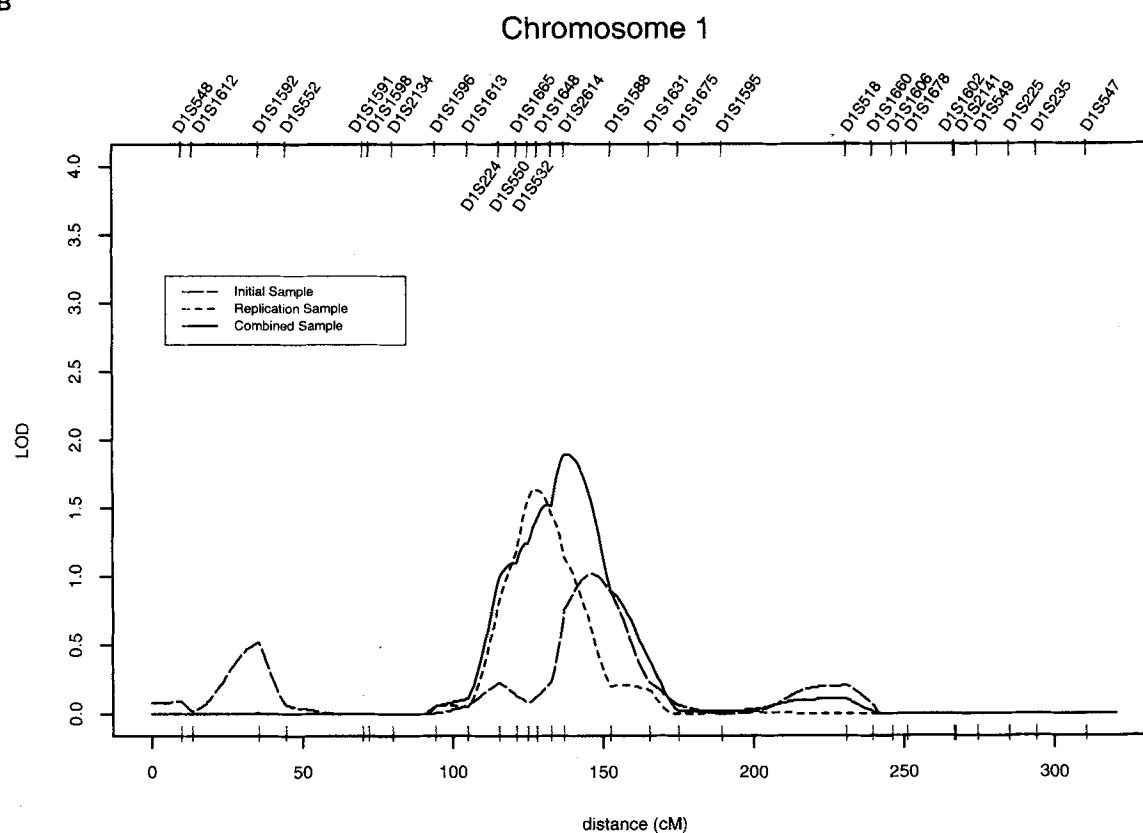
#### DISCUSSION

One of the key elements of the design used in this study was the two-stage approach to the genetic search for alcoholism susceptibility loci. This allowed us to use the initial

