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The Search for Genes Related to a Low-Level Response to Alcohol Determined by Alcohol Challenges

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Background: A low level of response (LR) to alcohol seems to relate to a substantial proportion of the risk for alcoholism and to have significant heritability.

Methods: This report describes the results of a genome-wide segregation analysis for the first 139 pairs of full siblings by using an alcohol challenge protocol as a direct measure of LR. Subjects from 18 to 29 years old were selected if the original screen indicated they had an alcohol-dependent parent, reported a personal history of drinking but had no evidence of alcohol dependence, and had a full sibling with similar characteristics. Body sway and Subjective High Assessment Scale scores were measured at baseline and at regular intervals after the administration of a measured dose of alcohol. Participants and available parents were genotyped for 811 microsatellite markers, and resulting data were analyzed with a variance component method.

Results: Nine chromosome regions with logarithm of the odds ratio (LOD) between 2.2 and 3.2 were identified; several had previously been implicated regarding phenotypes relevant to alcoholism and the LR to alcohol. Several regions identified in the previous linkage study by using a retrospective self-report questionnaire were potentially confirmed by this study. The strongest evidence was on chromosomes 10, 11, and 22.

Conclusions: Several chromosomal areas seem to relate to the low LR to alcohol as a risk factor for alcohol dependence.

Key Words: Alcohol, Linkage, Genetics, Level of Response.

A VARIETY OF GENETICALLY influenced characteristics seem to contribute to the risk for alcohol dependence (Schuckit, 2002). Thus, the 60% of the variance for alcoholism vulnerability that is likely to be explained by genes (Prescott and Kendler, 1999) may relate to separate sets of alleles tied to variations in alcohol-metabolizing enzymes (Li, 2000), high levels of disinhibition or impulsivity (Begleiter and Porjesz, 1999), and heavy problematic drinking observed in the context of several independent psychiatric disorders, such as schizophrenia and bipolar disease (Schuckit, 2002; Schuckit et al., 1997). Even after controlling for the potential effect of these characteristics (Schuckit et al., 2000), a substantial proportion of the risk for alcoholism also seems to relate to an additional phenotype: a person's level of response (LR) to alcohol.

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A low LR to alcohol can be established by demonstrating relatively little effect at a given blood alcohol concentration or through a self-report of a relatively high number of drinks required for specific alcohol effects (Schuckit and Smith, 1996, 2000; Schuckit et al., 1997). Through either method, a low LR has been noted by most, but not all (Vogel-Sprott and Chipperfield, 1987), studies to relate to a variety of risk factors for alcoholism, including a family history of alcohol dependence, Native American heritage, and, among Asians, a Korean background (Ehlers et al., 1999; Erblich and Earleywine, 1999; Wall et al., 1999). Most of the studies regarding familial alcoholism have evaluated the offspring of alcoholics by using an alcohol challenge, usually determining LR after the intake of three to five standard drinks consumed over approximately 10 min (Schuckit and Smith, 1996, 2000). The subsequent reaction to alcohol has historically been measured by changes in subjective feelings of intoxication and standing steadiness or body sway, along with corroborative information from more expensive or intrusive testing procedures, including electrophysiological measures and alcohol-related changes in hormones (Schuckit et al., 1983; Schuckit and Gold, 1988; Schuckit and Smith, 2000).

To date, all four follow-up studies have reported a relationship between a low LR, as established from an alcohol challenge earlier in life and before acquired tolerance would be likely to be prominent, and the subsequent risk

for alcohol-related problems, including alcohol dependence (Heath et al., 1999; Rodriguez et al., 1993; Schuckit and Smith, 1996; Volavka et al., 1996). We conclude that, at least for the subset of individuals with LRs at the extremes when initially tested, a low reaction to alcohol mediates a significant proportion of the relationship between a family history of alcoholism and alcoholic outcome in these subjects (Schuckit and Smith, 1996).

