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Suggestive Linkage on Chromosome 1 for a Quantitative Alcohol-Related Phenotype

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Background: Alcohol dependence is a clinically and etiologically heterogeneous disorder. Accordingly, a variety of subtypes of alcohol-dependent individuals have been proposed, and multiple operational definitions of alcohol use, abuse, and dependence have been used in linkage analyses directed toward detecting genes involved in alcohol use and problems. Here, we develop quantitative phenotypes that characterize drinking patterns among both alcoholic and nonalcoholic subjects, and use these phenotypes in subsequent linkage analyses.

Methods: More than 9000 individuals from alcoholic and control families were administered a semistructured interview and personality questionnaire as part of the initial stage of the Collaborative Study on the Genetics of Alcoholism (COGA). A principal component analysis was conducted on items that captured many of the dimensions of drinking and related behaviors, including aspects of alcohol use, antisocial behavior and affective disturbance when drinking, and personality. Factor scores were computed for all individuals. Nonparametric linkage analyses were conducted on these factor scores, in the initial COGA sample consisting of 987 individuals from 105 extended families, and in a replication sample consisting of 1295 individuals from 157 extended families.

Results: Three factors were identified, accounting for 68% of the total variance. The most promising regions of linkage appeared for factor 2, on which higher scores indicate a later age of onset of regular drinking and higher harm avoidance. Chromosome 1 yielded consistent evidence of linkage in both samples, with a maximum lod score of 3.3 when the samples were combined for analysis. Consistent suggestion of linkage also was found to chromosome 15.

Conclusions: Developing novel phenotypes that more accurately model the effect of influential genes may help efforts to detect genes involved in complex disorders. Applying principal component analysis in the COGA sample provided support for some regions of linkage previously reported in COGA, and identified other new, promising regions of linkage.

Key Words: Phenotype Development, Principal Component Analysis, Linkage Analyses, Drinking Patterns, Anxiety.

DETECTING GENES INVOLVED in complex behavioral and psychiatric disorders remains a daunting task. Despite significant evidence for the heritability of alcohol dependence (McGue, 1999), few consistently replicating areas of linkage have emerged (Foroud and Li, 1999). It is widely accepted that alcohol dependence is a clinically and etiologically heterogeneous disorder, varying in age of onset, clinical presentation, developmental course, and severity (Finn et al., 1997; Sher and Trull, 1994). Interrelated risk factors and comorbid disorders further complicate attempts to characterize etiologically relevant factors and to understand the process(es) leading to dependence. Defining phenotypes that accurately reflect the underlying genetic susceptibility is critical for the detection of dispositional genes. Uncertainty regarding how best to define alcohol problems likely contributes to the difficulty in consistently detecting genes involved in alcohol dependence.

The Collaborative Study on the Genetics of Alcoholism (COGA) has addressed this problem by exploring the use of several existent diagnostic criteria for alcohol dependence. In the initial genome scan, individuals were diagnosed as alcohol dependent if they met Feighner criteria for alcoholism *and* DSM III-R criteria for alcohol dependence (Reich et al., 1998). In replication analyses, a narrower, more restrictive, modified ICD-10 diagnosis also was analyzed (Foroud et al., 2000). Defining affection using

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The Collaborative Study on the Genetics of Alcoholism (COGA) (H. Begleiter, SUNY HSCB, Principal Investigator; T. Reich, Washington University, Co-Principal Investigator) includes eight different centers where data collection, analysis, and/or storage take place. The eight sites and Principal Investigator and Co-Investigators are: Indiana University (T.-K. Li, J. Nurnberger Jr., P.M. Conneally, H.J. Edenberg); University of Iowa (R. Crowe, S. Kuperman); University of California at San Diego (M. Schuckit); University of Connecticut (V. Hesselbrock); State University of New York, Health Sciences Center at Brooklyn (B. Porjesz, H. Begleiter); Washington University in St. Louis (T. Reich, C. R. Cloninger, J. Rice, A. Goate); Rutgers University (J. Tischfield); and Southwest Foundation (L. Almasy).

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multiple criteria is common in genetic studies of psychiatric disorders: it has been employed in studies of bipolar disorder (Berrettini et al., 1997; Nurnberger et al., 1997) and schizophrenia (Kendler et al., 1996), underscoring the current lack of consensus regarding how best to operationally define psychiatric disorders for genetic studies.

