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Evidence for Genetic Linkage to Alcohol Dependence on Chromosomes 4 and 11 From an Autosome-Wide Scan in an American Indian Population

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To identify specific genes affecting vulnerability or resistance, we performed a whole-autosomal genome scan for genetic linkage to alcohol dependence in a Southwestern American Indian tribe. Genotypes at 517 autosomal microsatellite loci and clinical evaluations were available for 152 subjects belonging to extended pedigrees and forming 172 sib-pairs. Highly suggestive evidence for linkage emerged for two genomic regions using two- and multipoint sib-pair regression methods; both regions harbored neurogenetic candidate genes. The best evidence is seen with *D11S1984* (nominal P = 0.00007, lod ≅ 3.1) on chromosome 11p, in close proximity to the *DRD4* dopamine receptor and tyrosine hydroxylase (*TH*) genes. Good evidence is seen with *D4S3242* (nominal P = 0.0002, lod ≅ 2.8) on chromosome 4p, near the β1 GABA receptor gene. Interestingly, three loci in the alcohol dehydrogenase gene cluster on chromosome 4q showed evidence for linkage with two-point analyses, but not multipoint analysis. Am. J. Med. Genet. (Neuropsychiatr. Genet.) 81: 216–221, 1998. © 1998 Wiley-Liss, Inc.†

INTRODUCTION

Alcohol dependence is the most severe diagnostic category of alcoholism. It indicates that an individual continues drinking despite significant problems related to tolerance, withdrawal, and compulsive drinking behavior [American Psychiatric Association, 1987]. Family [Aston and Hill, 1990; Crum and Harris, 1996], twin [Kendler et al., 1984; Pickens et al., 1991], and adoption [Cloninger et al., 1981; Sigvardsson et al., 1996] studies reveal that alcohol dependence is causally complex, but in part hereditary. According to epidemiologic studies, the prevalence of alcoholism is higher in men than in women and differs among ethnic groups. Regardless of sex or ethnicity, prevalence is negatively associated with education, income, and occupational status, and positively associated with marital instability [Bucholz, 1992; Helzer and Pryzbeck, 1988; Helzer et al., 1991]. Extremely high rates of alcoholism have been observed in some American Indian populations, i.e., approximately 80% in men and 50% in women. Nevertheless, no evidence suggests that alcoholism in American Indians departs from the basic sex/socioeconomic trends observed in other ethnic groups [Brown et al., 1993; Kinzie et al., 1992].

Well-defined populations such as American Indian tribes are advantageous for identifying the causes underlying complex diseases [Lander and Schork, 1994]. Such populations are often more genetically and environmentally homogeneous than the general population; they are geographically restricted, and large families are common. The members of the American Indian tribe studied here have shared a common heritage of biology, culture, language, and location since pre-Columbian times. The tribal name and exact location are not given here because they are unnecessary for the analyses presented.

In a large sample (N = 582) from this American Indian tribe [Long et al., 1996], the odds for alcoholism in a subject are significantly increased by having an

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alcoholic first-degree relative. While the male-to-male pattern of risk is not statistically significant, the odds for alcoholism in males are increased about 1.6-fold by having an alcoholic male first-degree relative. In females, there is a statistically significant 2.2-fold increase in odds for alcoholism afforded by having an affected first-degree female relative. Similar results have been observed in a large U.S. metropolitan sample. Interestingly, the odds for alcoholism in females in this sample are also significantly increased by having alcoholic female second- and even third-degree relatives. The risk pattern in females translates to a 40% heritability of alcoholism, which approximates that seen in female twin studies [Kendler et al., 1994; Pickens et al., 1991]. It is also consistent with the hypothesis that for multifactorial disorders, the subgroup with the lower frequency requires a higher genetic loading for expression [Reich et al., 1972, 1979]. More importantly, these findings suggest that genetic factors determining alcoholism in this population are not overwhelmed by a high environmental loading. To find specific genes predisposing to alcohol dependence, we performed a genome-wide scan using typings at 517 autosomal microsatellite loci spaced at an average distance of 6.9 cM. Genotypes were available for 152 subjects clinically evaluated for DSM-III-R alcohol dependence [American Psychiatric Association, 1987].

MATERIALS AND METHODS

Sampling Strategy

Elder tribal members who are regarded as matriarchs or patriarchs and who possess a good knowledge of family structures provided information on large multigenerational genealogies. Three of these were selected for analysis on the basis of availability of adult members, geographic accessibility, and willingness of at least one family member to advocate for the study. Participants were genealogy members, age ≥21 years, and eligible for tribal enrollment (≥¼ tribal heritage). Due to the high prevalence of alcoholism there was no need to ascertain subjects through affected probands, and recruitment was blind to the clinical histories of subjects or their relatives.

Focus groups comprised of tribal staff and community members reviewed testing instruments and questionnaires for potential cultural biases and general suitability to the population. They also made recommendations on our final selection of testing instruments and on the conduct of interviews. Informed consent was obtained under a human subjects research protocol approved by the Tribal Council and the Institutional Review Board of NIAAA. Our final sample consisted of 582 subjects, and the participation rate was 93% of the eligible persons contacted.

Testing Instruments, Interviews, and Psychiatric Diagnoses

Research diagnoses for alcohol dependence were based on: 1) a semistructured psychiatric interview using the Schedule for Affective Disorders and Schizophrenia-Lifetime Version (SADS-L), 2) medical, educational, court, and other records, and 3) corroborative information from family members. The SADS-L was administered to all subjects by a psychologist experienced in psychiatric assessment with this tribe and other American Indian populations.

