Published in final edited form as:

Drug Alcohol Depend. 2011 January 15; 113(2-3): 125–132. doi:10.1016/j.drugalcdep.2010.07.017.

Linkage Scan of Alcohol Dependence in the UCSF Family Alcoholism Study

Ian R. Gizer^{1,2}, Cindy L. Ehlers³, Cassandra Vietan⁴, Kimberly L. Seaton-Smith⁵, Heidi S. Feiler⁶, James V. Lee⁷, Samantha K. Segall¹, David A. Gilder³, and Kirk C. Wilhelmsen^{1,2,*}

¹ Department of Genetics, University of North Carolina, Chapel Hill, NC 27599

² Bowles Center for Alcohol Studies, University of North Carolina, Chapel Hill, NC 27599

³ Department of Molecular and Integrative Neurosciences, The Scripps Research Institute, La Jolla, CA 92037

⁴ California Pacific Medical Center, San Francisco, CA 94115

⁵ Arizona College of Osteopathic Medicine, Midwestern University, Glendale, AZ 85308

⁶ Department of Laboratory Medicine, University of California, San Francisco, CA 94143

⁷ School of Molecular and Cell Biology, University of Illinois, Urbana-Champaign, IL 61801

⁸ Department of Neurology, Carolina Genome Center, University of North Carolina, Chapel Hill, NC 27599

Abstract

Ample data suggest alcohol dependence represents a heritable condition, and several research groups have performed linkage analysis to identify genomic regions influencing this disorder. In the present study, a genome-wide linkage scan for alcohol dependence was conducted in a community sample of 565 probands and 1080 first-degree relatives recruited through the UCSF Family Alcoholism Study. The Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) was used to derive DSM-IV alcohol dependence diagnoses. Although no loci achieved genome-wide significance (i.e., LOD score > 3.0), several linkage peaks of interest (i.e., LOD score > 1.0) were identified. When the strict DSM-IV alcohol dependence diagnosis requiring the temporal clustering of symptoms served as the phenotype, linkage peaks were identified on chromosomes 1p36.31–p36.22, 2q37.3, 8q24.3, and 18p11.21–p11.2. When the temporal clustering of symptoms was not required, linkage peaks were again identified on chromosomes 1p36.31–p36.22 and 8q24.3 as well as novel loci on chromosomes 1p22.3, 2p24.3–p24.1, 9p24.1–p23, and 22q12.3–q13.1. Follow-up analyses were conducted by performing linkage analysis for the 12 alcohol dependence symptoms assessed by the SSAGA across the support intervals for the observed linkage peaks. These analyses demonstrated that different collections of symptoms often assessing distinct aspects of alcohol dependence (e.g., uncontrollable drinking and withdrawal vs. tolerance and drinking despite health problems) contributed to each linkage peak and often yielded LOD scores exceeding that reported for the alcohol dependence diagnosis. Such findings provide insight into how specific genomic regions may influence distinct aspects of alcohol dependence.

Corresponding Author: Kirk C. Wilhelmsen, M.D., Ph.D., University of North Carolina at Chapel Hill, 120 Mason Farm Road, Room 5015 Genetic Medicine Building CB 7264, Chapel Hill, NC 27599-7264, Phone: Office (919) 966-1373, Fax (919) 966-3630, kirk@med.unc.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Keywords

alcoholism; DSM-IV alcohol dependence; addiction; substance dependence; linkage

1. Introduction

The lifetime prevalence rate of alcohol dependence has been estimated at 12.5% to 13.2% (American Psychiatric Association, 1994; Hasin et al., 2007). Numerous family, twin, and adoption studies (e.g., Allgulander et al., 1991; Goodwin et al., 1973; Heath et al., 1997; Kendler et al., 1997; Reich et al., 1988) have suggested that alcohol dependence represents a heritable condition, and a growing number of studies have performed linkage analysis to identify genomic regions associated with the disorder (e.g., Bergen et al., 2003; Gelernter et al., 2008; Hill et al., 2004; Prescott et al., 2006). Nonetheless, few loci have shown replicable evidence of association with alcohol use phenotypes across studies (Dick and Bierut, 2006; Edenberg and Foroud, 2006). Thus, further studies are needed to identify genomic regions that confer risk for alcohol dependence.

The most widely replicated linkage finding for alcohol dependence has been to a region on chromosome 4q containing the alcohol dehydrogenase (ADH) gene cluster. This region was originally implicated in the Collaborative Studies on the Genetics of Alcoholism (COGA) sample (Reich et al., 1998) and was replicated in two subsequent studies (Ehlers et al., 2004; Prescott et al., 2006). A second linkage region has been reported on chromosome 4p that contains a GABA_A receptor subunit gene cluster (Long et al., 1998; Porjesz et al., 2002; Reich et al., 1998). Notably, candidate gene studies of the ADH and GABA_A gene clusters have yielded significant evidence of association to alcohol dependence (Edenberg et al., 2004; Mulligan et al., 2003) further validating these linkage findings. Finally, linkage to a region of chromosome 1p has been reported in multiple studies (Hill et al., 2004; Reich et al., 1998). These results represent important progress in understanding the genetic influences that contribute to alcohol dependence, but it is important to note the difficulties in identifying such relations as there have been failures to replicate the evidence for linkage to each of these regions as noted in several reviews (e.g., Dick and Bierut, 2006; Gelernter and Kranzler, 2009).

The most commonly used definitions of alcohol dependence are delineated in the Diagnostic and Statistical Manual of Mental Disorders, 4th edition (American Psychiatric Association, 1994) (DSM-IV) and International Classification of Disease and Related Health Problems, 10th edition (World Health Organization, 1992) (ICD-10), which require endorsement of 3 out of 6 to 7 symptom groups. Importantly, this allows two individuals with non-overlapping symptoms to meet criteria for alcohol dependence and can result in significant phenotypic heterogeneity. It is likely that such heterogeneity is responsible in part for the difficulties in detecting susceptibility loci for alcohol dependence. Notably, Prescott and colleagues (2006) utilized an approach to linkage with the potential to explain such heterogeneity. Specifically, they evaluated which alcohol dependence symptoms were contributing to each of the linkage signals reported in their sample. For example, they noted that the symptoms of tolerance, binge drinking, and inability to quit drinking provided the strongest contributions to an observed linkage peak on chromosome 4.