One approach to the heterogeneity that characterizes alcohol dependence has been to subtype individuals with alcohol problems (Babor, 1996; Hesselbrock, 1995). Cloninger (1987a) distinguished between type 1 alcoholics, characterized by a later age of onset of alcohol problems, guilt and anxiety about their drinking, high harm avoidance, and high reward dependence, and type 2 alcoholics, characterized by an earlier age of onset of problems, antisocial behavior, and high novelty seeking. The genetic architecture of these subtypes appears to differ, as there is differential risk to family members of each subtype depending on gender and co-occurring environmental risk factors (Cloninger et al., 1981). An extension of this work has been the type A and type B distinction (Babor et al., 1992; Schuckit et al., 1995). Type A alcoholics are characterized by a later onset, fewer childhood problems, less severe dependence and course, and fewer comorbid problems; type B alcoholics are characterized by an earlier onset of problems, more childhood risk factors, more severe dysfunction, and a more chronic course. The Babor and Cloninger subtypes share several features, including the later onset of problems in type A/type 1 alcoholics, and the manifestation of antisocial traits in type B/type 2 alcoholics. Personality profiles are also similar between type A/type I alcoholics and type B/type 2 alcoholics (Babor et al., 1992). However, noticeable differences exist between the two typologies; for example, type A alcoholics are not characterized by anxiety and depression, as are Cloninger's type I alcoholics. While the type 1/type 2 and type A/type B subtypes have gained substantial attention, it is important to note that a number of other subgroups also have been proposed (Finn et al., 1997; Jellinick, 1960; Zucker, 1986).

Thus, subgroups of alcoholics have been found to differ on a number of dimensions, including severity, levels of familial alcoholism, personality traits, and comorbid psychopathology (e.g., anxiety, depression, and antisocial behavior). Furthermore, studies have found that children of different alcoholic subtypes display differential behavioral outcome (although all generally have elevated patterns of alcohol use), lending support to the hypothesis of different underlying vulnerabilities for different subtypes of alcoholics (Babor et al., 1992; Finn et al., 1997). Therefore, there is substantial evidence that alcoholism is heterogeneous, although there remains controversy about how best to subtype alcoholics.

The rationale behind subtyping *for genetic studies* is that identifying more homogeneous groups of alcoholics will increase the likelihood that the sample will have greater genetic homogeneity and therefore will be segregating fewer susceptibility genes. Latent class analysis previously was used to subtype subjects in the COGA sample. Interestingly, unlike previous subtyping efforts, the subtypes identified in the COGA sample did not differ on symptomatic profile, but rather on degree of severity; four classes emerged: an unaffected group, a mildly affected group, a moderately affected group, and a severely affected group (Bucholz et al., 1996; Foroud et al., 1998). Sibling pairs that were either in the moderately- or severely affected groups were subsequently used in genetic analyses, suggesting a region on chromosome 16 that conferred risk to this multivariate phenotype (Foroud et al., 1998). These genetic analyses suggest that subtyping the alcohol dependence phenotype can provide additional information for identifying genes contributing to different aspects of alcohol use/abuse.

Thus far, most subtyping efforts have focused on defining patterns among alcohol-dependent individuals. However, behavior genetic studies have demonstrated that dimensions of normal alcohol use, such as frequency and persistence of use, are under substantial genetic influence (Heath, 1995; Heath and Martin, 1988; Rose et al., 2001). This suggests that it should be possible to identify genes contributing to quantitative patterns of alcohol use, in addition to the diagnosis of alcohol dependence. Support for this idea can be found in a report of strong linkage to chromosome 4 for the quantitative phenotype "maximum number of drinks in a 24-hr period" in the COGA sample (Saccone et al., 2000). Additionally, personality traits are under genetic influence (Plomin and Caspi, 1998; Plomin and Caspi, 1999). Numerous studies have documented a relationship between personality and alcohol use; individuals who score higher on novelty-seeking and sensation-seeking scales are more likely to use and abuse alcohol, and there is some suggestion that individuals who demonstrate low harm avoidance and reward dependence are also at a greater risk (Cloninger et al., 1988; Earlywine and Finn, 1991; Howard et al., 1997). This suggests that genes contributing to personality may also (indirectly) confer risk for alcohol use.