The heritability of aspects of LR has been estimated to be between 0.4 and 0.6 (Heath et al., 1999; Schuckit et al., 2001), although the specific relevant genes are not yet known. A pilot, case-control allelic association study suggested a possible role in the LR to alcohol for the γ-aminobutyric acid receptor A6 (GABRA6) and alleles for the promoter for the serotonin transporter (Schuckit et al., 1999). A family-based genome-wide chromosome segregation analysis was performed by using the indirect self-report measure of the usual number of drinks a person believed were required to achieve various levels of intoxication early in the drinking career: the Self-Report of the Effects of Ethanol (SRE) questionnaire (Schuckit et al., 2001). Nine areas of chromosomes were identified with logarithm of odds (LOD) scores greater than 2.0, including regions on chromosomes 1, 2, 10, 11, 13, 20, and 21. Areas of chromosomes 11, 13, 20, and 21 had LOD scores greater than 3.0, a traditional threshold for significance for a genome-wide analysis. These results encouraged us to perform a family-based genome-wide chromosome segregation analysis for the alcohol challenge, a more direct measure of an LR. This report describes data from the first 139 pairs of full siblings (sib pairs) as part of a new, ongoing study using LR determined from a traditional alcohol challenge protocol.

METHODS

The protocol described here was approved by the Human Subjects Protection Committee at the University of California in San Diego and used written, informed consent. The procedures are similar to those used in prior alcohol challenge studies (Schuckit and Smith, 1996, 2000). Subjects were initially chosen from among students attending two San Diego universities through a questionnaire mailed each year to more than 5000 randomly selected individuals. These men and women, 18 to 29 years old, answered questions on demography, substance use and problems, psychiatric histories, and family patterns of disorders according to items extracted from the Schedule of Affective Disorders and Schizophrenia (Spitzer and Endicott, 1977) and the Semi-Structured Assessment for the Genetics of Alcoholism (Hesselbrock et al., 1999). Potential subjects were paid US$5 for completed questionnaires, which were used as an initial screen of large numbers of individuals to identify relevant sib pairs. Mailings were limited to the original questionnaire and several reminder letters and generated response rates of between 30% and 40%. Staff members reviewed the questionnaires to identify subjects who fulfilled the following criteria: were not alcohol dependent; had consumed alcohol at some time; never met criteria for dependence on an illicit substance; were in good health and did not take chronic medications; indicated that a parent seemed to have enough alcohol problems to meet DSM-IV alcohol dependence; if the alcohol-dependent parent was the mother, the onset of alcoholism occurred after the birth of the subject; at least one parent was still alive; and there was an 18-to 29-year-old full sibling with similar characteristics regarding a history of drinking, no evidence of alcohol dependence, and health status.

Potential subjects were then telephoned to check the information from the questionnaire and to see whether they was interested in participating in an alcohol challenge protocol. The approximately 90% who answered in the affirmative were asked to speak with their parents and relevant siblings to see whether they would participate and to ask their permission to be contacted.

A face-to-face meeting was held with each subject and separately with each full sibling. The session began with the administration of the Semi-Structured Assessment for the Genetics of Alcoholism interview, which reviews family history, 19 axis I diagnoses from the DSM-IV, and symptoms of antisocial personality disorder (American Psychiatric Association, 1994; Hesselbrock et al., 1999). Appropriate subjects were invited to the laboratory for acclimation to the testing environment and to practice tests, and women were screened for pregnancy (an exclusion criterion). Each person also filled out the SRE and gave permission for future follow-ups. Subjects were then scheduled for the alcohol challenge and asked to refrain from food for 10 hr and from alcohol and all other drugs for 24 hr before testing.

The test session began at 7:30 AM with a light breakfast of juice and buttered toast. A blood sample was drawn for tests to corroborate the health status, along with a 40-ml sample of whole blood for genetic analysis. Baseline scores were next obtained for relevant tests, including body sway and the Subjective High Assessment Scale (SHAS; Schuckit and Gold, 1988). Body sway was measured by an apparatus consisting of a harness worn at the level of the axilla from which two perpendicular ropes extended forward and to the left side (Schuckit and Gold, 1988). These ropes were attached to pulleys so that the number of centimeters of movement per minute could be recorded, and a value was calculated as the mean of three 1-min sessions, each separated by 60 sec of rest. The SHAS included ratings of 13 subjective feelings of both pleasant effects of alcohol (e.g., high, intoxicated, and cheerful) and negative aspects of the drug (e.g., confused thinking, nauseated). Each item was measured as a 36-point Likert scale ranging from 0 (not at all) to 36 (extremely).

