Identification of Susceptibility Loci for Alcohol-Related Traits in the Irish Affected Sib Pair Study of Alcohol Dependence


**Background:** Alcoholism is a phenotypically and probably genetically heterogeneous condition. Thus, one strategy for finding genes influencing liability to alcoholism is to study the components of alcoholism, which may be more directly related to the underlying pathophysiology than is clinical diagnosis. The goal of this study was to identify genomic regions containing susceptibility loci for alcohol-related traits.

**Methods:** A 4-cM dense whole-genome linkage study was conducted in the Irish Affected Sib Pair Study of Alcohol Dependence. Probands, affected siblings, and parents were evaluated by structured interview. Variance component linkage analysis was applied to data from 485 families for 5 measures: initial sensitivity and tolerance (based on scales from the self-report of the effects of ethanol; maximum drinks within 24 hours, an empirically derived factor score based on withdrawal symptoms, and age at onset of alcohol dependence.

**Results:** Evidence for linkage ($p < 0.005$) was found on 9 chromosomes. For age at onset, 2 regions were found on chromosome 9 (highest Lod $= 2.3$, $p = 0.0005$). For initial level of response to alcohol, suggestive regions were on chromosomes 1 and 11 (highest Lod $= 2.9$, $p = 0.0001$ on chromosome 11), while those for tolerance signals were on chromosomes 1, 6, and 22. Maximum drinking was associated with regions on chromosomes 12 and 18. For withdrawal symptoms, the highest peak was on chromosome 2 (Lod $= 2.2$, $p = 0.0007$).

**Conclusions:** Using quantitative measures of components of alcohol dependence, we identified several regions of the genome that may contain susceptibility loci for specific alcohol-related traits and merit additional study.

**Key Words:** Age at Onset, Maximum Drinking, Response to Ethanol, Withdrawal, Molecular Genetics.

Evidence from family, adoption, and twin studies indicates strong familial aggregation of alcoholism, and this appears to be significantly genetically influenced (McGue, 1997). However, identification of specific genes that contribute to an individual's susceptibility for alcohol dependence (AD) has progressed slowly. This may in part be due to the difficulty of defining etiologically homogeneous subtypes of alcoholism for genetic studies. Additionally, finding genes for complex traits has proved to be challenging because such genes may each contribute a small effect, requiring large sample sizes to detect them (Glazer et al., 2002).

Alcoholism is a clinically and etiologically heterogeneous condition, with affected individuals varying in age of onset, clinical presentation, comorbid psychopathology, developmental course, and severity. For example, DSM-IV (Diagnostic and Statistical Manual of Mental Disorder, version IV, American Psychiatric Association, 1994) diagnosis of AD requires any combination of 3 of 7 symptoms. Individuals can therefore meet criteria with a wide variety of presentation. Evidence from twin studies suggests the existence of a genetic heterogeneity associated with comorbidity and gender (McGue et al., 1992; Prescott and Kendler, 1999; Prescott et al., 2005a). This heterogeneity may in part be responsible for the relatively few replications of linkage and candidate gene results (Sanders et al., 2004; Schuckit et al., 2004).
Identification of susceptibility genes may be aided by studying genetically relevant phenotypes. The component processes that form AD may be more directly related to the underlying pathophysiology than is the clinical disorder. Results from animal quantitative trait locus (QTL) studies suggest that there are specific loci for different alcohol-related behaviors, including acute/chronic withdrawal, preference drinking, stimulated activity, and blood level after acute dose (see Crabbe et al., 1999). However, it is only recently that genetic studies have used alcohol-related traits in humans, such as alcohol craving (Ehlers and Wilhelmsen, 2005), response to ethanol (Schuckit et al., 2001; Wilhelmsen et al., 2003; Schuckit et al., 2005), and maximum number of drinks (Saccone et al., 2000; Bergen et al., 2003). Meanwhile, linkage results obtained from these alcohol-related traits often did not identify similar genetic regions, which also suggested looking for specific genetic regions for different components of alcoholism.