Previous research has suggested that using quantitative phenotypes in linkage analyses can be more powerful than using dichotomous categories (e.g., affected/unaffected) (Duggirala et al., 1997). Subtyping necessarily reduces one's sample size for linkage analyses because subjects are grouped into different clusters, with individuals assigned as "affected" or "unaffected" under each cluster. Furthermore, because the clusters identified in the COGA sample did not differ on symptomatic profile, but rather on severity, it should be possible to develop quantitative phenotypes that capture aspects of drinking patterns and personality traits that contribute to these patterns, but apply to *all* drinking individuals in the COGA sample. Here we describe the development of these quantitative phenotypes, and the results of linkage analyses of these quantitative traits.

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METHODS

Sample

The Collaborative Study on the Genetics of Alcoholism is a multi-site project, in which families were collected at six centers across the United States: Indiana University, State University of New York Health Science Center, University of Connecticut, University of Iowa, University of California/San Diego, and Washington University, St. Louis. Probands identified through inpatient/outpatient alcohol treatment programs at these six sites were invited to participate if they had a sufficiently large family (usually sibships ≥3 with parents available) with two or more members in a COGA catchment area (Reich, 1996). Probands were considered affected by COGA criteria if they met requirements for lifetime DSM-III-R alcohol dependence and Feighner definite alcoholism. A total of 1227 families of alcohol-dependent probands were recruited for the first stage of the study. Additionally, a sample of 234 control families was assessed, consisting of two parents and at least three children over the age of 14, and obtained through random sources such as driver's license registries and dental clinics. All individuals were administered the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA) interview (Bucholz et al., 1994; Hesselbrock et al., 1999), and the Tridimensional Personality Questionnaire (Cloninger, 1987b). At the time these analyses were conducted, >9000 individuals had been assessed in stage I of COGA recruitment, approximately 85% of whom were from families of alcoholics. The institutional review boards of participating institutions approved the study.

Proband families that were not bilineal and had at least two affected first degree relatives in addition to the proband were invited to participate in the more intensive stage of the study, which involved a blood draw, EEG, and a battery of auditory and visual evoked potentials, and neuropsychological assessments. Second and third degree relatives in the families were assessed when they were considered to be informative for the genetic linkage studies. A total of 987 individuals from 105 extended families are included in the initial genotyped data set (Reich et al., 1998); 46% of these individuals were considered affected using COGA criteria (Reich, 1996). A replication sample has also been ascertained and genotyped following identical procedures; it consists of 1295 individuals from 157 extended families (Foroud et al., 2000).

Analyses

A principal component analysis (PCA) was conducted using the statistical package SPSS (1993) on items from the SSAGA that captured many of the dimensions of alcohol use incorporated into previous models of subtyping. In these preliminary analyses, we chose to focus on alcohol-related symptoms and personality characteristics, with the primary goal of characterizing patterns of alcohol use across individuals in the COGA sample. In future analyses, we plan to incorporate aspects of additional comorbid disorders. In the PCA, we included the maximum number of drinks ever consumed in a 24 hr period, log-transformed to minimize distribution skew and to reduce the impact of extreme outliers, and a tally of total alcohol-related problems (28 items) from the DSM-III-R symptom count. We also included self-reported age of onset of regular drinking, as defined by drinking at least once a month for 6 months or more. We measured aggressive behavior when drinking by tallying five items asking about arguments, hitting things or people, and physical fights when drinking, and an item regarding arrest due to drunk behavior (other than drunk driving). This yielded a 6-point scale, with higher scores indicating a wider variety of antisocial acts. Affective disturbance associated with drinking was included by totaling five items measuring emotional or psychological problems resulting from drinking, such as feeling depressed, jumpy or easily startled, and trouble thinking clearly, and one item regarding guilt about one's drinking. Again, this yielded a 6-point scale, with higher scores indicative of greater affective disturbance resulting from drinking. Additionally, harm avoidance, novelty seeking, and reward dependence scores from the TPQ were included in the PCA. Harm avoidance measures the extent to which one is cautious, apprehensive, inhibited, and shy; novelty-seeking measures the extent to which an individual is impulsive and excitable; and reward dependence measures the extent to which an individual is emotionally dependent on others and sensitive to social cues.