The current study conducted a genome-wide linkage scan for alcohol dependence in the UCSF Family Alcoholism Study to support and extend previous findings of genetic linkage. First, we report the results of a linkage scan for alcohol dependence using DSM-IV diagnoses requiring a participant exhibit 3 out of 7 alcohol dependence symptom clusters during the same 12 month period. Second, we report the results of a linkage scan allowing

for a broader alcohol dependence phenotype in which the same DSM-IV criteria were used but without requiring that the symptom clusters exhibit temporal clustering or overlap. The latter definition corresponds more closely to the DSM-III-R criteria (APA, 1987), which provides a more inclusive definition of alcohol dependence relative to DSM-IV (e.g., Schuckit et al., 1994). This approach is justified by previous studies that have used alternative definitions of alcohol dependence (Reich et al., 1998; Saccone et al., 2000; Wilhelmsen et al., 2003) to more fully test for susceptibility loci contributing to alcohol misuse. We then conducted follow-up analyses for identified linkage peaks by conducting linkage analysis for each of the 12 alcohol dependence symptoms assessed by the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) (Bucholz et al., 1994) to determine which symptoms were responsible for the linkage signal. Finally, exploratory linkage analyses were conducted for symptom clusters assessing withdrawal, severe drinking, and medical/mental health problems.

2. Methods

2.1 Participants

Data for this report were obtained from the UCSF Family Alcoholism Study, a nationwide study on the genetics of alcoholism and other substance dependence (Seaton et al., 2004; Vieten et al., 2004). In brief, probands were sampled from the community through semi-targeted direct mail, a web site, press releases, advertisements and from alumni of treatment centers across the nation. Probands were invited to participate if they met screening criteria for alcohol dependence at some point in their lifetime and had at least one sibling or both parents available to participate in the study. With the permission of the proband, relatives were invited by mail to participate.

A modified version of the SSAGA (Bucholz et al., 1994), an interview developed by COGA, was used to make DSM-IV alcohol and other substance misuse and psychiatric diagnoses. Probands with serious drug addictions (e.g., stimulants, cocaine, or opiates) and those who reported any history of intravenous substance use were excluded. Probands were excluded if, upon screening, they reported a current or past diagnosis of schizophrenia, bipolar disorder, or other psychiatric illness involving psychotic symptoms (those with depressive and anxiety disorders were accepted); a life-threatening illness; or an inability to speak and read English.

Two thousand five hundred and twenty-four individuals were enrolled in the UCSF Family Alcoholism Study of which 1647 were included in the reported linkage analysis. These participants had a mean age of 49.9 ± 12.8 years, a mean educational level of 14.6 ± 2.9 years, and a mean annual income of $\$59,663 \pm \$55,542$ (median, \$45,000). Racial distribution was 93% Caucasian, 3% Hispanic American, 2% African-American, and 1% each Native American and other. No attempt was made to exclude or over sample minorities. Probands were 62% female. Relatives of probands were 37% alcohol dependent.

An unselected general population sample of 147 individuals with similar demographic characteristics to the family sample (58% female, 92% Caucasian) was recruited to assess phenotype base rates. Letters were sent to residents of the same geographical areas as the family samples, requesting participation in a study on "health behaviors and characteristics" to avoid a sample biased toward participation in a study on alcoholism. No inclusion/exclusion criteria were applied. Twenty-four (16%) control participants met criteria for DSM-IV alcohol dependence, which is similar to the prevalence rate for the disorder reported in larger epidemiological studies (e.g., Hasin et al., 2007).

2.2 DNA Collection and Genotyping

The DNA extraction procedure and genotyping protocol have been previously described (Wilhelmsen et al., 2003). Briefly, DNA was isolated from whole blood using a commercial kit (Gentra, Minneapolis, MN), and genotypes for a panel of microsatellite polymorphisms were generated using fluorescently labeled polymerase chain reaction (PCR) primers (HD5, version 2.0; Applied Biosystems, Foster City, CA). The HD5 panel set consisted of 811 markers with an average marker-to-marker distance of 4.6 cM (maximum, 14 cM) and an average heterozygosity of greater than 77%. A small subset of markers was omitted from the panel because of null alleles, irregular allele spacing or other problems with reproducibility. None of the omitted markers were adjacent to other omitted markers.

The sizes of marker amplicons were determined (blinded to pedigree structure and subject characteristics) from electropherograms produced with an ABI 3700 (Applied Biosystems, Foster City, CA) using the Genotyper software package (ABI). All electropherograms were visually inspected and exported from Genotyper in base pair sizes relative to the standard measured to one hundredth of a base pair. Fragment sizes were binned to alleles by using an automated algorithm developed by one of the authors (KCW) which assumes that the distribution of allele sizes will have a sine-squared distribution with a fitted periodicity near two base pairs. The program determines the best periodicity and phase for the modeled distribution relative to the observed distribution. Fragments that are distributed between minimums of the modeled distribution are assumed to be the same allele. Allele frequencies observed in the full sample were used for all analysis. The sex-averaged marker map order obtained from the manufacturer was used and verified with the family data from the current sample.

The genotypes for all of the autosomal markers were analyzed using Pedigree Relationship Statistical Test (PREST) (McPeck and Sun, 2000) to detect sample and pedigree structure errors. DNA was reisolated from a stored frozen blood specimen and the genotyping repeated for any individual with a probable error. If re-genotyping failed to resolve the error, the problematic genotype was subsequently treated as missing. Fifteen families were identified with pedigree structure errors. Five were resolved following re-genotyping. The program Pedcheck was used to detect non-Mendelian inheritance (O'Connell and Weeks, 1998). Markers with a high frequency of Mendelian segregation errors were excluded from analysis, and for isolated Mendelian errors, the genotypes for the entire family were excluded for the specific marker that yielded the error. Pedcheck identified 3104 Mendelian errors resulting in 7714 lost genotypes and the exclusion of one marker. To further reduce errors, the probability that each genotype was correct was assessed using the error-checking algorithm implemented in MERLIN, and as suggested by the authors, genotypes that had a probability of less than 0.025 of being correct were removed from further consideration. (Abecasis et al., 2002). A total of 1867 problematic genotypes were identified and removed by MERLIN. Following these quality control procedures, a total of 1,269,708 genotypes were accepted with a success rate of 99.6%.

2.3 Analysis

Both genotype and phenotype data were available for 1647 individuals, and phenotype but not genotype data was available for an additional 875 individuals. Seven hundred and thirteen families were considered genetically informative for linkage analysis. Families that contained sibling, half-sibling, avuncular or cousin pairs were included as being potentially genetically informative. When considering all participants, these families ranged in size from 3 to 20 subjects (average 2.90 ± 2.44). The data includes: 1085 sibling, 40 half sibling, 17 grandparent-grandchild, 238 avuncular, and 32 cousin genetically-informative relative pairs. An additional 177 families contained only a single individual with phenotype data.