One of the most extensively studied alcohol-related traits in animal genetic research is response to ethanol. A similar construct used in human alcohol studies, level of response (LR) to ethanol (i.e., needing high does of alcohol to produce an effect), has been reported to be genetically influenced (Heath et al., 1999; Schuckit et al., 2001). A low LR to ethanol has been shown to be more common among people with a family history of alcoholism (Schuckit and Smith, 2000). Level of response at the time of initial use is a risk factor for the development of alcoholism (Schuckit et al., 2001). Previous studies have identified several gene regions associated with the initial LR to alcohol (Schuckit et al., 2001; Wilhelmsen et al., 2003).

Another important measure used in animal research on genetic risk for AD is the volume of ethanol consumed. In humans, this is typically measured as the reported maximum number of drinks ever consumed in a 24-hour period. This phenotype is complex as it combines individual differences in alcohol metabolism capacity, lack of control over drinking, and probably personality traits such as risk taking. Maximum drinking has been found to have a heritability estimate of about 30% (Slutske et al., 1999). It is also an indicator of the severity of alcoholism and differs between alcoholic patients with and without tolerance and/or withdrawal symptoms (Schuckit et al., 1998). Several regions of human genome have been reported to be associated with maximum drinking (Bergen et al., 2003; Saccone et al., 2000).

Another domain of human alcohol problems is reflected by the physiological adaptation to chronic alcohol use. The physiological mechanisms required for maintaining a stable homeostasis of ethanol in body and brain provides the basis for tolerance and the withdrawal syndrome. Once the body and brain adapt to a certain amount of ethanol, the absence of this level leads to the withdrawal syndrome. These physiological symptoms have been shown to have a genetic component and are indicators of the severity of alcoholism (Schuckit et al., 1998). Withdrawal is a pivotal symptom for AD diagnosis (Langenbucher et al., 2000) and is also associated with worse prognosis (Hasin et al., 2000). A review of animal studies reported genetic correlations between predisposition to high levels of drinking and alcohol withdrawal severity (Crabbe, 2001), as well as susceptibility loci for acute and chronic withdrawal phenotypes. One human study has reported linkage evidence for withdrawal and drinking severity (Ehlers et al., 2004a).

Variation in age at onset of AD may also reflect differences in magnitude of the underlying risk for AD. Early onset is associated with a more severe course (Johnson et al., 2000). Prior research suggests genetic heterogeneity associated with the onset of regular alcohol use and the transition from regular alcohol use to AD diagnosis (Liu et al., 2004). In another study, additive genetic influences have been found to be significantly greater in early-onset than in late-onset male alcoholism (McGue et al., 1992).

In view of the extensive literature on genetic influences on alcohol-related traits from animal QTL studies, we conducted this study to identify susceptibility loci for alcohol-related traits in humans. The present report is based on a large-scale affected sibling pair sample collected in Ireland. We performed autosomal linkage analysis of 5 quantitative alcohol-related traits, including age at onset, initial sensitivity, tolerance, maximum drinks, and withdrawal severity to ethanol. Use of these quantitative alcohol-related traits, which may be more etiologically homogeneous, could increase the chances for detecting susceptibility genes for AD.

MATERIALS AND METHODS

Subjects

Participants in this study were recruited in Ireland and Northern Ireland between 1998 and 2002. More details of the study design, sample ascertainment, and clinical characteristics of this sample are described elsewhere (Prescott et al., 2005b). In brief, ascertainment of probands was mainly conducted in community alcoholism treatment facilities and public and private hospitals. Probands were eligible for study inclusion if they met the current American Psychiatric Association (1994, DSM-IV) criteria for AD and if all 4 grandparents had been born in Ireland, Northern Ireland, Scotland, Wales, or England. After a prospective family was identified through probands, parents, and potentially affected siblings whom the probands provided permission to contact were recruited. We attempted to enroll all living, biological parents.

Phenotype Measurement

Probands, siblings, and parents were interviewed by clinically trained research interviewers, most of whom had extensive clinical experience with alcoholism. The assessment included demographic characteristics, lifetime history of AD, and other comorbid conditions, alcohol-related traits, personality features, and clinical records. We used the same inclusion criteria for both probands and

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In the remainder of this article, we use Ireland and Irish to refer to both Ireland (sometimes called the Republic of Ireland) and Northern Ireland.
sibings, and DSM-IV AD was assessed in probands and siblings using the SSAGA (Semi-Structured Assessment of the Genetics of Alcoholism) interview (version 11, Bucholz et al., 1994), modified to reduce assessment time (e.g., we deleted items assessing onset ages of most individual symptoms). Among parents using an adapted version of the Structured Clinical Interview for DSM-IV (SCID) interview (Spitzer and Williams, 1985), there were 10 siblings who, upon interview, did not meet the full criteria of AD.