The PCA was computed on a variety of samples within the COGA data. Initially, we computed the PCA using all individuals with SSAGA data, with the exception of those who skipped out of the alcohol section. [These individuals skipped out of the alcohol section of the SSAGA because they (a) never had a drink of alcohol, (b) never drank more than three drinks in a 24-hr period, or (c) never drank regularly and never became intoxicated]. Thus, all individuals assessed in the initial stage of COGA recruitment were used to compute the PCA. We also ran the PCA using (1) only individuals who were genotyped, a sample consisting of multiplex alcohol-dependent families, (2) only control families, which would represent individuals and their relatives recruited from the general population and thus not oversampled for alcohol-dependent individuals, and (3) all non-control COGA families, a sample of kindreds recruited through an alcohol-dependent proband. The solution was very stable and the components were virtually unchanged across samples. Thus, we report results from the PCA computed using the initial sample of all individuals with SSAGA data. This sample comprised the largest data set, with the broadest spectrum of drinking patterns represented (although heavier drinkers are likely over-represented in the sample, as most individuals were relatives of alcoholics). A total of 6103 individuals had complete data on all items entered into the PCA. Factors with eigenvalues greater than 1.0 were interpreted and used in linkage analyses.

Genome-wide, multipoint nonparametric linkage analyses were conducted using individuals from the initial, replication, and combined samples, recruited as part of the more intensive stages of the COGA project. Genotyping was performed in laboratories at Indiana University and Washington University using radioactive and fluorescence-based detection systems, as described previously (Reich et al., 1998). The initial genome scan of COGA pedigrees included 292 markers with an average intermarker distance of 13.8 cM (Reich et al., 1998); regions with gaps >20 cM were typed more extensively, as were regions with suggestive linkage in the initial genomic scan. Thus, a total of 351 markers were used in the current analyses.

Pedigrees were checked for non-Mendelian inheritance using the GeneMaster database and the programs CRIMAP (Green, 1990) and USERM13 (Boehnke, 1991). Recombination based marker maps were generated from the sample using CRIMAP. Maximum likelihood estimates of marker allele frequencies were computed from the data using USERM13. The program Mapmaker/SIBS (Kruglyak and Lander, 1995) was used to analyze sibling data using the maximum likelihood method and the Haseman-Elston method. Extended pedigrees were split into nuclear family units for sibling analyses. When families have more than two affected siblings, the test statistic may be biased by families contributing a large number of sibships. Conversely, using only independent sibling pairs may be an overly conservative technique; thus, both methods of analysis were employed. Therefore, analyses were conducted using all possible sibling pairs and using only independent sibling pairs (n-1; n = number of siblings in a nuclear family). A total of 1516 genotyped individuals from the initial and replication samples (66% of the total genotyped sample) had factor scores and were used in linkage analyses. These individuals comprised 1366 all possible sibling pairs and 705 independent sibling pairs. This sample has 90% power to detect a QTL accounting for 20% of the trait variance (Purcell and Sham, 2001; http://statgen.iop.kcl.ac.uk/gpc/).

RESULTS

Three factors with eigenvalues greater than 1.0 were identified. Together, these factors accounted for 68% of the total variance. Factor loadings for each of the components, and the amount of variance accounted for by each, are shown in Table 1. The first factor yielded positive loadings for maximum number of drinks in a 24 hr period, total drinking problems, total antisocial behavior while

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Table 1. Factor Loadings, Eigenvalues, and Percent of Variance Accounted for by each Component

Characteristic	Component 1	2	3
eigenvalues:	3.31	1.15	1.01
% variance explained:	(41%)	(14%)	(13%)
Age onset regular drinking	−0.44	0.41	0.12
Maximum number of drinks	0.81	−0.13	−0.18
Total drinking problems	0.92	0.12	0.09
Total antisocial behavior	0.83	−0.03	−0.02
Total emotional/psychological problems	0.81	0.25	0.2
Harm avoidance	0.22	0.72	0.35
Novelty seeking	0.44	−0.53	0.21
Reward dependence	−0.22	−0.31	0.86

Table 2. Percentage of Individuals who Meet Diagnostic Criteria for Alcoholism Among those Above and Below the Mean for each Factor

	Factor 1		Factor 2		Factor 3	
	DSMIV	ICD10	DSMIV	ICD10	DSMIV	ICD10
Above mean	88	77	47	40	45	37
Below mean	12	5	41	30	43	33