These individuals were included within some analyses to the extent that they contribute information about trait means and variance and the impact of covariates.

Initial analyses were conducted using SOLAR (Almasy and Blangero, 1998) to test the alcohol dependence diagnosis with and without the temporal clustering of symptoms for evidence of familial resemblance that would be consistent with genetic transmission. Similar analyses were conducted for the 12 alcohol dependence symptoms assessed by the SSAGA (see Table 1 for list of symptoms). SOLAR estimates an h^2 parameter by partitioning the trait relative pair covariance into additive genetic and environmental contributions while correcting for any covariates included in the model. Participant's age at the time of evaluation and sex were evaluated as potential covariates and retained if they accounted for at least 5% of the total variance. The probability that h^2 was greater than zero was determined using a Student's t-test for each scale. This test of significance was used to evaluate the potential genetic transmission of the alcohol dependence phenotypes and constituent symptoms. Corrections for multiple testing were not made given the correlated nature of the alcohol dependence symptoms.

The variance components method implemented in SOLAR was then used to calculate multipoint LOD scores across the genome at 1 cM intervals for alcohol dependence diagnoses with and without the clustering criterion. Linkage peaks exceeding a LOD score of 2.2 were reported as yielding suggestive evidence for linkage as described by Lander and Kruglyak (1995). In addition, linkage peaks exceeding a LOD score of 1.0 were reported as regions of interest. While these latter peaks represent weaker evidence for linkage, reporting such findings may aid future studies and meta-analytic reviews. To ensure that identified linkage peaks were not the result of a small subset of pedigrees, homogeneity tests were performed using the HLOD (Goring, 2002) function available in SOLAR. Specifically, this test contrasts a null model in which families belong to a single distribution exhibiting genetic linkage to the tested locus against an alternative model in which families belong to one of two distributions only one of which shows evidence of genetic linkage to the tested locus.

Follow-up analyses were then conducted for 8 identified linkage peaks by conducting linkage analysis for each of the 12 alcohol dependence symptoms assessed by the SSAGA across the support intervals for the identified peaks with each symptom at each peak representing a unique statistical test. Support intervals were defined as the region surrounding a linkage peak yielding a LOD score that was greater than the maximum LOD – 1 in each direction. As a final step, exploratory linkage analyses were conducted for the presence of one or more withdrawal symptoms (“experienced uncontrollable shaking due to drinking” or “experienced withdrawal symptoms”), one or more symptoms of severe drinking (“unable to reduce drinking,” “wanted to quit drinking 3 or more times,” “little time for non-drinking related activities,” or “drinking interfered with work or social responsibilities”), and one or more symptoms related to medical or mental health problems (“continued drinking despite psychological problems” or “Continued drinking despite physical health problem”). These symptom grouping were used previously to identify genetic loci that confer risk to alcohol dependence in a Native American population (Ehlers et al., 2004). The follow-up and exploratory analyses were considered exploratory and descriptive in nature, LOD scores were only provided as a measure of magnitude and were not used to determine significance. Because the current sample was selected for alcohol dependence, it should be noted that prevalence rates for the alcohol dependence diagnoses and respective symptoms in the unselected control sample were estimated and included in the tested models to correct for ascertainment bias when calculating h^2 and linkage.

3. Results

The h^2 estimate for the alcohol dependence diagnosis was significant whether or not the temporal clustering of symptoms was required ($h^2 = .278 \pm .068$, $p < .001$ and $h^2 = .173 \pm .058$, $p = .007$, respectively). In addition, the h^2 estimates for 11 of the 12 alcohol dependence symptoms were also significant ($p < .05$). The 12th symptom (Continued drinking despite psychological problems) did not reach significance ($p = .18$). Despite the nonsignificant result, this final symptom was included in the follow-up linkage analyses to provide a complete examination of the symptom-level linkage data in relation to the observed linkage peaks for alcohol dependence (see Table 1 for complete results).

Alcohol Dependence with Temporal Clustering of Symptoms

Linkage analysis of the alcohol dependence diagnosis requiring the temporal clustering of symptoms failed to yield any peaks that achieved genome-wide significance, but four peaks of interest were identified (see Table 2 and Figure 1). The first peak was located on chromosome 1 at approximately 11 cM (LOD = 1.41) with a support interval that extended from the p terminus of the chromosome to 39 cM. The second peak was located on chromosome 2 at 287 cM (LOD = 1.15) with a support interval that extended from 248 cM to the q terminus. The third peak was located on chromosome 8 at 163 cM (LOD = 1.47) with a support interval that extended from 158 cM to the q terminus at 175 cM. The fourth peak was located on chromosome 18 at approximately 48 cM (LOD = 1.27) with a support interval that extended from 26 – 60 cM. Notably, there was no evidence of heterogeneity in LOD scores between families as each pedigree had an estimated alpha, which can be interpreted as the probability of linkage for a given family, that was >0.99 for each peak as estimated by HLOD (Goring, 2002).

Linkage analysis of the 12 alcohol dependence symptoms assessed by the SSAGA was then conducted for the support intervals of the 4 linkage peaks. Both the chromosomes 1 and 2 peaks showed evidence of linkage to a broad range of symptoms from 4 of the 7 DSM-IV symptom clusters. In contrast, the chromosomes 8 and 18 peaks showed evidence of linkage to a much narrower range of symptoms with only 2 DSM-IV symptom clusters represented for each peak. The linked symptoms and the associated LOD scores for each peak are shown in Table 3.

Alcohol Dependence without Temporal Clustering of Symptoms

Linkage analysis of the alcohol dependence diagnosis when the temporal clustering of symptoms was not required also failed to yield any peaks that achieved genome-wide significance, but six peaks did exceed a LOD score of 1.0 (see Table 2 and Figure 1). The first peak overlapped with that described for the alcohol dependence diagnosis when the temporal clustering criterion was used (chromosome 1 at 12 cM, LOD = 1.24). The second peak was also located on chromosome 1 though closer to the centromere at 114 cM (LOD = 1.19) with a support interval that extended from 103 – 127 cM. The third peak was found on chromosome 2 at approximately 32 cM (LOD = 2.42) with a support interval that extended from 17 – 36 cM. The fourth peak overlapped with the peak on chromosome 8 described for the alcohol dependence diagnosis when the temporal clustering criterion was used (LOD = 1.11). The fifth peak was located on chromosome 9 at 18 cM (LOD = 1.70) with a support interval that extended from 6 – 27 cM, and the sixth peak was located on chromosome 22 at approximately 36 cM (LOD = 1.78) with a support interval that extended from 29 – 47 cM. Notably, there was no evidence of locus heterogeneity as each pedigree had an estimated alpha of >0.99 for each peak as estimated by HLOD (Goring, 2002).