Subjects were given 8 min to consume a beverage administered from a closed container, which helped to disguise the taste and impaired a person's ability to estimate the number of drinks (Mendelson et al., 1984). The beverage was a 20% by volume solution of 0.75 ml/kg of 95% ethanol for women and 0.90 ml/kg for men, a differential based on gender differences in the percentage of body water and the initial rate of metabolism of alcohol. Breath alcohol levels were determined at both baseline and regular intervals Alco-Sensor III (Intoximeters Inc., St. Louis, MO). Body sway, SHAS, and breath alcohol levels were then repeated at approximately 15 and 30 min and every half-hour thereafter during the 3-hr test protocol.

Changes in body sway and SHAS values from baseline to 60 min after alcohol consumption were used as the dependent variables for the genetic analyses performed on samples sent to the University of California at San Francisco (UCSF) laboratory. DNA was isolated from whole blood by using a commercial kit (Gentra, Minneapolis, MN), and genotypes were generated for a panel of microsatellite polymorphisms (Weber and May, 1989) by using fluorescently labeled polymerase chain reaction (PCR) primers (HD5, version 2.0; Applied Biosystems, Foster City, CA). The HD5 panel set had 811 markers with an average marker-to-marker distance of 4.6 cM (maximum, 14 cM) and an average heterozygosity of greater than 77%. Some additional markers in the commercial panel had been omitted because of null alleles, irregular plus "A" effects, and irregular allele spacing or other problems with reproducibility. None of the omitted markers was adjacent to other omitted markers. The sizes of the PCR products for the markers were determined from electropherograms produced with an ABI 3700 (Applied Biosystems, Foster City, CA), a 96-channel capillary electrophoresis device. A single-assay protocol was developed in which a subject's DNA was amplified for 811 markers, with PCR reactions from an individual combined into 96 pools. The sizes of the 811 amplimers for a subject were then measured relative to internal standards in a single run of the ABI 3700. Repeated analysis of a control

sample at intervals over a period of several years suggested that the size of amplimers to standards varied less than 0.1 base pairs for more than 95% of the markers.

From 1 to 5% of electropherograms from a particular run were unusable because of poor sample or matrix loading. These electropherograms were identified through an automated algorithm, and robotic protocols were used for resampling the PCR reactions for reanalysis on the ABI 3700. Fifteen samples failed to give usable genotypes for at least 90% of the markers, usually because of poor DNA quality, and were regenotyped. Comparison of the genotypes from these repeated samples, along with 86 samples from other projects, which were regenotyped blinded to previous results, produced a 0.7% rate of genotype discrepancies. This error rate represents an upper limit and should be contrasted with an error rate of less than 0.05% for the control samples that were genotyped more than 40 times over the course of several years.

The sizes of marker amplimers were determined (blinded to pedigree structure and subject characteristics) from the electropherogram by 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 a one hundredth of a base pair. Fragment sizes were binned to alleles by using an automated algorithm developed in the UCSF laboratory which assumes that the distribution of allele sizes will have a sine-squared distribution with a periodicity about the 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, and allele frequencies observed in the founders 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 UCSF laboratory.