Table 3. Summary of Linkage Results Across all Samples and Factors

		Sample					
		Initial		Replication		Combined	
Factor	Chromosome	Lod score	Location	Lod score	Location	Lod score	Location
1	1	0.01	117cM	**2.1**	**91cM**	1.5	124cM
2	1	**2.3**	**235cM**	1.7	206cM	**3.3**	**209cM**
2	3	0.9	219cM	**2.6**	**214cM**	1.9	216cM
2	4	**2.7**	**106cM**	0.4	127cM	0.3	103cM
2	5	**2.5**	**6cM**	1.0	33cM	**2.0**	**25cM**
2	15	1.4	75cM	1.7	66cM	**2.0**	**67cM**
3	1	1.2	0cM	**2.5**	**63cM**	1.3	62cM
3	5	0.5	45cM	**3.2**	**194cM**	1.3	194cM

Note: Results from linkage analyses performed using all possible sibling pairs using the maximum likelihood method of analysis. All cM distances are based on the combined map. Linkage results with lod scores ≥2.0 are shown in bold. For comparison purposes, the nearest maximum lod score in the other samples is shown for regions yielding lod scores ≥2.0 in at least one sample.

drinking, total emotional/psychological problems resulting from drinking, and novelty seeking; and a negative loading for age of onset of regular drinking. Thus higher scores reflect greater alcohol use, beginning from an earlier age, and related problems. Scores on factor 1 are also strongly related to alcohol dependence diagnoses, both using DSMIV and ICD10 criteria; see Table 2. The second factor was largely defined by harm avoidance, novelty seeking (negatively associated), and age of onset of drinking; higher scores indicate a later age of onset of drinking, higher harm avoidance, and lower novelty seeking. These individuals also had a somewhat elevated risk of alcohol dependence diagnoses (Table 2); drinking patterns of these individuals were more reminiscent of Cloninger's type 1 alcoholics. The third component was primarily defined by high reward dependence. There was very little relationship between this factor and alcoholism diagnoses (Table 2).

Chromosomal regions yielding lod scores ≥2.0 are listed in Table 3 for each of the factors. Results reported both in text and tables are for the maximum likelihood method of analysis for all possible sibling pairs. The use of independent sibling pairs and the Haseman-Elston method of analysis had the expected effects of slightly decreasing the lod scores, as these are more conservative methods of analysis, but the results from the more conservative analyses were consistent with the results reported here.

Factor 2 provided the strongest evidence of linkage, with two chromosomal regions providing consistent evidence of linkage across the samples. Chromosome 1 yielded the strongest evidence of linkage across the samples, with a maximum lod score of 3.3 in the combined sample near the marker D1S518 (Fig. 1). Additionally, chromosome 15 yielded consistent evidence of linkage across the samples (Fig. 2), with a maximum lod score of 2.0 in the combined sample between markers D15S143 and GATA153, which are 8 cM apart.

Other suggestive linkage findings were evident in only one of the samples (Table 3). For factor 1, lod scores of 2.0 and 2.1 were obtained at markers D1S1596 and D1S1613, respectively, in the replication sample; however, there was no evidence of linkage to this region in the initial or combined samples. For factor 2, on chromosome 3, a lod score of 2.6 was obtained at marker D3S2418 in the replication sample; however, there was little evidence of linkage to this region in the initial sample. Conversely, there was evidence of linkage to

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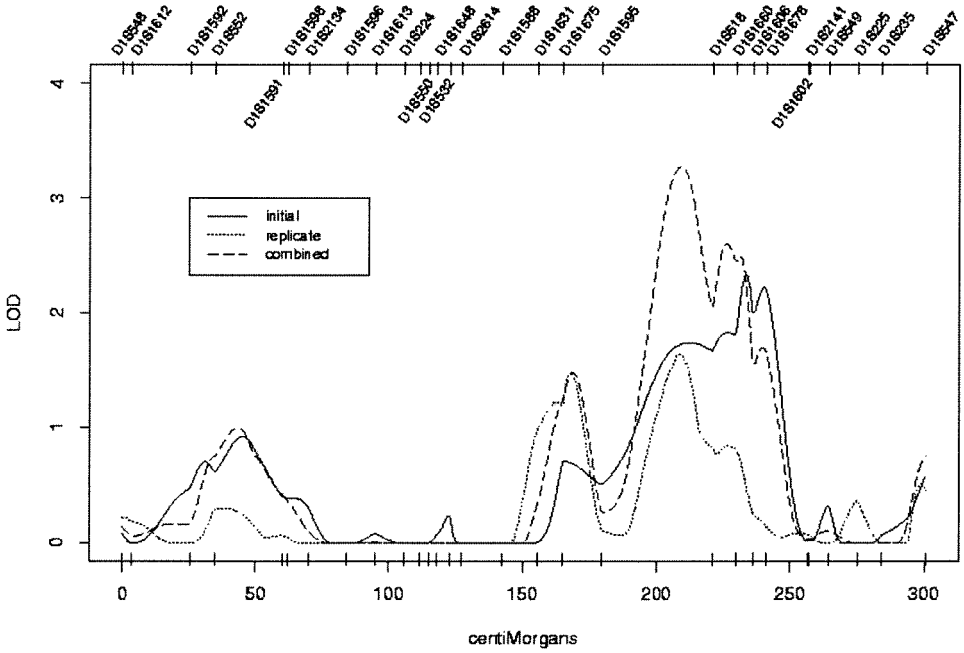