Follow-up linkage analyses of the 12 alcohol dependence symptoms assessed by the SSAGA were then conducted for the reported linkage peaks. For the chromosome 1 peak at 114 cM, a broad range of symptoms assessing 4 of the 7 DSM-IV symptom clusters yielded evidence of linkage within the support interval. Both the chromosome 2 peak at 32 cM and the chromosome 22 peak showed evidence of linkage with symptoms that fell into 3 of the 7 symptom clusters. Finally, the chromosome 9 region yielded evidence for linkage with a narrower range of symptoms with only 2 of the 7 DSM-IV symptom clusters represented. The symptom-specific LOD scores are shown in Table 3.

To investigate potential explanations for the limited overlap in linkage findings when the temporal clustering of symptoms was and was not required for the alcohol dependence diagnosis, we conducted a post hoc analysis comparing the average age-of-onset of those symptoms associated with linkage peaks for the alcohol dependence diagnosis when the temporal clustering of symptoms was and was not required. Regions that showed linkage to both phenotypes (i.e., chromosome 1 at 12 cM and chromosome 8 at 163 cM) were attributed to the phenotype that showed the higher LOD score. A nonparametric, rank-order Mann-Whitney U test suggested that symptoms linked to peaks for the alcohol diagnosis when the temporal clustering of symptoms was required showed a later age-of-onset than symptoms linked to peaks when the temporal clustering of symptoms was not required ($U = 48.50$, $p = .038$).

3.1 Exploratory Analysis of Alcohol Dependence Symptoms

The full results of the exploratory linkage analysis of withdrawal, severe drinking, and medical/mental health symptoms are presented in Table 4. The strongest result was observed for symptoms of severe drinking on chromosome 1 at 20 cM (LOD = 3.08). LOD scores greater than 2.0 were also observed for severe drinking symptoms on chromosome 2 at 41 cM (LOD = 2.12) and chromosome 19 at 33 cM (LOD = 2.62) and for withdrawal symptoms on chromosome 9 at 161 cM (LOD = 2.06).

4. Discussion

The primary aim of the current study was to conduct a genome-wide linkage scan of alcohol dependence in the UCSF Family Alcoholism Study to support and extend the findings of previous linkage studies. When alcohol diagnoses were derived from the SSAGA using the full DSM-IV criteria including the requirement that presentation of symptoms overlap or cluster temporally, the strongest linkage peak was identified at chromosome 8q24.3. Though somewhat distant from the present peak, a region of chromosome 8 (60 cM centromeric) has been implicated in alcohol dependence in two previous studies (Bergen et al., 2003; Corbett et al., 2005). A novel locus was identified at chromosome 1p36.31–p36.22. Although no previous linkage studies of alcohol dependence have identified this region as a susceptibility locus, previous studies have linked this region to depression (McGuffin et al., 2005; Nash et al., 2004) and conduct disorder (Dick et al., 2004), which are highly co-morbid with alcohol dependence (Hasin et al., 2007). A third locus was identified at chromosome 18p11.21–p11.2, and notably, multiple studies from four independent samples have linked this region to alcohol dependence-related phenotypes (Hill et al., 2004; Kuo et al., 2006; Prescott et al., 2006; Schuckit et al., 2001; Schuckit et al., 2005; Wilhelmsen et al., 2005).

In a second linkage analysis, alcohol diagnoses were derived from the SSAGA using the DSM-IV criteria with the exception of the criterion requiring the temporal overlap or clustering of symptoms to provide a broader alcohol misuse phenotype as suggested by previous studies (Reich et al., 1998; Saccone et al., 2000; Wilhelmsen et al., 2003). Four additional linkage peaks were identified in this analysis. The strongest peak was identified at chromosome 2p24.3–p24.1. This region has been implicated in alcohol misuse phenotypes in

a previous study (Wilhelmsen et al., 2005), and two additional studies identified a region approximately 60 cM centromeric of the current locus (Foroud et al., 2000; Reich et al., 1998). Further, previous linkage studies have suggested this region is involved in aspects of antisocial behavior (Ehlers et al., 2008; Kendler et al., 2006) and reward dependence (Cloninger et al., 1998), which are thought to influence drinking behavior. The three additional loci identified in the present study, 1p22.3 (Corbett et al., 2005; Foroud et al., 2000; Guerrini et al., 2005; Hill et al., 2004; Kuo et al., 2006; Reich et al., 1998), 9p24.1-p23 (Gelernter et al., 2008; Long et al., 1998; Prescott et al., 2006) and 22q12.3-13.1 (Bergen et al., 2003; Foroud et al., 2000; Prescott et al., 2006), have all been previously identified in linkage studies of alcohol misuse phenotypes. Thus, the findings reported in the present study provide further evidence supporting several genomic regions as harboring susceptibility loci for alcohol dependence.

The limited overlap in linkage findings when the temporal clustering of symptoms was and was not required for the alcohol dependence diagnosis was somewhat unexpected, and merits further investigation. In a previous study, we estimated the average age-of-onset of the 36 'alcohol related life events' assessed by the SSAGA, including the 12 alcohol dependence symptoms described in the present study, in the UCSF Family Study (Ehlers et al., 2010). Applying those data to the present study, we observed that linkage evidence for the alcohol dependence diagnosis when the temporal clustering of symptoms *was* required was driven by symptoms with a later age-of-onset that were more characteristic of the long-term consequences of persistent drinking, whereas linkage evidence for the alcohol dependence diagnosis when the temporal clustering of symptoms *was not* required was driven by symptoms with an earlier age-of-onset that were more characteristic of the short-term consequences of drinking. This provides a demonstration of how differences in ascertainment or phenotyping strategies can result in biases toward specific genetic liabilities involved in alcohol misuse and away from others.

Linkage analysis of the 12 alcohol dependence symptoms also provided insights into the relations between specific genomic regions and the alcohol dependence diagnosis. For example, the linkage evidence for the chromosome 1 locus at 11 cM was driven by a broad range of symptoms including spending significant time drinking, an inability to reduce drinking, experiencing withdrawal symptoms, and reduced social and occupational functioning due to drinking. In contrast, the linkage evidence for the chromosome 8 locus was driven by a much narrower range of symptoms including an inability to reduce drinking and experiencing withdrawal symptoms. Further, several of the individual symptoms showed higher LOD scores at a given locus than the alcohol dependence diagnosis itself (max LOD score for either alcohol dependence diagnosis = 2.42 vs. max LOD score among alcohol dependence symptoms = 4.22).