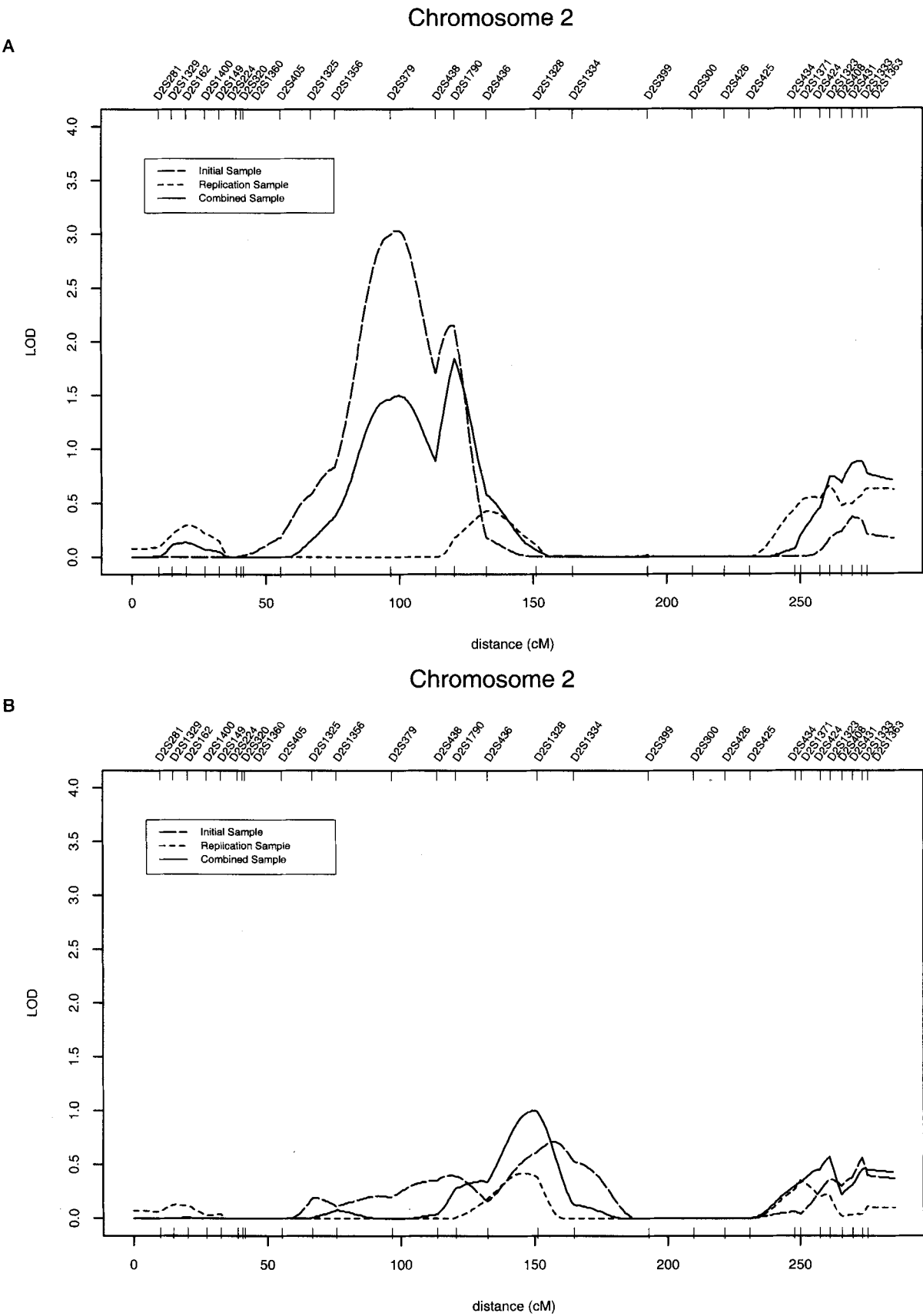
A



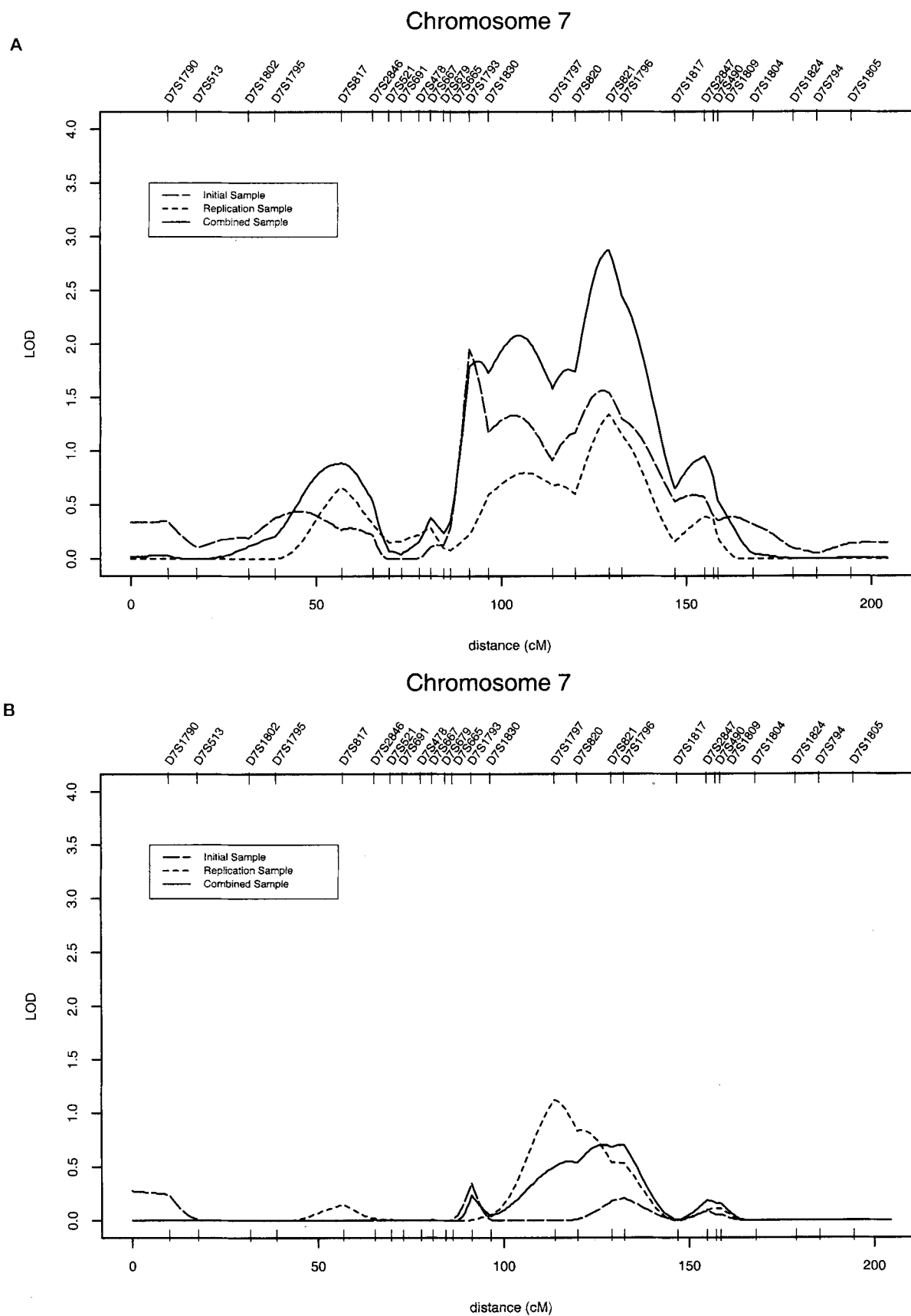
B



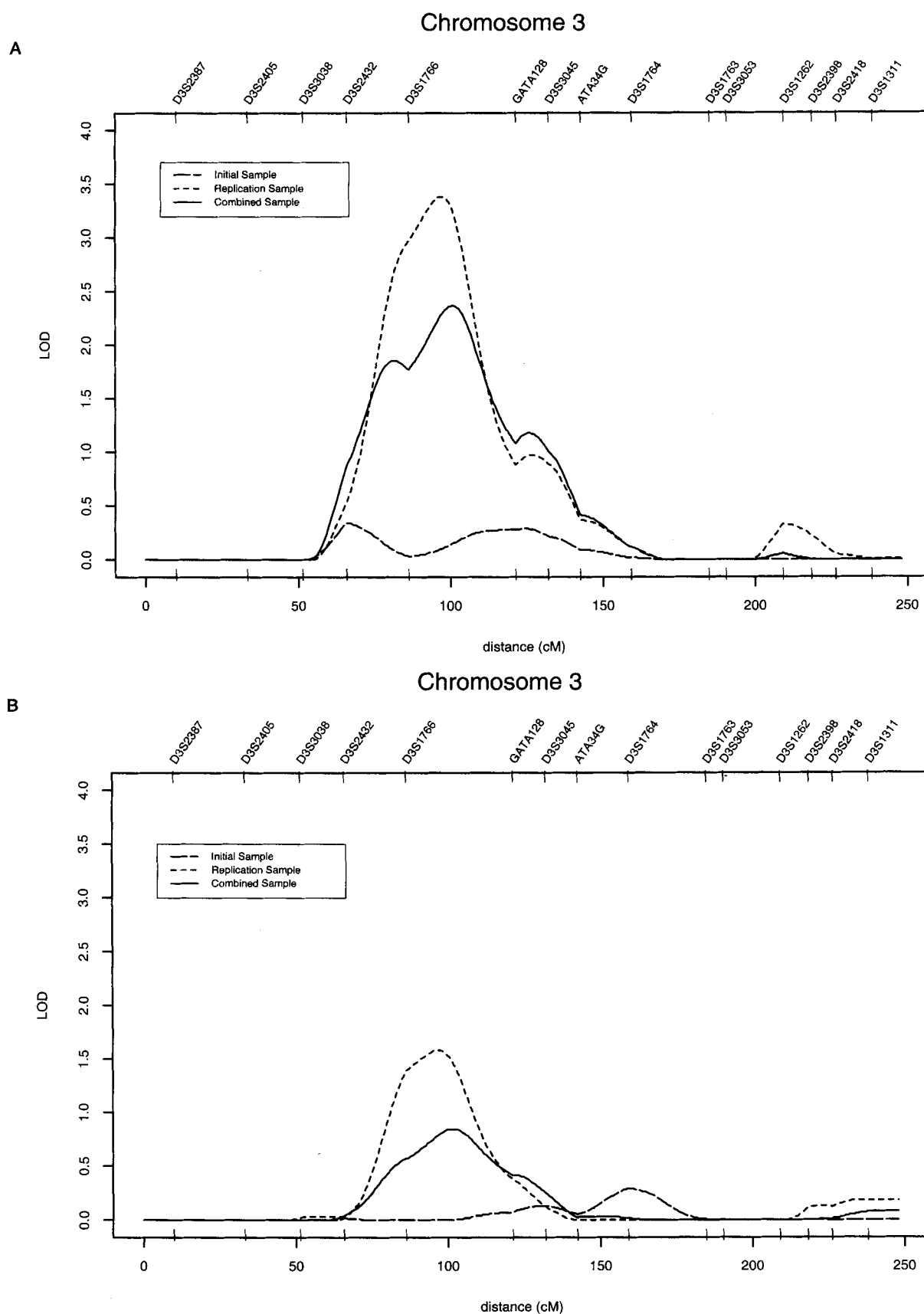
**Fig. 1.** Multipoint linkage analysis on chromosome 1 which used (a) sibling pairs that met COGA diagnostic criteria for alcoholism and (b) sibling pairs that met ICD-10 diagnostic criteria for alcoholism. Linkage analyses were performed by using only those affected sibling pairs with genotyped parents (Sib/BD).



**Fig. 2.** Multipoint linkage analysis on chromosome 2 which used (a) sibling pairs that met COGA diagnostic criteria for alcoholism and (b) sibling pairs that met ICD-10 diagnostic criteria for alcoholism. Linkage analyses were performed by using only those affected sibling pairs with genotyped parents (SibIBD).



**Fig. 3.** Multipoint linkage analysis on chromosome 7 which used (a) sibling pairs that met COGA diagnostic criteria for alcoholism and (b) sibling pairs that met ICD-10 diagnostic criteria for alcoholism. Linkage analyses were performed by using only those affected sibling pairs with genotyped parents (SibIBD).