Fig. 1. Multipoint lod score graph for chromosome 1, factor 2. Shown are the results for the initial, replication, and combined samples.

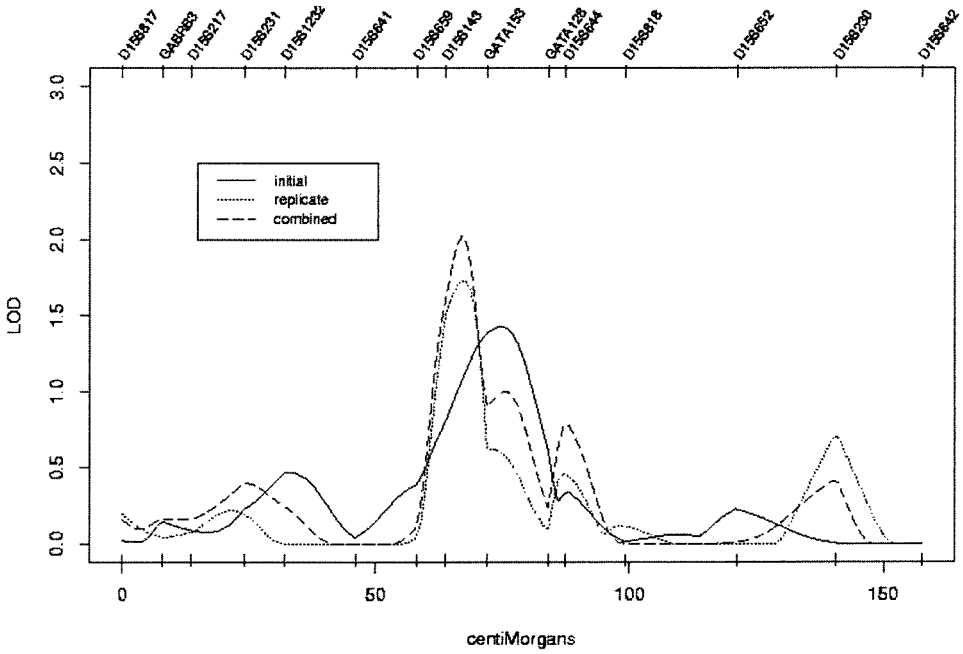

Fig. 2. Multipoint lod score graph for chromosome 15, factor 2. Shown are the results for the initial, replication, and combined samples.

chromosome 4 in the initial sample, with a maximum lod score of 2.7 near the marker D4S1544; however, this was not replicated in the second sample. Similarly, chromosome 5 yielded evidence of linkage in the initial sample, with a maximum lod score of 2.5 near the marker D5S807. However, there was little evidence of linkage in the replication sample; accordingly, combining the initial and replication samples for analysis reduced the evidence of linkage, although the combined sample still yielded a lod score of 2.0 between markers D5S807 and D5S1473. For factor 3, lod scores suggestive of linkage were found on chromosomes 1 and 5 in the replication sample only. On chromosome 1, markers D1S1591 and D1S1598 yielded lod scores of 2.0 and 2.5, respectively; on chromosome 5, a lod score of 3.2 was observed at GABRA1 in the replication sample.