The genotypes for all of the autosomal markers were analyzed for each family by using Pedigree RElationship Statistical Test (PREST) (McPeek and Sun, 2000) to detect sample and pedigree structure errors. DNA was reisolated from a stored frozen blood specimen, and the genotyping was repeated for any individual for whom PREST detected a probable error. Data were removed from further consideration for two samples because of what seemed to be errors in either the laboratory procedures or the pedigree structure. The program Pedcheck was used to detect non-Mendelian inheritance (O'Connell and Weeks, 1998), revealing that fewer than 0.5% of genotypes were inconsistent with other family members. Regenotyping of a fraction of these markers indicated that most of these results were reproducible, suggesting somatic mutations, mosaicism, or null alleles. Six markers were excluded from analysis because of a high frequency of Mendelian segregation errors, and in other cases, the genotypes for the entire family were excluded for the specific marker with an Mendelian error. To further reduce errors, the probability that each genotype was correct was assessed by using the error-checking algorithm implemented in Merlin (Abecasis et al., 2002), in which genotypes that had a probability of less than 0.025 of being correct were removed from further consideration. Less than 0.2% of the genotypes were identified as being unlikely when compared with other marker data for the families.

Variance component estimate methods were used to calculate LOD scores by using Genhunter version 2.1 (Kruglyak et al., 1996) and Merlin, with similar results. Subsequent analysis with Sequential Oligogenic Linkage Analysis Routines(SOLAR) version of variance component analysis gave similar results without assuming a dominant component for the trait (Almasy and Blangero, 1998). Including gender as a covariate had no appreciable affect on the analysis. Data were also secondarily analyzed by using the nonparametric methods implemented in Merlin and Genhunter for quantitative traits.

RESULTS

Alcohol challenge data and genotypes incorporated into Table 1 and Fig. 1 were available from 90 nuclear families and included 70 families with 2 children, 17 with 3 children, and 3 with 4 children, generating 139 sib pairs and 8 cousin pairs. The Pearson product-moment correlations for LR measures in sib pairs was 0.36 ($p < 0.001$), and for unrelated individuals it was 0.02 ($p = 0.87$).

The siblings included 43.7% males and 56.3% females; 56.1% of the pairs involved same-sex individuals. At the time of testing, the 203 sibs had an average age of 22.4 (standard deviation of 3.26) years and 14.2 (standard deviation of 1.74) years of education. Most were Caucasian (72.2%), 20.0% were nonblack Hispanic, and 7.8% were African-American. At the time of testing, 14.8% of the subjects were married, 83.8% were single, and 1.4% were separated or divorced. For 85.0% of the subjects, the alcohol-dependent parent was the father, whereas for 4.4% it was the mother, in 4.0% it was both parents, and in 6.6% the more intensive interview revealed that neither parent met full criteria for dependence.

Table 1 and Fig. 1 present the multipoint variance component LOD scores for the SHAS and for body sway. Table 1 offers more detailed information, whereas Fig. 1 presents an overview of all chromosomal findings. Bars in Fig. 1 above the LOD score plot for each chromosome refer to areas associated with findings from additional studies regarding the SRE, alcoholism and related phenotypes, and several specific genes (Almasy et al., 2001; Dick et al., 2002; Foroud et al., 2000; Long et al., 1998; Nurnberger et al., 2001; Schuckit et al., 1999, 2001).

Nine chromosome regions gave multipoint LOD scores between 2.2 and 3.2, including several areas previously implicated in phenotypes relevant to alcoholism. The highest multipoint LOD scores (3.2 and 2.4) for the SHAS were on chromosomes 10 and 13, and those for body sway were on chromosomes 11, 22, 2, and 18, with LOD scores of 2.9, 2.9, 2.6, and 2.6, respectively.

DISCUSSION

This genome-wide linkage analysis found evidence for loci related to SHAS and body sway in several chromosome locations, with the strongest evidence for chromosomes 10, 11, and 22. These regions reach traditional conventional levels of significance for a genome-wide scan (Morton, 1955) but would be classified as only suggestive by the more stringent criteria suggested by Lander and Kruglyak (1995). The high density of markers used for this genome-wide scan extracted most of the available segregation data (analysis not shown). To confirm these findings, we continue to recruit subjects toward a goal of a final sample of 500 sib pairs.