All participants provided informed consent. There were 1,248 individuals who completed the SSAGA and provided interview data used in the present study, including 591 probands, 610 affected siblings, 27 affected individuals from 10 complex families, and 20 questionable siblings (10 were diagnosed as AD later and 10 did not meet the full criteria of AD upon interview, details in Prescott et al., 2005b). Because parents were interviewed using the SCID, they did not have information for these quantitative traits.

We selected 5 alcohol-related phenotypes hypothesized to have a heritable basis for use in linkage analysis. These include the following:

- age at onset of AD (ONSET) – the age of a subject when he/she first met the criteria for a DSM-IV AD.
- subjective response to ethanol – responses to the self-rating of the effects of alcohol (SRE) (Schuckit et al., 1997) were used to form 2 measures for analysis: initial sensitivity (ISENS) and tolerance/maximum drinking (TOLMX). The SRE asks how many drinks were required for an individual to have effects (e.g., feel dizzy, begin stumbling, or pass out) from alcohol consumption at different alcohol-use stages in life. The ISENS is based on “the first 5 times you ever drank” and items contributing to TOLMX concern the “period when you drank the most.” The score of each measure was computed by summing the number of drinks required to produce an effect and dividing by the numbers of effects endorsed. The SRE has been shown to have good internal consistency and test–retest reliability, provide good validity to identify people who had a low response to alcohol in a laboratory challenge test, and has been associated with AD diagnosis in several populations (Schuckit et al., 2001). The ISENS measure has been used previously in linkage analyses of the COGA (Collaborative Study on the Genetics of Alcoholism) sample (Schuckit et al., 2001), where it was called LR.
- maximum drinking within 24 hours (MAX24) – the largest number of drinks an individual reported having consumed in 24 hours. Because units in Ireland differ from those in the United States, participants were asked to describe the type of beverage and volume consumed and this was converted into standard drinks (i.e., 12 oz of beer, 4 oz of wine, or 1.5 oz of spirits).
- withdrawal symptoms factor score (WDSFS) – the SSAGA interview included 10 symptoms of withdrawal (hands trembling, unable to sleep, feeling anxious, depressed or irritable, tachycardia, sweating, nausea, feeling physically weak, hallucinations, and feeling fidgety or restless) following cessation or reduction in drinking. Because each symptom may not equally contribute to withdrawal severity, an exploratory factor analysis of these dichotomous symptoms was conducted using Mplus (Muthen and Muthen, 2004). A single factor accounted for 70% of the variance in the symptoms. Factor loadings were greater than 0.6 for each symptom and were especially high (≥ 0.9) for the “feel anxious” and “fidgety or restless” symptoms. We calculated a factor score for each individual to represent the varying contribution of each symptom to withdrawal severity based on the loadings on the single factor.

Laboratory Methods

All interviewed probands and parents (except for 1 mother) provided DNA samples. Among 610 affected siblings, only 6 individuals did not provide DNA samples. Individuals not willing to donate blood and those interviewed by telephone were asked to contribute buccal epithelial cells collected with 4 cytology brushes. 85% of the subjects provided blood or blood plus brush samples; 15% gave brush samples only. Blood samples from 66 volunteer control subjects recruited in Ireland were used to obtain allele frequency estimates. Controls were screened and excluded if they reported a history of heavy drinking or problem alcohol use.

Details of the laboratory methods and error checking are provided elsewhere (Prescott et al., 2006). DNA was extracted using standard techniques. A 1020-marker autosomal genomewide screen was conducted by deCODE Genetics (http://www.decode.com/) using their standard panel of polymorphic microsatellite markers. The average marker spacing was 4 cm, and the average heterozygosity in our sample was 72.5% (min 6.3%, max 91.8%). Genotyping reliability was assessed in 17 individuals for whom we submitted 2 blinded samples for genotyping. The between-sample agreement ranged from 98.44% to 100%, with an average of 99.68%.