DISCUSSION

The success of a genetic study is, in part, determined by the use of a phenotype that accurately reflects the cluster of

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symptoms affected by the gene or genes that are to be identified. While there are many reasons to use clinically defined disorders as phenotypes in genetic studies, these classification systems were developed primarily for clinical practice (Diagnostic and Statistical Manual of Mental Disorders, 1994), and there is reason to believe that clinical diagnoses may not always reflect the constellation of symptoms that most accurately reflect the expression of a particular gene or genes. For example, major depression and generalized anxiety disorder are classified and diagnosed as distinct disorders in the Diagnostic and Statistical Manual of Mental Disorders (1994); however, data from the Virginia Twin Register suggests that the genes that influence these disorders are largely shared, meaning that certain genes predispose to both depression and anxiety and whether an individual manifests one disorder or the other is based on the environmental stressors that they encounter (Kendler et al., 1992). Thus, while the distinction between these disorders may be useful for clinical purposes, it would be unwise to treat these disorders as separate and distinct entities in genetic studies.

PCA provides a potentially useful tool for genetic analyses. It creates phenotypes that are statistical combinations of naturally occurring correlations between variables. Thus, it is possible that these groupings of symptoms may result from the expression of a particular gene or gene(s), making them interesting alternative phenotypes for use in genetic studies. Additionally, PCA creates quantitative phenotypes that potentially maximize the power of a sample by using information from all available individuals. It seems especially appropriate to apply this technique to the alcohol domain, because normative patterns of alcohol use are under genetic influence (Rose et al., 2001), as are alcohol disorders (McGue, 1999).

The most promising regions of linkage appeared for factor 2, the factor for which higher values reflect a later age of onset of drinking, higher harm avoidance and lower novelty seeking. Our finding on chromosome 1 for factor 2 is distal to the findings for alcohol dependence (Foroud et al., 2000) and the phenotype "alcoholism or depression" (Nurnberger et al., 2001), previously reported in the COGA sample. Interestingly, the region on chromosome 1 where the factor 2 finding is located has been implicated in a variety of studies of anxiety-related phenotypes. Smoller and colleagues (Smoller et al., 2001) found suggestive evidence of linkage (peak NPL = 2.05, $p = 0.035$) to the same markers that provide linkage to factor 2, using nonparametric analyses of an anxiety "diathesis-type" phenotype characterized by three or more of the following: panic disorder/agoraphobia, comorbid anxiety disorders, childhood-onset anxiety disorders, and/or continuous anxiety problems since before age 13. There is also reported evidence of possible linkage to panic disorder on chromosome 1q in two independent genome scans using parametric linkage methods specifying a dominant disease model (Crowe et al., 2001; Gelertner et al., 2001). Together, these findings are of interest since factor 2 is characterized largely by high harm avoidance, another anxiety-related trait. We subsequently examined the relationship between factor 2 and anxiety disorders in our sample. The SSAGA provides diagnoses for agoraphobia, social phobia, and panic disorder; 12% of individuals with factor 2 scores above the mean were diagnosed with at least one of these anxiety disorders, whereas only 5% of individuals below the mean had an assessed anxiety disorder. We are currently in the process of exploring additional anxiety related phenotypes in the COGA sample.

The region on chromosome 15 with linkage to factor 2 is also a chromosomal region with significant EEG findings in the COGA sample. We obtained a maximum lod score of 2.0 for factor 2 less than 10 cM from a region with a lod score >3.0 for the beta 2 EEG component (16–20 Hz). Additionally, there is suggestive evidence of linkage for beta 3 (20–28 Hz) and for theta in this region (Bernice Porjesz, personal communication).

Notably, we did not find linkage of factor 2 to chromosome 8, where Cloninger reported evidence of linkage to harm avoidance in the initial sample of COGA (Cloninger et al., 1998). That may suggest that this quantitative, more comprehensive phenotype reflects a different genetic vulnerability than that involved purely in the expression of the personality trait harm avoidance.

The linkage reported for factor 1 to chromosome 1 in the replication sample was an area with modest linkage (>1.5) in the initial COGA sample; however, the peak lod score on chromosome 1 for the phenotype alcohol dependence in the COGA sample was substantially stronger, and nearly 60 cM upstream, from this finding. This suggests that a potential susceptibility gene in this region is related primarily to alcohol dependence, rather than to drinking patterns in general, as the linkage was stronger when the sample consisted of alcohol-dependent families, rather than all alcohol users.

Suggestion of linkage for factor 2 to chromosome 4 in the initial sample is of particular interest because it is in the same region as the linkage reported for unaffected sibling pairs in the original COGA genome scan (Reich et al., 1998) and for the phenotype "maximum number of drinks in a 24-hr period" (Saccone et al., 2000). Thus, the analyses reported here provide additional modest support for a gene influencing patterns of alcohol use in this region.