Comparison of these findings with those of other studies searching for loci that relate to the LR to alcohol and the diagnosis of alcoholism suggests reasons for being optimistic that even some of the weaker findings will be replicated in a larger dataset. In the previously reported genome-wide linkage study that used the indirect evaluation of LR—the

Table 1. Summary of Multipoint LOD Scores for SHAS and Body Sway (BS) Measures During the Alcohol Challenge

Trait	Chromosome	Locus	Maximum LOD	Linkage findings	Candidate genes[a]
BS	1	20	1.7		
BS	1	50	1.9		Serotonin receptor 1D; cannabinoid receptor 2; opioid receptor δ
BS	1	190	1.2	SRE[b]	
SHAS	1	225	1.8	COGA factor 2[c]	
BS	2	140	2.6	COGA dependence[d]	
BS	4	60	1.3	COGA β EEG power[e] NIH dependence[f] COGA dependence[d] Maximum drinks in 24 hrs[g]	GABA receptor subunit B1, A2, A4, and G2; nicotine receptor A9
BS	4	170–190	1.4	COGA P3[h]	
BS	5	145	1.2		*GABRA6*; aldehyde dehydrogenase 7A1
BS	7	60	1.7	COGA dependence[d]	L-dihydroxyphenylalanine decarboxylase; pituitary adenylate cyclase–activating polypeptide receptor type I; adenylyl cyclase 1
BS	7	100	1.8	COGA dependence[d]	Metabotrophic glutamate receptor 3
SHAS	10	120	1.7	SRE[b]	Alpha-2A-adrenergic receptor; vesicular monoamine transporter 2; serotonin receptor 7
BS	10	140	2.6		
SHAS	10	170	3.2		*CYP2E*
SHAS	11	70	1.8	SRE[b]	
BS	11	145	2.9		
BS	12	20	1.8		
SHAS	13	90	2.4		
SHAS	14	50	1.7		
BS	16	60	2.2		
BS	18	70	2.6		
BS	18	105	1.6		
BS	19	20	2.3		
BS	22	20–30	2.9		Adenosine A2 receptor

[a] Location of genes as reported in http://www.ncbi.nlm.gov and http://www.celera.com/.
[b] Self-Rating of the Effect of Ethanol (Schuckit et al., 2001).
[c] Principal component analysis factor 2 that is weighted for late-onset drinking and harm avoidance (Dick et al., 2002).
[d] Alcohol dependence from the COGA project replication study (Foroud et al., 2000).
[e] Beta frequency EEG power in participants from the COGA project (Porjesz et al., 1997).
[f] Alcohol dependence in Native Americans (Long et al., 1998).
[g] Response of participants in the COGA project to the maximum number of drinks consumed in a 24-hr period (Nurnberger et al., 2001).
[h] P3 evoked potential of participants in the COGA project (Almasy et al., 2001).

SRE—nine chromosome regions were observed to have a LOD score of greater or equal to 2.0, including scores of 4.0 for chromosomes 11 and 21 (Schuckit et al., 2001). Although no significant LOD scores were detected in this study for chromosome 21, a maximum LOD score of 1.8 was observed for the SHAS trait for the region on chromosome 11. Several additional regions identified in the previous linkage study for the SRE were also supported by this work.

The diagnosis of alcoholism has been used as a phenotypic trait in two large genome-wide family-based chromosome segregation studies, including the COGA investigation (Almasy et al., 2001; Dick et al., 2002; Foroud et al., 2000; Nurnberger et al., 2001) and the NIH study of Native Americans (Long et al., 1998). Because we believe that some of the susceptibility to the diagnosis of alcoholism can be due to the LR to alcohol, we investigated whether any of the regions implicated in these studies was supported by the current analyses. Among five regions cited by COGA regarding a possible linkage for alcohol dependence, four may relate to our SHAS or body sway findings. An additional factor on chromosome 1 was identified in the COGA dataset as related to the later onset of drinking and to higher harm avoidance in the region where we saw a LOD score of 1.8 for the SHAS.

Both the NIH Native American Project and COGA reported findings related to alcoholism at 60 to 80 cM on chromosome 4 in an area that contains the GABA receptor gene, *GABR1*, and that may relate to β electroencephalogram frequency (Foroud et al., 2000; Long et al., 1998; Porjesz et al., 1997). This study observed a maximum LOD score of 1.3 in this region. An additional area highlighted in our work on chromosome 4 at approximately 170 to 190 cM has been reported to relate to the positive deflection brain wave (P3) of the event-related potential (Almasy et al., 2001).