Similar results were obtained for the exploratory analysis of withdrawal, severe drinking, and medical/mental health symptoms. For example, both the withdrawal and severe drinking symptoms showed evidence of linkage to chromosome 1p, and the linkage evidence for the severe drinking symptoms was stronger than that observed for the alcohol dependence diagnosis (LOD = 3.08 vs. 1.41). Additionally, the exploratory analyses also yielded novel evidence for linkage not detected for alcohol dependence such as the evidence of linkage to chromosome 19 for the severe drinking symptoms and chromosome 9 for the withdrawal symptoms.

The results of these follow-up and exploratory analyses are not surprising given that alcohol dependence is hypothesized to represent a multi-faceted disorder that is likely due to the convergence of partially distinct biological mechanisms (e.g., sensitivity to alcohol and personality characteristics related to addiction), which may be measured to differing degrees

by each of the DSM-IV alcohol dependence symptoms (Schuckit, 1994). While it is important to emphasize that these analyses were conducted in an exploratory manner thus requiring replication before substantive conclusions can be made, these findings provide an important illustration of how a subset of symptoms may show increased evidence for linkage relative to the overarching diagnostic category. As a result, it is an important empirical question to ask which collection of symptoms within a diagnosis is responsible for the observed genetic linkage or association. This approach to linkage analysis of alcohol dependence was previously utilized by Prescott et al. (2006), and together with the findings reported herein, provide important insights for future studies seeking to further our understanding of the genetic influences underlying alcohol dependence.

The findings reported in the present study have important implications for molecular genetic studies of alcohol dependence, but there are limitations that should be noted. First, the present study was designed to detect loci with moderate effects, and a power analysis suggested reasonable power (0.50–0.60) to detect (logarithm of odds score ≥ 1) a locus that accounts for 20% of the phenotypic variation in the sample. Thus, it is likely that additional genetic loci that exhibited only a small effect on alcohol dependence in the UCSF sample were missed, and it may also explain the lack of support in the present study for loci previously linked to alcohol dependence such as chromosome 4q (Ehlers et al., 2004; Prescott et al., 2006; Reich et al., 1998). Second, multiple statistical tests were conducted in the present study given that we evaluated evidence for linkage using each of the 12 alcohol dependence symptoms assessed by the SSAGA. Corrections were not made to the reported LOD scores to account for multiple testing, but we attempted to control for this in two ways. First, we conducted follow-up analyses of the 12 symptoms only in those regions identified by the initial genome scans of the alcohol dependence diagnoses and limited the exploratory analyses to 3 symptoms clusters. Second, we interpreted the results of these analyses in a descriptive manner rather than using them to determine statistical significance. It is important to note that while this approach significantly reduced the number of statistical tests conducted, we likely missed some potentially interesting linkage findings between individual symptoms and genomic regions that fall outside of the initial linkage peaks.

In conclusion, the current study adds to the literature by supporting evidence of genetic linkage to alcohol dependence for several chromosomal regions. This study also provides evidence suggesting that different chromosomal regions may be associated with alcohol dependence when using the full DSM-IV criteria versus a broader definition of the diagnosis. Finally, the present study provides insight into how distinct genomic regions may underlie different alcohol dependence symptoms and how such data might be used to inform future molecular genetic studies of alcohol dependence.

References

- Abecasis GR, Cherny SS, Cookson WO, Cardon LR. Merlin--rapid analysis of dense genetic maps using sparse gene flow trees. *Nat Genet.* 2002; 30:97–101. [PubMed: 11731797]
- Allgulander C, Nowak J, Rice JP. Psychopathology and treatment of 30,344 twins in Sweden. II Heritability estimates of psychiatric diagnosis and treatment in 12,884 twin pairs. *Acta Psychiatr Scand.* 1991; 83:12–15. [PubMed: 2011949]
- Almasy L, Blangero J. Multipoint quantitative-trait linkage analysis in general pedigrees. *Am J Hum Genet.* 1998; 62:1198–1211. [PubMed: 9545414]
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders: DSM-III-R.* American Psychiatric Association; Washington, DC: 1987.
- American Psychiatric Association. *Diagnostic and Statistical Manual of Mental Disorders: DSM-IV.* American Psychiatric Association; Washington, DC: 1994.