The genome scan was conducted with samples from 1,423 individuals, including 1,357 family members and 66 control subjects. We used GRR (Abecasis et al., 2001) to check for familial relationships/ nonpaternity and marker error. There were 39 families whose samples were excluded from analyses because 1 or more individuals had genotypes inconsistent with the reported family structure. After data cleaning, 1,289 samples were included in the analyses. The genotyping success rate among these individuals was 90.2%, reflecting 1,185,544 of a possible 1,314,780 genotypes (1,289 subjects × 1,020 markers).

Statistical Methods

The final sample used for linkage analyses included 485 informative families ranging from 2 to 12 members, with the majority of a family size of 2 (affected siblings, 81.1%), 3 (14.2%), or 4 (1.9%) persons. We performed genomewide multipoint linkage analyses using the variance component method in the Merlin program (Abecasis et al., 2002) to analyze the 5 quantitative traits. Because of missing parental data, the allele frequency estimates derived from 66-screened controls were applied in Merlin. This procedure produced linkage results very similar to those obtained when we used founders to estimate allele frequencies (results not shown). To compare a polygenic model with or without a QTL under the variance component framework, we use likelihood ratio test statistics, which are distributed under the null hypothesis of no linkage with a 0.5:0.5 mixture of a chi-square distribution with 1 df and a point mass at 0 (Self and Liang, 1987). Theoretically, the Lod score can be calculated according to dividing the log-likelihood differences by log(10). Because we are testing multiple phenotypes, we chose a marker significance level of p < 0.005, which corresponds to a Lod score of approximately 1.5 in our sample.

Because the variance component method is sensitive to nonnormality, before performing linkage analysis, each phenotype was transformed to normalize its distribution using the RANK procedure in SAS (SAS, 2002). We calculated intertrait correlations for the 5 quantitative traits with and without transformation. We also examined the correlations between each transformed quantitative trait with gender and body mass index.

RESULTS

Sample characteristics for the alcohol-related traits are shown in Table 1. There are about twice as many males as females in our sample, consistent with sex differences in the prevalence of AD. On average, men had significantly earlier onset of AD than women and exhibited more serious drinking problems (e.g., higher amount of maximum drinks in 24 hours and more withdrawal symptoms, etc.)
Table 1. Descriptive Statistics for Alcohol-Related Phenotypes Among Probands and Affected Siblings Whose Provided Interviewed Information (N = 1248)

<table>
<thead>
<tr>
<th>Male (n=699)</th>
<th>Female (n=439)</th>
<th>Correlations</th>
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<tr>
<td>ONSET</td>
<td>ISSENS</td>
<td>TOLMX</td>
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<tr>
<td>24.9 (8.0)</td>
<td>28.7 (10.2)</td>
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<tr>
<td>6.5 (3.1)</td>
<td>5.5 (2.6)</td>
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<td>13.6 (6.6)</td>
<td>10.4 (5.3)</td>
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<td>43.3 (19.6)</td>
<td>29.1 (14.6)</td>
<td>-0.14</td>
</tr>
<tr>
<td>7.3 (0.1)</td>
<td>6.7 (0.1)</td>
<td>-0.04</td>
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Note: ONSET, ranked age at onset for AD; ISSENS, ranked drinks in the first 5 times; TOLMX, ranked drinks in the drink the most period; MAX24, ranked maximum drinking in 24 hours; and WDSFS, ranked factor score of withdrawal symptoms. In the correlation matrix, the upper diagonal represents correlations between original phenotypes; the lower diagonal represents correlations between ranked phenotypes.

Gender differences were significant for all 5 original phenotypes (p<0.0001).

both p<0.01). Gender was significantly associated with the ISSENS, TOLMX, and MAX24 (r = 0.17–0.40), but body mass index was slightly associated only with TOLMX (r = –0.07). The same patterns of gender differences still existed after adjusting for body mass index. We therefore incorporated gender as a covariate in the variance component linkage analysis, but did not attempt to adjust for individual differences in body mass (note: after adjusting for gender, residuals of 5 transformed traits were still approximately normally distributed with skewness and kurtosis less than an absolute value of 1). The transformed phenotypes in Table 1 had the same pattern of correlation in the original phenotypes. The cross-trait correlations are low (r<0.15) for transformed phenotypes, except for the correlations between ISSENS and TOLMX, ISSENS and MAX24, and TOLMX and MAX24.