**Fig. 4.** Multipoint linkage analysis on chromosome 3 which used (a) sibling pairs that met COGA diagnostic criteria for alcoholism and (b) sibling pairs that met ICD-10 diagnostic criteria for alcoholism. Linkage analyses were performed by using only those affected sibling pairs with genotyped parents (SibIBD).



sample to identify candidate regions, whereas the larger replication sample was used to confirm the initial linkage findings as well as to identify other loci (Holmans and Craddock, 1997; Suarez et al., 1994a). In addition, the independent development of two samples allowed us to create marker maps independently and confirm marker order and distances between the two data sets, as well as to use the combined data set to estimate the most accurate maps and marker distances for the genetic analyses.

There are inherent differences in the two data sets that likely will arise in such a split sample design when the families are ascertained over an extended period of 10 years. As proposed by Suarez et al. (1994a), these differences could account for some of the likely variability in the frequency of the alcoholism susceptibility genes and thus result in some of the unique loci that have been identified in each sample. For example, the number of individuals genotyped per family in the initial sample was greater than that in the replication sample (9.5 vs. 8.3). This is due in part to the greater density of alcoholic individuals in the initial sample as compared with the replication sample. An average of 4.2 COGA affected individuals per pedigree were in the initial sample compared with 3.8 per pedigree in the replication sample. The number of ICD-10 alcoholics per pedigree was relatively constant between the two data sets (2.7 vs. 2.6). Thus, the density of COGA affected individuals per pedigree was greater in the initial sample compared with the replication sample and may result in the identification of unique susceptibility loci in each data set.

Another factor that may influence the linkage results from each sample is the pedigree structure. In the initial sample, 61% of COGA affected sibling pairs had both parents genotyped and another 29% had one parent genotyped. In the replication sample, 46% of the COGA affected sibling pairs had both parents genotyped and 34% had one parent genotyped. Thus, despite the larger number of sibling pairs in the replication sample, when analyses are limited to those sibpairs with both parents genotyped (SibIBD), the effective sample size is nearly identical in the two samples.

Despite the differences between the initial and replication sample, consistent evidence of linkage to chromosome 1 was identified in both data sets separately, as well as when the two data sets were combined. Reported initially by Reich et al. (1998), linkage to chromosome 1 was pursued further by the genotyping of additional markers in the critical region. The finding in the initial sample, which used sibling pairs that met COGA diagnostic criteria, remained robust with a LOD score of 2.5 and 64% allele sharing near the markers D1S2614 and D1S1588. In this same marker interval in the initial sample, sibling pairs affected by the more stringent ICD-10 criteria have their maximum allele sharing (63%). Multipoint analysis of the replication data set also supported linkage to chromosome 1 with a LOD score of 1.6 and 61% allele sharing among sibling pairs who met the ICD-10 criteria for alcoholism. The maximum

LOD score in this sample is slightly more proximal, near the markers D1S550 and D1S1648, but it is less than 20 cM from the maximum observed in the initial sample.

Not surprisingly, analysis of the combined sample provided evidence for linkage to chromosome 1 when both the ICD-10 and COGA criteria for alcoholism were used. In both analyses, the maximum LOD score is between the markers D1S2614 and D1S1588, the same interval in which the initial sample had its maximum. However, the 1-LOD support interval in the combined data set is substantially larger (40 cM) than that for either of the individual data sets (22–25 cM). The increase in the 1-LOD confidence interval in the combined data set may reflect the presence of more than one locus that influences alcoholism susceptibility in this chromosomal region, or it may be related to the mapping difficulties inherent in the analysis of traits dichotomized by using a diagnostic threshold.

Several notable candidate genes are within the chromosome 1 region linked to the COGA and ICD-10 phenotypes. These include several genes that encode channels such as the calcium-activated chloride channel and the voltage-gated potassium channel. Other candidate genes in the chromosome 1 region include adenosine 3':5'-cyclic monophosphate-specific, phosphodiesterase 4B, gamma-5 guanine nucleotide-binding protein, and protein kinase C-like 2.