- Bergen AW, Yang XR, Bai Y, Beerman MB, Goldstein AM, Goldin LR. Genomic regions linked to alcohol consumption in the Framingham Heart Study. *BMC Genet.* 2003; 4(Suppl 1):S101. [PubMed: 14975169]
- Bucholz KK, Cadoret R, Cloninger CR, Dinwiddie SH, Hesselbrock VM, Nurnberger JI Jr, Reich T, Schmidt I, Schuckit MA. A new, semi-structured psychiatric interview for use in genetic linkage studies: a report on the reliability of the SSAGA. *J Stud Alcohol.* 1994; 55:149–158. [PubMed: 8189735]
- Cloninger CR, Van Eerdewegh P, Goate AM, Edenberg HJ, Blangero J, Hesselbrock VM, Reich T, Nurnberger JI Jr, Schuckit MA, Porjesz B, Crowe R, Rice JP, Foroud T, Przybeck TR, Almasy L, Bucholz KK, Wu W, Shears S, Carr K, Crose C, Willig C, Zhao J, Tischfield JA, Li TK, Conneally PM. Anxiety proneness linked to epistatic loci in genome scan of human personality traits. *Am J Hum Genet.* 1998; 81:313–317.
- Corbett J, Saccone NL, Foroud T, Goate A, Edenberg H, Nurnberger J, Porjesz B, Begleiter H, Reich T, Rice JP. A sex-adjusted and age-adjusted genome screen for nested alcohol dependence diagnoses. *Psychiatr Genet.* 2005; 15:25–30. [PubMed: 15722954]
- Dick DM, Bierut LJ. The genetics of alcohol dependence. *Curr Psychiatry Rep.* 2006; 8:151–157. [PubMed: 16539893]
- Dick DM, Li TK, Edenberg HJ, Hesselbrock V, Kramer J, Kuperman S, Porjesz B, Bucholz K, Goate A, Nurnberger J, Foroud T. A genome-wide screen for genes influencing conduct disorder. *Mol Psychiatry.* 2004; 9:81–86. [PubMed: 14699444]
- Edenberg HJ, Dick DM, Xuei X, Tian H, Almasy L, Bauer LO, Crowe RR, Goate A, Hesselbrock V, Jones K, Kwon J, Li TK, Nurnberger JI Jr, O'Connor SJ, Reich T, Rice J, Schuckit MA, Porjesz B, Foroud T, Begleiter H. Variations in GABRA2, encoding the alpha 2 subunit of the GABA(A) receptor are associated with alcohol dependence and with brain oscillations. *Am J Hum Genet.* 2004; 74:705–714. [PubMed: 15024690]
- Edenberg HJ, Foroud T. The genetics of alcoholism: identifying specific genes through family studies. *Addict Biol.* 2006; 11:386–396. [PubMed: 16961766]
- Ehlers CL, Gilder DA, Slutske WS, Lind PA, Wilhelmsen KC. Externalizing disorders in American Indians: Comorbidity and a genome wide linkage analysis. *Am J Med Genet B Neuropsychiatr Genet.* 2008
- Ehlers CL, Gilder DA, Wall TL, Phillips E, Feiler H, Wilhelmsen KC. Genomic screen for loci associated with alcohol dependence in Mission Indians. *Am J Med Genet B Neuropsychiatr Genet.* 2004; 129:110–115. [PubMed: 15274051]
- Ehlers CL, Gizer IR, Vieten C, Gilder A, Gilder D, Stouffer GM, Lau P, Wilhelmsen KC. Age at regular drinking, clinical course, and heritability of alcohol dependence in the San Francisco Family Study: A gender analysis. *Am J Addict.* 2010; 19:101–10. [PubMed: 20163381]
- Foroud T, Edenberg HJ, Goate A, Rice J, Flury L, Koller DL, Bierut LJ, Conneally PM, Nurnberger JI, Bucholz KK, Li TK, Hesselbrock V, Crowe R, Schuckit MA, Porjesz B, Begleiter H, Reich T. Alcoholism susceptibility loci: confirmation studies in a replicate sample and further mapping. *Alcohol Clin Exp Res.* 2000; 24:933–945. [PubMed: 10923994]
- Gelernter J, Kranzler HR. Genetics of alcohol dependence. *Hum Genet.* 2009; 126:91–9. [PubMed: 19533172]
- Gelernter J, Kranzler HR, Panhuysen C, Weiss RD, Brady K, Poling J, Farrer L. Dense genomewide linkage scan for alcohol dependence in African Americans: significant linkage on chromosome 10. *Biol Psychiatry.* 2008; 65:111–115. [PubMed: 18930185]
- Goodwin DW, Schulsinger F, Hermansen L, Guze SB, Winokur G. Alcohol problems in adoptees raised apart from alcoholic biological parents. *Arch Gen Psychiatry.* 1973; 28:238–243. [PubMed: 4684290]
- Goring, HH. Hlod (Homo v. 0.2) documentation. SOLAR online user's manual. 2002. URL: http://bioinfo.hku.hk/solar/95.appendix_5.txt
- Guerrini I, Cook CC, Kest W, Devitgh A, McQuillin A, Curtis D, Gurling HM. Genetic linkage analysis supports the presence of two susceptibility loci for alcoholism and heavy drinking on chromosome 1p22.1-11.2 and 1q21.3-24.2. *BMC Genet.* 2005; 6:11. [PubMed: 15740611]

- Hasin DS, Stinson FS, Ogburn E, Grant BF. Prevalence, correlates, disability, and comorbidity of DSM-IV alcohol abuse and dependence in the United States - Results from the National Epidemiologic Survey on Alcohol and Related Conditions. *Arch Gen Psychiatry*. 2007; 64:830–842. [PubMed: 17606817]
- Heath AC, Bucholz KK, Madden PA, Dinwiddie SH, Slutske WS, Bierut LJ, Statham DJ, Dunne MP, Whitfield JB, Martin NG. Genetic and environmental contributions to alcohol dependence risk in a national twin sample: consistency of findings in women and men. *Psychol Med*. 1997; 27:1381–1396. [PubMed: 9403910]
- Hill SY, Shen S, Zezza N, Hoffman EK, Perlin M, Allan W. A genome wide search for alcoholism susceptibility genes. *Am J Med Genet B Neuropsychiatr Gen*. 2004; 128:102–113.
- Kendler KS, Kuo PH, Webb BT, Kalsi G, Neale MC, Sullivan PF, Walsh D, Patterson DG, Riley B, Prescott CA. A joint genomewide linkage analysis of symptoms of alcohol dependence and conduct disorder. *Alcohol Clin Exp Res*. 2006; 30:1972–1977. [PubMed: 17117961]
- Kendler KS, Prescott CA, Neale MC, Pedersen NL. Temperance board registration for alcohol abuse in a national sample of Swedish male twins, born 1902 to 1949. *Arch Gen Psychiatry*. 1997; 54:178–184. [PubMed: 9040286]
- Kuo PH, Neale MC, Riley BP, Webb BT, Sullivan PF, Vittum J, Patterson DG, Thiselton DL, van den Oord EJ, Walsh D, Kendler KS, Prescott CA. Identification of susceptibility loci for alcohol-related traits in the Irish Affected Sib Pair Study of Alcohol Dependence. *Alcohol Clin Exp Res*. 2006; 30:1807–1816. [PubMed: 17067344]
- Lander E, Kruglyak L. Genetic dissection of complex traits: guidelines for interpreting and reporting linkage results. *Nat Genet*. 1995; 11:241–247. [PubMed: 7581446]
- Long JC, Knowler WC, Hanson RL, Robin RW, Urbanek M, Moore E, Bennett PH, Goldman D. Evidence for genetic linkage to alcohol dependence on chromosomes 4 and 11 from an autosomal-wide scan in an American Indian population. *Am J Med Genet*. 1998; 81:216–221. [PubMed: 9603607]
- McGuffin P, Knight J, Breen G, Brewster S, Boyd PR, Craddock N, Gill M, Korszun A, Maier W, Middleton L, Mors O, Owen MJ, Perry J, Preisig M, Reich T, Rice J, Rietschel M, Jones L, Sham P, Farmer AE. Whole genome linkage scan of recurrent depressive disorder from the depression network study. *Hum Mol Genet*. 2005; 14:3337–3345. [PubMed: 16203746]
- McPeck MS, Sun L. Statistical tests for detection of misspecified relationships by use of genome-screen data. *Am J Hum Genet*. 2000; 66:1076–1094. [PubMed: 10712219]
- Mulligan CJ, Robin RW, Osier MV, Sambuughin N, Goldfarb LG, Kittles RA, Hesselbrock D, Goldman D, Long JC. Allelic variation at alcohol metabolism genes (ADH1B, ADH1C, ALDH2) and alcohol dependence in an American Indian population. *Hum Genet*. 2003; 113:325–36. [PubMed: 12884000]
- Nash MW, Huezio-Diaz P, Sterne A, Purcell S, Hoda F, Cherny SS, Abecasis GR, Prince M, Gray JA, Ball D, Asherson P, Mann A, Goldberg D, McGuffin P, Farmer A, Plomin R, Craig IW, Sham PC. Genome-wide linkage analysis of a composite index of neuroticism and mood-related scales in extreme selected sibships. *Hum Mol Genet*. 2004; 13:2173–2182. [PubMed: 15351774]
- O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. *Am J Hum Genet*. 1998; 63:259–266. [PubMed: 9634505]
- Porjesz B, Begleiter H, Wang KM, Almasy L, Chorlian DB, Stimus AT, Kuperman S, O'Connor SJ, Rohrbaugh J, Bauer LO, Edenberg HJ, Goate A, Rice JP, Reich T. Linkage and linkage disequilibrium mapping of ERP and EEG phenotypes. *Biol Psychol*. 2002; 61:229–248. [PubMed: 12385677]
- Prescott CA, Sullivan PF, Kuo PH, Webb BT, Vittum J, Patterson DG, Thiselton DL, Myers JM, Devitt M, Halberstadt LJ, Robinson VP, Neale MC, van den Oord EJ, Walsh D, Riley BP, Kendler KS. Genomewide linkage study in the Irish affected sib pair study of alcohol dependence: evidence for a susceptibility region for symptoms of alcohol dependence on chromosome 4. *Mol Psychiatry*. 2006; 11:603–611. [PubMed: 16534506]
- Reich T, Cloninger CR, Van Eerdewegh P, Rice JP, Mullaney J. Secular trends in the familial transmission of alcoholism. *Alcohol Clin Exp Res*. 1988; 12:458–464. [PubMed: 3056065]