Table 2 presents the marker names and positions of suggestive linkage regions for each trait. Figure 1 shows the linkage signals for 9 chromosomes that corresponded to the peaks listed in Table 2. The top 3 figures display results mainly for ONSET and MAX24; the middle 4 figures display results for ISSENS and TOLMX; and the bottom 2 figures display results for WDSFS. For ONSET, 2 regions were found on chromosome 9, with a peak Lod score of $2.33\ (p = 0.0005)$. There were weaker signals for MAX24 on chromosome 12 (Lod = 1.52, p = 0.004) and 18 (Lod = 1.61, p = 0.003). For ISSENS, strong evidence for linkage was found on regions of chromosomes 11 (Lod = 2.87, p = 0.00014) and 1 (Lod = 1.89, p = 0.002). For TOLMX, suggestive regions were on chromosomes 1 (Lod = 1.78, p = 0.002), 6 (Lod = 1.51, p = 0.004), and 22 (Lod = 2.05, p = 0.0011). Regions on chromosome 2 (peak Lod = 2.2, p = 0.0007) and 15 (Lod = 1.69, p = 0.003) were associated with WDSFS.

Among several suggestive regions for these quantitative traits, there was little overlap across traits (see Fig. 1). The only exception was chromosome 1, where there were peaks for ISSENS and TOLMX at positions 100 and 130 cM, respectively.

**DISCUSSION**

Complex psychiatric disorders like alcoholism are diagnosed by subjective report symptoms or behaviors, e.g., tolerance or unsuccessful attempts to cut down on alcohol use, sometimes supplemented by hospital or other official records but not by biological markers or known indices of pathophysiology. Although results from adoption and twin studies have shown a substantial genetic influence on AD, what and where those genes are and how they act to influence AD symptoms are largely unknown. Most of the early published linkage studies focused on the diagnosis of AD (Foroud et al., 2000; Long et al., 1998; Reich et al., 1998), while some others adjusted for covariates or used narrower diagnostic definitions to define potentially more homogeneous groups for genetic analysis (Corbett et al., 2005; Guerrini et al., 2005; Hill et al., 2004; Nurnberger et al., 2001; Williams et al., 1999). Aware of the heterogeneity of AD diagnosis and inconsistent findings from previous linkage studies of alcoholism, several recent studies have reported linkage results for alcohol-related quantitative traits that contribute to the risk of AD, such as LR to ethanol (Schuckit et al., 2001; Wilhelmsen et al., 2003).

In the present study, we adopted 2 approaches to reduce heterogeneity. We focused on quantitative components of alcoholism, which may represent etiologically more homogeneous phenotypes, and we conducted the study using a culturally and genetically homogeneous population of alcoholic individuals from Ireland. Using this Irish sample, we previously identified 1 significant linkage peak located between 4q22-4q32 (Lod $=4.6, p = 2 \times 10^{-6}$ at D4S1611) for the phenotype of number of AD symptoms (Prescott et al., 2006), suggesting the importance of genetic variation in this region for the severity of alcoholism. Although this region has been consistently reported in linkage studies for AD diagnosis, only 1 previous linkage study for components of alcoholism reported the same region for severity (Ehlers et al., 2004a).

In the current study, we found suggestive linkage regions on chromosomes 1, 2, 6, 9, 11, 12, 15, 18, and 22, for a variety of narrowly defined alcohol-related traits, but not at our previously reported chromosome 4 region (see Fig. 2). However, several of our suggestive linkage regions overlapped with those identified in previous linkage studies, as well as some minor findings shown in Prescott et al., (2006) using the same sample, including 2q37, 9q21, 9q33, and 22q11. This again highlights the importance of genetic variation in this region for the severity of alcoholism. Although this region has been consistently reported in linkage studies for AD diagnosis, only 1 previous linkage study for components of alcoholism reported the same region for severity (Ehlers et al., 2004a).