Analyses of pilot data showed a trend suggesting an association between a low LR to alcohol and alleles of the serotonin transporter (*SLC6A4*) and the GABA receptor subunit α6 (*GABRA6*; Schuckit et al., 1999). In this work, a LOD score of 1.2 was observed for body sway for the segment that contained the *GABRA6* locus on chromosome 5, but no association evidence of a locus affecting LR to alcohol was seen in the area relevant to the *SLC6A4* locus. It is possible that the effect of *SLC6A4* on the LR to alcohol could be obscured by epistasis.

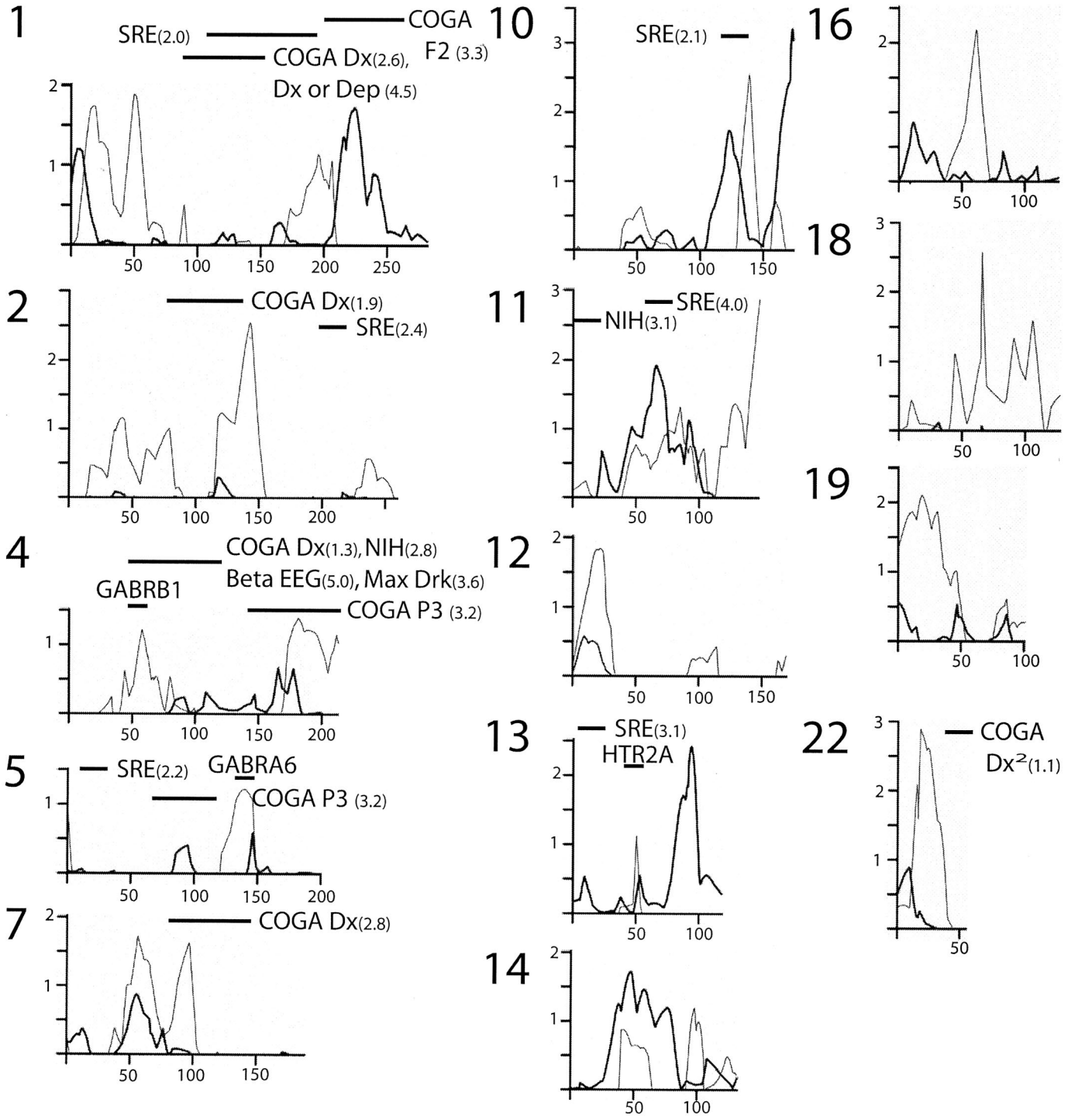

Fig. 1. Multipoint LOD scores for SHAS and body sway measures during the alcohol challenge. Multipoint LOD scores are shown for all chromosomes for which a LOD score greater than 1.5 was observed for either the SHAS (thick lines) or body sway (thin lines) and for chromosomes 4 and 5. In addition, the location of candidate genes relative to the linkage maps are shown with small bars for candidate genes that have been assessed for allelic association with a low level of response to alcohol, including GABAalpha (*GABRA6*) and serotonin$_{2A}$ (*HTR2A*) receptors (Schuckit et al., 1999). The locations of the serotonin transporter and serotonin receptor 2C from the same study are not shown because meaningful LOD scores were not observed for these chromosomes. Some of the chromosomes shown have had interesting findings reported by other studies investigating alcohol-related phenotypes. The bars indicate the approximate support interval for these findings. SRE, Self-Report of the Effects of Ethanol (Schuckit et al., 2001); COGA Dx, COGA diagnosis of alcohol dependence (Foroud et al., 2000); COGA F2, second factor from a principal components analysis of the COGA project subjects that is weighted for late-onset drinking and harm avoidance (Dick et al., 2002); Dx or Dep, COGA diagnosis of alcohol dependence or depression (Nurnberger et al., 2001), respectively; NIH, alcohol dependence from a study of Native Americans (Long et al., 1998); Beta EEG, power of the β frequencies in electroencephalograms for the COGA project participants; Max Drk, maximum number of drinks consumed in a 24-hr period by the COGA project participants (Saccone et al., 2001); COGA P3, evoked potentials (Porjesz et al., 1997). LOD scores are shown in parentheses. The location of the GABA receptor Beta 1 on chromosome 4 is indicated by *GABRB1* because polymorphisms in this gene seem to be responsible for the β frequency EEG power linkage results (Porjesz et al., 2002).