Genotyping of additional markers on chromosome 2 in the initial sample increased the evidence of linkage to this chromosomal region. Among COGA affected sibling pairs, allele sharing was nearly 65% and the LOD score was 3.0. Similar evidence of linkage to this region was not observed in the replication sample and, as a result, analysis of the combined sample of COGA affected sibling pairs had allele sharing of 57% in the same chromosomal region. These findings suggest that the locus on chromosome 2 has greater effect on disease susceptibility or is present at higher frequency in the initial sample as compared with the replication sample. The increase in allele sharing, which was observed in the initial sample after the genotyping of additional markers, suggests that this is not a spurious linkage finding in the initial sample. The candidate region is still relatively large based on a 1-LOD support interval (25 cM) and includes the genes for protein kinase C epsilon and acyl-CoA binding protein.

Linkage to chromosome 3 was not observed in the initial sample and thus is a novel finding identified in the replication data set. In fact, linkage to chromosome 3 in the replication data set was the most significant finding in that sample, with a LOD score of 3.4 and allele sharing among COGA affected sibling pairs that reached 72.5%. Allele sharing of nearly 72% also was observed in the same chromosomal region among ICD-10 affected sibling in the replication sample. Due to the lack of linkage in the initial sample, analysis of the combined sample resulted in decreased, although still elevated, allele sharing among COGA affected sibling pairs (64%). These results suggest

that linkage to chromosome 3 is a unique finding in the replication sample. Genotyping of additional markers in this chromosomal region will be essential to assess the robustness of this linkage finding as well as to further limit the critical region.

The third region reported by Reich et al. (1998) with evidence of linkage in the initial data set was chromosome 7. Genotyping of additional markers in this chromosomal region decreased the LOD score and allele sharing in this chromosomal region to 2.0 and 58%, respectively. A second region, about 40 cM distal to the initial linkage finding, also provided evidence of linkage with a LOD score of 1.6 and 58% allele sharing. Analyses in the replication data set provide some evidence of linkage to the more distal region (at 128 cM), with allele sharing among COGA affected sibling pairs that exceeds 57%. As a result, analysis of the combined data set increased the LOD score in this region to 2.9 with corresponding allele sharing of 58%. The region identified in the initial sample continued to provide evidence of linkage with a maximum LOD score of 2.1 and 58% allele sharing. Review of these findings suggests that more than one locus may influence alcoholism susceptibility on chromosome 7. Genotyping of additional markers on chromosome 7 has broadened the chromosomal region linked to the COGA phenotype; however, the 1-LOD support interval is only 17 cM around the primary linkage finding at 128.5 cM. Further marker genotyping will be necessary to dissect this linkage result and determine whether there are indeed multiple susceptibility loci in this chromosomal region.

Linkage has been reported in the alcohol-preferring and nonpreferring rats to chromosome 4, which is syntenic to this region of human chromosome 7 (Carr et al., 1998). The coding regions of the candidate gene neuropeptide Y have been sequenced without finding a mutation (Bice et al., 1998); nevertheless, this gene remains an excellent candidate gene, and variation in upstream regulatory regions may account for differences in alcohol preference. Other candidate genes in this region of chromosome 7 include adenylate cyclase 1, syntaxin 1A, as well as several receptors such as a glutamate (GRM3) and a cholinergic (CHRM2) receptor.

To estimate the effect of the putative loci on chromosomes 1, 2, 3, and 7, we compared the estimated proportion of allele sharing among affected sibling pairs to that expected under the null hypothesis by using a formula proposed by Risch (1990). Assuming no recombination between the marker with the maximum LOD score and the disease locus, the risk ratio due to the loci was estimated in those data sets that demonstrated evidence of linkage. In the combined sample, the chromosome 1 locus had an estimated risk ratio for the COGA diagnosis of alcoholism of 1.5, although in the initial sample the risk ratio was estimated to be 1.9. When the data for ICD-10 affection was used, the initial sample had a risk ratio of 1.7, the replication sample a risk ratio of 1.6, and the combined

data set a risk ratio of 1.5. The locus on chromosome 2 in the initial sample had an estimated risk ratio of 1.9. The chromosome 3 linkage had an estimated risk ratio of 3.1 for both the COGA and ICD-10 alcoholism diagnoses in the replication sample. The locus on chromosome 7 provided consistent evidence of a risk ratio between 1.4 and 1.5 regardless of the sample in which it was estimated and by using both the COGA and ICD-10 diagnosis of alcoholism. These results are consistent with those estimated by Reich et al. (1998) and represent only a portion of the total sibling risk ratio for alcohol dependence, which has been estimated to be between 3 and 8 in the COGA sample (Reich et al., 1998).