In the current study, we found suggestive linkage regions on chromosomes 1, 2, 6, 9, 11, 12, 15, 18, and 22, for a variety of narrowly defined alcohol-related traits, but not at our previously reported chromosome 4 region (see Fig. 2). However, several of our suggestive linkage regions overlapped with those identified in previous linkage studies, as well as some minor findings shown in Prescott et al., (2006) using the same sample, including 2q37, 9q21, 9q33, and 22q11. This again highlights the genetic heterogeneity of AD that involves different clusters of genes or implies that different classes of gene action (i.e., the susceptibility genes or the modifier genes) were detected in different genome regions (Fanous and Kendler, 2005). It may also result from the limited power of conducting linkage for a discrete trait. Using quantitative methods to combine genetic variation across the genome, our results suggest that increased power is needed to detect the genetic heterogeneity of AD.
components of alcoholism, we might have more power to
detect susceptibility loci in the whole genome for the risk
of AD.

Three of our measures were associated with response to
ethanol: initial sensitivity (ISENS), chronic tolerance
(TOLMX), and maximum consumption (MAX24). The
results obtained for these overlapped with those from
several other studies using AD diagnosis or similar pheno-
types based on self-report and laboratory procedures.

Linkage peaks for ISENS (130 cM) and TOLMX (100
cM) on chromosome 1 overlap with findings in the COGA
sample for AD (Guerrini et al., 2005; Foroud et al., 2000;
Reich et al., 1998), sex- and age-adjusted AD (Corbett
et al., 2005), and AD/major depression (Nurnberger et al.,
2001). More interestingly, on chromosome 1 the peak of
our quantitative ISENS overlapped with the linkage signal
obtained by Schuckit et al. (2001) for their LR measure
(a dichotomous variable based on the ISENS measure). As
the ISENS to ethanol is a risk factor for developing AD,
the consistent evidence from the COGA sample in this
region supports for searching candidate genes predispos-
ing to ethanol response under this peak. In addition, the
weak signals around 220 cM on chromosome 1 for both
ISENS and TOLMX traits are consistent with the suggest-
ive region in other linkage studies for similar measures of
alcohol response, including scores on the Subjective High

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**Table 2. Lod Score and p Value for Suggestive Linkage Regions of Quantitative Alcohol-Related Traits**

<table>
<thead>
<tr>
<th>Locus</th>
<th>Position (cM)a</th>
<th>ONSET Lod</th>
<th>p Value</th>
<th>ISENS Lod</th>
<th>p Value</th>
<th>TOLMX Lod</th>
<th>p Value</th>
<th>MAX24 Lod</th>
<th>p Value</th>
<th>WDSFS Lod</th>
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*Note:* ONSET, ranked age at onset for AD; ISENS, ranked drinks in the first 5 times; TOLMX, ranked drinks in the drink the most period; MAX24, ranked maximum drinking in 24 hours; and WDSFS, ranked factor score of Withdrawal symptoms.

*aThe genetic position uses the deCODE map.*
Assessment Scale for response to ethanol (Wilhelmsen et al., 2003) and maximum single-day alcohol consumption (Bergen et al., 2003). These overall results indicate that there may be some genes influencing the LR to ethanol in these chromosome 1 regions.

The region of chromosome 11 for which we obtained a peak for ISENS was also identified in other linkage studies for AD (Long et al., 1998; Reich et al., 1998) and body sway during an alcohol challenge procedure (Wilhelmsen et al., 2003), but did not overlap with the linkage peak (11p14) obtained by Schuckit et al. (2001) using the same measurement of quantitative ISENS as in this study. This discrepancy may result from stochastic variation using different samples or identification of indeed different genes corresponding to this trait. For TOLMX, the linkage peak we found on chromosome 6 is near the peak reported for maximum alcohol consumption (Bergen et al., 2003), and the peak on chromosome 22 is closed by the peaks for maximum alcohol consumption (Bergen et al., 2003) and for body sway (Wilhelmsen et al., 2003).

For MAX24, suggestive regions were on chromosomes 12 and 18. These are close to areas identified for AD (Hill et al., 2004; Long et al., 1998; Reich et al., 1998) and for other quantitative traits, including alcoholism severity on chromosome 12 (Ehlers et al., 2004a) and body sway on chromosome 18 (Wilhelmsen et al., 2003). However, linkage results for a similar measure, MAX24, using a different transformation method (Bergen et al., 2003) were not found on either chromosome 12 or 18 but did overlap or were close to our suggestive peaks for another measure, TOLMX, on chromosomes 6 and 22. Although several studies have applied different assessments of response to alcohol, the linkage regions are somewhat consistent across studies.