Although not reported in Table 1 or Fig. 1, LOD scores substantially higher and lower than those shown were occasionally seen when other analytical methods were used, with a good overall correlation between the Hasman-Elston–based algorithms and the variance component analysis shown here. The maximum likelihood quantitative trait linkage variance estimation gave higher LOD scores at most of the locations identified in Table 1, along with additional regions with LOD scores between 3 and 4.5 that were not supported by the variance component or Hasman-Elston analysis. Because there is no consensus as to which is the best analytic approach, the ultimate gold standard is to be able to demonstrate the reproducibility that allows for positional cloning.

Table 1 lists some candidate genes that map to the regions identified, including several that might play a role in the biology of an LR to alcohol. A potentially promising example is *CYP2E* on chromosome 10, which encodes a cytochrome P450 and which is induced by, and metabolizes, ethanol (Upadhya et al., 2000). Alleles of genes related to other alcohol-metabolizing enzymes contribute to more intense alcohol reactions, such as flushing, which are associated with lower alcohol consumption (Li, 2000), and an allele of *CYP2E* may be seen more frequently among heavy-drinking Japanese men (Sun et al., 1999).

CONCLUSION

In conclusion, these data represent the first family-based genome-wide chromosome segregation analyses using alcohol challenges to measure the LR phenotype. The results corroborated the possible importance of several chromosomal regions highlighted in prior segregation studies that used an indirect measure of LR and reinforced several areas of interest regarding alcohol-related behaviors or alcoholism. The consistency of these results will be re-evaluated as the number of sib pairs grows.

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