The remaining linkage findings with LOD scores greater than 1.5 were on chromosomes 8 and 22, occurred in only the initial or replication data set, and did not continue to demonstrate increased allele sharing in the combined analysis. The likelihood that these linkage findings are spurious is certainly greater than that for linkages previously described that remained interesting ( $\text{LOD} > 1.5$ ) in the combined data set. Some additional markers will be genotyped in these chromosomal regions; however, the focus of our genotyping efforts will remain on chromosomes 1, 2, 3, and 7.

One of the difficulties in a genetic study of a complex disorder is determining what constitutes replication of a linkage finding. The first issue to address is whether the linkage findings in the two data sets represent the same gene, even if the maximum LOD score is not at the same chromosomal position. Roberts et al. (1999), in a simulation study that addressed the accuracy of gene localization based on the maximum LOD score, found that the precision of localization was directly influenced by sample size. In samples that consist of 200 families, a slightly larger sample than either of the two COGA data sets, there is great variation in the most likely placement of the susceptibility gene based on the maximum LOD score, which suggests that in modest sample sizes, the same gene may be identified even if the maximum LOD score in each data set is 20 or even 30 cM apart.

A second issue to consider is the significance threshold to determine replication of a linkage result. According to the criteria proposed by Lander and Kruglyak (1995), our initial results do not constitute significant linkage findings. Therefore, it could be argued that we do not have linkage results to replicate. However, our initial linkage results did warrant further evaluation because they were the results of the largest study designed to identify alcoholism susceptibility loci and they were the most significant linkage findings reported to date; thus, we were forced to develop our own criteria for replication of our initial results. We considered a linkage finding to be replicated if, in the second data set, a LOD score greater than 1.5 was obtained. This is a more stringent replication criteria than that proposed by Lander and Kruglyak (1995) for replication of linkage and thus decreases the likelihood of a false positive result.

By using this criterion, we have replicated our initial

linkage finding on chromosome 1. Another means to evaluate the evidence of linkage is to determine whether the analysis of the combined data sets increases the evidence of linkage. By using this criterion, the linkage finding on chromosome 7 appears to be more robust with the inclusion of families from both data sets. The linkage results on chromosomes 2 and 3 are the most difficult to interpret because each is observed in only one of the two data sets, and the combined analysis does not improve the linkage finding. Two conclusions are possible: The susceptibility locus has its effect primarily in one data set and therefore is not detectable by currently available linkage methods in the second data set, or the linkage finding is spurious. Only further testing of markers in these chromosomal regions can clarify these results.

The regions reported by the COGA study do not correspond with those identified in another genome-wide study of alcoholism performed in a sample of alcoholics from a Southwestern Native American population (Long et al., 1998), who were likely to be genetically more homogeneous than the sample of U.S. White families collected by COGA. Linkage to chromosomes 4 and 11 were reported in the Southwestern Native American population. Further analyses of the chromosome 11 candidate gene, tyrosine hydroxylase, supported linkage of this locus in a sample of Finnish offenders with alcoholism and comorbid antisocial personality (Nielsen et al., 1998).

To identify the genetic loci that influence susceptibility for alcoholism, we and others have used a genome-wide approach to identify linked chromosomal regions. Although genotyping of additional markers may narrow the critical chromosomal interval, it is likely that only through the concerted study of both human and animal models of alcoholism, as well as the use of recently developed microarray techniques, will genes that influence the risk for alcoholism be identified and characterized.

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