- Reich T, Edenberg HJ, Goate A, Williams JT, Rice JP, Van Eerdewegh P, Foroud T, Hesselbrock V, Schuckit MA, Bucholz K, Porjesz B, Li TK, Conneally PM, Nurnberger JI Jr, Tischfield JA, Crowe RR, Cloninger CR, Wu W, Shears S, Carr K, Crose C, Willig C, Begleiter H. Genome-wide search for genes affecting the risk for alcohol dependence. *Am J Med Genet.* 1998; 81:207–215. [PubMed: 9603606]
- Saccone NL, Kwon JM, Corbett J, Goate A, Rochberg N, Edenberg HJ, Foroud T, Li TK, Begleiter H, Reich T, Rice JP. A genome screen of maximum number of drinks as an alcoholism phenotype. *Am J Med Genet.* 2000; 96:632–637. [PubMed: 11054770]
- Schuckit MA. A clinical model of genetic influences in alcohol dependence. *J Stud Alcohol.* 1994; 55:5–17. [PubMed: 8189726]
- Schuckit MA, Edenberg HJ, Kalmijn J, Flury L, Smith TL, Reich T, Bierut L, Goate A, Foroud T. A genome-wide search for genes that relate to a low level of response to alcohol. *Alcohol Clin Exp Res.* 2001; 25:323–329. [PubMed: 11290841]
- Schuckit MA, Wilhelmsen KC, Smith TL, Feiler HS, Lind P, Lange LA, Kalmijn J. Autosomal Linkage Analysis for the Level of Response to Alcohol. *Alcohol Clin Exp Res.* 2005; 29:1976–1982. [PubMed: 16340454]
- Seaton KL, Cornell JL, Wilhelmsen KC, Vieten C. Effective strategies for recruiting families ascertained through alcoholic probands. *Alcohol Clin Exp Res.* 2004; 28:78–84. [PubMed: 14745304]
- Vieten C, Seaton KL, Feiler HS, Wilhelmsen KC. The University of California, San Francisco Family Alcoholism Study. I Design, methods, and demographics. *Alcohol Clin Exp Res.* 2004; 28:1509–1516. [PubMed: 15597083]
- Wilhelmsen KC, Schuckit M, Smith TL, Lee JV, Segall SK, Feiler HS, Kalmijn J. The search for genes related to a low-level response to alcohol determined by alcohol challenges. *Alcohol Clin Exp Res.* 2003; 27:1041–1047. [PubMed: 12878909]
- Wilhelmsen KC, Swan GE, Cheng LS, Lessov-Schlaggar CN, Amos CI, Feiler HS, Hudmon KS, Ring HZ, Andrews JA, Tildesley E, Benowitz NL, Hops H. Support for previously identified alcoholism susceptibility Loci in a cohort selected for smoking behavior. *Alcohol Clin Exp Res.* 2005; 29:2108–2115. [PubMed: 16385180]
- World Health Organization. Draft International Classification of Diseases and Related Health Problems. Geneva, Switzerland: 1992.

Figure 1. Multipoint linkage analysis for Alcohol Dependence with and without the clustering criterion for the entire genome. Chromosome numbers are represented on the x -axis, and LOD scores are represented on the y -axis. Results for each chromosome are aligned end to end with the p terminus on the left. Vertical lines indicate the boundaries between chromosomes.

Table 1

DSM-IV Alcohol Dependence Symptom Clusters and Prevalence Rates and h^2 Estimates for the 12 Corresponding SSAGA Alcohol Dependence Symptoms.

DSM-IV Symptom Cluster	SSAGA Symptoms	Prevalence Rates		Familial Transmission	
		Linkage Sample (n = 1647)	Control Sample (n = 147)	h^2 estimate and 95% CI	p-value
Tolerance to alcohol	- Developed tolerance to alcohol	930 (56%)	32 (21%)	.266 ± .149	<.001
Experienced withdrawal symptoms	- Experienced uncontrollable shaking due to drinking	448 (27%)	6 (4%)	.233 ± .170	.003
Alcohol is taken in larger amounts or for longer than intended	- Experienced withdrawal symptoms	618 (38%)	13 (9%)	.772 ± .443	<.001
	- Drank more than intended	945 (57%)	33 (23%)	.350 ± .198	.017
	- Drank when not intended	845 (51%)	23 (16%)	.459 ± .174	<.001
Persistent desire for or inability to reduce alcohol consumption	- Unable to reduce drinking	739 (45%)	9 (6%)	.751 ± .233	<.001
	- Wanted to quit drinking 3 or more times	834 (51%)	21 (14%)	.386 ± .174	<.001
Great deal of time spent consuming/recovering from effects of alcohol	- Little time for non-drinking related activities	634 (38%)	18 (12%)	.486 ± .335	<.001
	- Decreased important activities due to drinking	626 (38%)	15 (10%)	.231 ± .172	.026
Drinking impacts occupational, social, or recreational activities	- Drinking interfered with work or social responsibilities	686 (42%)	20 (14%)	.398 ± .233	<.001
	- Continued drinking despite psychological problems	622 (38%)	11 (8%)	.126 ± .276	.181
	- Continued drinking despite physical health problem	355 (22%)	6 (4%)	.329 ± .235	.008

Abbreviations: n – number of participants, h^2 – proportion of variation in a trait that is potentially due to genetic transmission, CI – confidence interval.