Our strongest evidence for linkage to age at onset (ONSET) of AD was found on chromosome 9. The same region was implicated by Schuckit et al. (2001) for ISENS to alcohol. In the same region, we observed a weak peak for ISENS, but this did not reach a suggestive level. These results could indicate that individuals who are less sensitive to alcohol may need to drink more to obtain effects and those who do so accelerate the development of AD.

Our strongest evidence for linkage to age at onset (ONSET) of AD was found on chromosome 9. The same region was implicated by Schuckit et al. (2001) for ISENS to alcohol. In the same region, we observed a weak peak for ISENS, but this did not reach a suggestive level. These results could indicate that individuals who are less sensitive to alcohol may need to drink more to obtain effects and those who do so accelerate the development of AD.

Only one previous linkage study reported findings for a withdrawal-related trait. Ehlers et al. (2004a) described...
4 suggestive regions on chromosomes 6, 15, and 16 for withdrawal severity. One peak on chromosome 15 in that study is within about 25 cM of the peak for our withdrawal factor. Because of the imprecision of linkage, these peaks may be close enough to be in the same region (Roberts et al., 1999). It is also noteworthy that several loci have been identified in linkage analyses of AD in the same or nearby regions as our findings for WDSFS on chromosomes 2 (Hill et al., 2004; Reich et al., 1998) and 15 (Reich et al., 1998). In addition, the linkage region on chromosome 2 for our withdrawal factor is the same as the linkage evidence found from other studies for ISENS to alcohol (Schuckit et al., 2001) and for a narrowly defined phenotype of comorbid alcoholism and major depression (Nurnberger et al., 2001). These findings suggest that this region is rich in alcohol-related genes as the evidence comes from a variety of traits.

In summary, some of our suggestive linkage regions overlapped with findings from previous linkage studies using AD diagnosis or similar quantitative traits. However, some of our findings were discrepant from other studies (e.g., the peak for ISENS on chromosome 11; Schuckit et al., 2001; and the peak for withdrawal on chromosome 15 Ehlers et al., 2004a). These differences may reflect genetic heterogeneity, methodological differences among studies (e.g., including unaffected siblings in the COGA sample but not in our sample), or sampling error.

Evidence supporting the involvement of these regions is also provided when they contain functional candidate genes, such as neurotransmitter-related genes. For instance, the chromosome 22 suggestive region for TOLMX in the present study includes the COMT (catechol-O-methyltransferase) gene, which is critical to help catalyze the formation of many neurotransmitters, including dopamine, epinephrine, and norepinephrine (http://www.ncbi.nlm.nih.gov, NCBI gene database). Another example are the dopamine receptor-binding genes, which have been found to be associated with addictive behavior in animal studies, such as sensitization to ethanol (Broadbent et al., 2005) and ethanol preference (Thanos et al., 2005). The dopamine receptor D2 gene is on chromosome 11 (11q21-23), in the same region as our linkage peak for ISENS. This gene plays a central role in the neuro-modulation of appetitive behaviors and is involved in reward-mediating mesocorticolimbic pathways, which has been investigated in many addictive behavior studies (reviewed by Bowirrat and Oscar-Berman, 2005).

Some evidence from animal studies shows the important role of central nervous system genes in alcohol-related traits. For instance, one study used inbred long-sleep and short-sleep strains of mice to study ISENS to alcohol and found 3 promising candidates genes (Znf142, Ptprn, and Znf133) in the central nervous system involved in the differential sensitivity to alcohol between the 2 strains of mice (Ehringer et al., 2002). The homologous genes for Znf142 and Ptprn in humans are located at chromosome 2 (2q35), in the vicinity of our peak for withdrawal severity at 2q34-37. In addition, results from animal QTL studies suggest that several genes associated with a variety of alcohol-related traits (including acute and chronic withdrawal, preference drinking, stimulate activity, loss of righting reflex, etc.) corresponded to this region of human chromosome 2 (see review articles by Crabbe, 1999; Crabbe et al., 2001).