Our finding of suggestive linkage for factor 2 to chromosome 5 is also of interest because this region has also been linked to alcohol response in the COGA sample (Schuckit et al., 2001). The marker D5S807 exhibiting linkage to factor 2 across the samples also yielded a multipoint lod score of 2.2 for self-ratings of individuals' response to alcohol. A low level of response to alcohol has been observed at an elevated rate in the children of alcoholics and predicts later alcohol abuse or dependence (Schuckit and Smith, 1996).

Linkage of factor 3, defined largely by reward depen-

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dence, to the *GABRA1* marker on chromosome 5 is also of interest. *GABRA1* encodes the alpha 1 subunit of the $GABA_A$ receptor. The $GABA_A$ receptor is involved in many of the effects of alcohol on the central nervous system, including altered cognition, motor activity, and sensory function (Feldman et al., 1997). Thus, it is plausible that variations in the $GABA_A$ receptor may be involved in altering risk of alcohol use, mediated through a reward dependence phenotype. Further support for the role of the inhibitory neurotransmitter GABA in alcohol use and abuse is provided by Long and colleagues' report of linkage for alcohol dependence in Southwestern American Indians near the GABRB1 gene, encoding the β1 subunit of the $GABA_A$ receptor (Long et al., 1998).

Despite interesting speculations, the linkage findings evident in only one of the samples should be viewed tentatively. There are several reasons why reports of linkage may vary among samples, including genetic heterogeneity, incomplete penetrance, and the presence of phenocopies. Additionally, as reported by Suarez et al. (1994), replication of a specific linkage result is considerably more difficult in a second sample, requiring a substantially larger sample size than was used in the original sample. Such apparent inconsistency across samples can result from varying frequencies of genes predisposing or protecting against disease. Therefore, lack of replication of a linkage finding does not necessarily lead to the conclusion that the initial linkage finding was spurious. Rather, ascertainment of an independent sample will often lead to differing population substructures. Because of the difficulties inherent in replication, we place greatest confidence in those findings that were present in both of the samples, on chromosomes 1 and 15, where a maximum lod score occurred in the combined sample.

The interpretation of the results of genome wide linkage analyses is an area of active discussion. Previously, Kruglyak and Lander (1995) suggested guidelines for interpreting linkage results from sibling studies of complex traits: a lod score $\geq$2.2 would be considered suggestive of linkage because a lod score of this magnitude would be expected only once by chance in a genome screen. Lod scores $\geq$3.6 would correspond to a p-value of 0.05 for a genome-wide scan and could be considered significant evidence of linkage. By these criteria, no significant lod scores were found for any of the factors analyzed in this study; however, several chromosomal regions indicative of suggestive linkage were identified. Importantly, the study of complex disease has resulted in few studies whose linkage results meet the stringent standards proposed by Kruglyak and Lander (1995). Therefore, we cautiously interpret our results and have reported all results for methods of analysis producing lod scores greater than 2.0.

CONCLUSION

In conclusion, we find suggestive evidence of linkage to chromosome 1 for a quantitative phenotype related to aspects of alcohol use and anxiety. This evidence is consistent across two independently ascertained samples, and a maximum lod score of 3.3 is obtained when these samples are combined for analysis. This finding is of particular interest because a number of other anxiety-related phenotypes provide suggestion of linkage to this area, as well. This phenotype also yields consistent, albeit modest, suggestion of linkage to chromosome 15, a region also implicated in EEG findings in the COGA sample. In the initial sample, there is also suggestion of linkage of this phenotype to chromosome 5 to a region with previously reported linkage to low alcohol response, and to chromosome 4 for the phenotype "maximum number of drinks in a 24-hr period." The diversity of phenotypes providing linkage to areas implicated in our quantitative phenotype suggests that this new phenotype may capture many elements of susceptibility to alcohol use and related behaviors, and may represent the expression of genes contributing to the overlap between these behaviors. Although we chose to focus these preliminary analyses on items from the SSAGA regarding alcohol use and patterns, future phenotype development will include aspects of diagnostic depression, anxiety, antisocial personality, and other disorders comorbid with alcohol problems. We are working on further exploring and refining this phenotype in the COGA sample.

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