Table 2

Chromosomal Regions with Evidence of Linkage to Alcohol Dependence.

Phenotype	Chromosomal Region	cM	LOD	Nearest Markers	Previous evidence for linkage to alcohol dependence (within 40 cM)
Alcohol Dependence with clustering	1p36.31–p36.22	11	1.41	D1S214/D1S450	-
	2q37.3	287	1.15	D2S140	Prescott et al., 2006 (265 cM) ^{*2} ; Kuo et al., 2005 (250 cM) ^{*3}
	8q24.3	163	1.47	D8S1743/D8S1836	-
Alcohol Dependence without clustering	18p11.21–p11.2	48	1.27	D18S453/D18S1107	Kuo et al., 2005 (83 cM) ^{*4} ; Schuckit et al., 2005 (21 cM) ^{*5} ; Hill et al., 2004 (41 cM) ^{*1} ; Wilhelmsen et al., 2003 (70 cM) ^{*5}
	1p22.3	114	1.19	D1S2766	Reich et al., 1998 (121 cM) ^{*1} ; Corbett et al., 2005 (99cM) ^{*1} ; Foroud et al., 2000 (120 cM) ^{*1} ; Guerrini et al., 2005 (121 cM) ^{*1} ; Kuo et al., 2006 (141 cM) ^{*6} ; Hill et al., 2004 (90 cM) ^{*1}
	2p24.3–p24.1	32	2.42	D2S149/D2S305	Wilhelmsen et al., 2005 (43 cM) ^{*7}
	9p24.1–p23	18	1.70	D9S286/D9S168	Long et al., 1998 (42 cM) ^{*1}
	22q12.3–q13.1	36	1.78	D22S283/D22S423	Prescott et al., 2006 (8 cM) ^{*1} ; Kuo et al., 2005 (8 cM) ^{*8} ; Bergen et al., 2003 (33 cM) ^{*4} ; Foroud et al., 2000 (58 cM) ^{*1} ; Wilhelmsen et al., 2003 (25 cM) ^{*5}

Abbreviations: cM – centimorgans,

* studies indicated by an asterisk within a chromosomal region were conducted using overlapping datasets, numbered superscript indicate the trait studied:

¹ Alcohol Dependence/Alcoholism,

² Alcohol Dependence symptom count,

³ withdrawal symptoms,

⁴ maximum drinks in 24 hours,

⁵ body sway following exposure to alcohol,

⁶ initial sensitivity to alcohol,

⁷ average drinks/week,

⁸ tolerance.

Table 3

Symptoms with LOD Scores >1 at Linkage Peaks for Alcohol Dependence Diagnosis with and without Temporal Clustering of Symptoms.

Linked Alcohol Dependence Dx. (LOD Score)	Chr.	Pos. (cM)	Nearest Marker	Trait	LOD Score
With (LOD = 1.41) & Without Clustering (LOD = 1.24)	1	0	D1S468	Little time for non-drinking related activities	1.40
		9	D1S214	Unable to reduce drinking	1.40
		39	D1S2644	Experienced uncontrollable shaking due to drinking	1.30
		24	D1S434	Wanted to quit drinking 3 or more times	1.28
Without Clustering (LOD = 1.19)	1	0	D1S468	Drinking interfered with work/social responsibilities	1.22
		114	D1S2766	Wanted to quit drinking 3 or more times	4.22
		114	D1S2766	Developed tolerance to alcohol	2.76
		103	D1S500	Little time for non-drinking related activities	1.42
Without Clustering (LOD = 2.42)	2	114	D1S2766	Continued drinking despite physical health problem	1.27
		32	D2S149	Drank when not intended	3.02
		17	D2S162	Little time for non-drinking related activities	1.86
		27	D2S149	Developed tolerance to alcohol	1.77
With Clustering (LOD = 1.15)	2	254	D2S338	Continued drinking despite psychological problems	3.13
		264	D2S125	Wanted to quit drinking 3 or more times	1.49
		248	D2S206/D2S2202	Drank more than intended	1.36
		248	D2S206/D2S2202	Experienced withdrawal symptoms	1.03
With (LOD = 1.47) & Without Clustering (LOD = 1.11)	8	175	D8S1836	Unable to reduce drinking	1.34
		175	D8S1836	Experienced withdrawal symptoms	1.28
Without Clustering (LOD = 1.70)	9	18	D9S286	Continued drinking despite psychological problems	1.56
		25	D9S269	Developed tolerance to alcohol	1.51
With Clustering (LOD = 1.27)	18	60	D18S56	Developed tolerance to alcohol	1.18
		34	D18S464	Continued drinking despite psychological problems	1.06
Without Clustering (LOD = 1.78)	22	43	D22S423	Drank more than intended	2.28
		32	D22S277	Unable to reduce drinking	2.14
		29	D22S280	Drinking interfered with work/social responsibilities	1.80
		34	D22S283	Drank when not intended	1.67
		29	D22S280	Decreased important activities due to drinking	1.10

Abbreviations: Dx. – Diagnosis, Chrom. – Chromosome, cM – centimorgans.

Table 4

Results from Linkage Analysis of Withdrawal, Severe Drinking, and Medical/Mental Health Symptoms yielding LOD Scores >1.

Symptom Group	Chromosomal Region	Position (cM)	Nearest Marker	LOD Score
Drank despite health problem	12p13.31	14	D12S99	1.10
Exhibited severe drinking symptoms	1p36.22	20	D1S2667	3.08
	2p23.2	41	D2S165	2.12
	2q37.1	247	D2S206	1.14
	3q21.3	137	D3S3606	1.50
	8q24.3	171	D8S1836	1.37
	9q34.3	178	D9S1838	1.06
	10p15.3	3	D10S1745	1.59
	12q24.33	160	D12S1659/D12S367	1.77
	19p13.2	33	D19S865	2.62
	20p12.1	38	D20S112	1.44
	22q12.3	36	D22S283	1.11
Exhibited withdrawal symptoms	1p36.12-11	47	D1S2864/D1S234	1.07
	6p22.3	35	D6S1660/D6S276	1.40

Abbreviations: cM - centimorgans.