One very important group of enzymes in the alcohol metabolism pathway is the ALDH (aldehyde dehydrogenase) family, located on several human chromosomes, including chromosomes 9, 12, 15, and 17. Several of our reported linkage regions for a variety of alcohol-related traits are in the same regions as these genes. ALDH is the second enzyme of the major oxidative pathway of alcohol metabolism. There are 2 major liver isoforms of this enzyme: cytosolic and mitochondrial. Although some polymorphisms of this gene have a higher frequency in Asian populations, some are highly polymorphic in both Caucasians and Orientals (Yoshida, 1992). Recent studies have suggested that this gene may also be relevant to the risk for AD in a Caucasian population (Agarwal, 1997). The gene that encodes a cytosolic isoenzyme, which has a high affinity for aldehydes, ALDH1A1, is located at 9q13-21 in the vicinity of our peak for ONSET. A polymorphism of this gene, ALDH1A1*2, has been found to be associated with protection from the development of alcohol and other substance use disorders in American Indians (Ehlers et al., 2004b). Another gene, ALDH2 plays a protective role by causing alcohol intolerance and is located at chromosome 12 (12q24.1-24.2) under the peak of MAX24 in the present study. Individuals lacking the enzyme undergo the alcohol-flush reaction when they drink alcohol, and this unpleasant symptom tends to reduce alcohol consumption. As pointed out by Crabb...
(1990), a single-base mutation in ALDH2 is responsible for the acute alcohol-flushing reaction in Asians and has become a well-characterized genetic factor influencing alcohol-drinking behavior. One empirical study conducted in Han Chinese living in Taiwan demonstrated that alcoholic patients had significantly lower frequencies of ALDH2*2 (Chen et al., 1999), and this association warrants further study in Caucasians. Another gene in the ALDH family, ALDH1A3, which locates at 15q26, is under the same region of our peak for WDSFS. In addition, in the nearby suggestive region of MAX24 on chromosome 18, a gene located at 18q22 called ZADH2, the zinc-binding alcohol dehydrogenase, is also involved in the alcohol metabolic pathway.

In a brief power calculation, based on our sample in linkage analysis (485 informative families), we have more than 95% power to detect a QTL accounting for 20% of the trait variance (with nonfamiliar factors accounting for 30% of the variance) and more than 60% power for a 10% QTL (http://pngu.mgh.harvard.edu/~purcell/gpc). In addition, we are aware of the normality issue in variance component linkage analysis that type I error may be affected with a nonnormally distributed trait especially for kurtosis (Blangero et al., 2000). After we performed transformation on 5 alcohol-related traits, the skewness and kurtosis were close to 0 for each trait, except for WDSFS, with a kurtosis of 0.3. We then performed 10,000 permutation runs for WDSFS to obtain the empirical \( p \) value under null hypothesis of no linkage. For 2 markers that have the highest Lod score on chromosomes 2 (Lod = 2.2, \( p = 0.0007 \) at D2s2973) and 15 (Lod = 1.69, \( p = 0.003 \) at D15S1014), the empirical \( p \) values are very significant (\( p = 0.00016 \) for D2s2973, and \( p = 0.00024 \) for D15S1014).

The current study has noteworthy strengths and several limitations. Its strengths include quantitative measures of components of AD and use of a genetically and culturally homogeneous population. Our use of an affected sib-pair design means that we do not need to make assumptions about the “unaffected” status of nondrinkers. However, this severely affected sample means that the range of these quantitative measures is limited and does not represent the full distribution of these measures in the general population. It is possible that the linkage regions that we identified include genes that influence the severity of affected status instead of loci being responsible for the proposed quantitative traits. A second limitation is our reliance on transformation to normalize the distributions of our trait scores. Although this procedure has been used in many studies, it is possible that this may have biased results. Third, our measures are subject to the usual measurement error and recall bias associated with self-report. Fourth, even with our large sample size, we had limited power to detect linkage to regions containing susceptibility loci with minor effects. Fifth, some results could be false positives arising from testing multiple phenotypes and relatively liberal significance levels.


Guerrini I, Cook CC, Kest W, Devigh A, McQuillan A, Curtis D, Gurling HM (2005) Genetic linkage analysis supports the presence of two susceptibility loci for alcoholism and heavy drinking on chromosome 1q21-1.1-2 and 1q21.3-2.42. BMC Genet 6:11–18.