Diagnostic and Statistical Manual of Mental Disorders, Third Edition-Revised (DSM-III-R) [American Psychiatric Association, 1987] diagnoses were made from the SADS-L by following operationally defined criteria and using the instructions of Spitzer et al. [1989]. Diagnoses were made from the SADS-L interview data by two blind raters: a clinical social worker and a clinical psychologist. Diagnostic differences were resolved in a consensus conference which included a senior psychiatrist experienced in diagnosis of American Indian people. All analyses reported hereafter in this presentation are based on a subsample of 152 subjects who also participated in a long-term epidemiologic study of chronic disease, and for whom genotypes at 517 microsatellite loci were available. The 152 clinically evaluated subjects belong to 32 interrelated nuclear families, and they form 172 sib-pairs. Among the sib-pairs, 18 were concordantly unaffected, 57 were discordant, and 97 were concordantly affected.

Genotypings

The typings were determined for 1,311 individuals as a part of a study on other diseases for which our research subjects substantially overlapped. Each locus was amplified by PCR, using either fluorescent- or radioactive-labeled primers, and most typings were performed following standard protocols by the Marshfield Medical Research Foundation (Marshfield, WI) [Dubovsky et al., 1995; Schwengel et al., 1994]. The loci analyzed cover all of the autosomes with an average separation of 6.9 cM and largest gap of 25.6 cM. The CRI-MAP program [Lander and Green, 1987] was used to create maps with distances estimated from these data. In most cases, the order of the marker loci was taken as that on maps provided by the Marshfield Medical Research Foundation. In a few instances (chromosomes 1, 4, 7, and 19), a slightly different marker order was used, as there was strong evidence to support such an order. Allele frequencies were estimated by gene counting on all 1311 individuals.

Linkage Analyses

Two-point linkage analysis was conducted using the nonparametric sib-pair regression method developed by Haseman and Elston [1972], as programmed in the SIBPAL module of the S.A.G.E. package [S.A.G.E., 1994]. While this sib-pair linkage method was originally explained for a continuous trait, it is also applicable to binary traits [Elston, 1997]. Among the chief advantages of the Haseman-Elston method are that it is conceptually based on allelic identity by descent; it is powerful with common disorders because it combines information from all affected/unaffected classes of sib-pairs; and it provides a significance test corrected for multiplex sibships. The statistical validity of *P* values produced by the Haseman-Elston test has been demonstrated for small samples [Wilson and Elston, 1993]. In addition, we performed limited computer simula-

tions in order to confirm the accuracy of *P* values produced by the SIBPAL program for this study. Based on a total of 50,000 trials using allelic configurations at five of the tested loci (10,000/locus) and the observed pedigrees and trait distributions, the overall agreement was good. Nevertheless, a slight anticonservative trend was noted (SIBPAL→empirical): (0.0500→0.05290), (0.0100→0.01402), (0.0010→0.00244), and (0.0001→0.00034). Since a computer simulation to definitively estimate *P* values, for all loci and allelic configurations, and for two-point and multipoint analysis, is beyond the scope of this study, we chose to report all *P* values as "nominal *P* values."

Genotypes from 29 relatives who were not psychiatrically interviewed were used for family structure testing and to improve estimates of allelic identity by descent. Our sample contained 172 clinically evaluated sib-pairs, but the families included several large multiplex sibships; therefore, significance tests were conducted with about 70 effective degrees of freedom as determined by the standard weighting formula employed in SIBPAL. While multiplex sibships reduce the number of degrees of freedom available for hypothesis tests, they are generally beneficial for analysis because they provide information on the genotypes of missing parents, and thereby improve estimates of the proportion of shared genes that are identical by descent. SIBPAL uses a likelihood method for nuclear families that fully uses this information. With respect to these analyses, it is important to note that the 517 locus typings allowed for extensive parentage testing, so that our family relationships were essentially confirmed. As a second check, we note that the proportions of identical-by-descent alleles shared by siblings at these loci do not differ systematically from the expected, i.e., 50%. SIBPAL also provides an estimate of the proportion of alleles shared by descent in all classes of affected/unaffected sib-pairs. These proportions are useful for demonstrating the effects underlying the regression results.

Multipoint linkage analysis was performed by the sib-pair interval mapping procedure of Fulker et al. [1995], using a SAS program (written by R.L.H.). This is an extension of the Haseman-Elston procedure that simultaneously takes into account all marker information available on a chromosome. It is particularly useful for dense maps with marker loci that vary with respect to information content.

RESULTS

As demonstrated in Table I, alcohol dependence is highly prevalent, it is more common in men than women, and the mean age of participating subjects is late relative to the onset of heavy drinking.

TABLE I. Sample Structure and Alcohol Dependence Phenotypes

Sex	Clinical status	N	Age ± SE[a]	Onset ± SE[b]
Female	Not affected	45	44.1 ± 2.5	
	Alcohol dependent	51	43.4 ± 1.6	23.8 ± 1.4
Male	Not affected	9	30.6 ± 3.0	
	Alcohol dependent	47	40.9 ± 1.9	17.5 ± 0.7

[a]Subject age (years) at clinical evaluation ± standard error.
[b]Age (years) at which heavy drinking began ± standard error.

The 517 Haseman-Elston sib-pair regression linkage analyses revealed the 30 loci listed in Table II with nominal *P* values below 0.05. The results are presented by regression *P* value because the Haseman-Elston method is the most powerful nonparametric linkage technique for nuclear families [Davis and Weeks, 1997]. Table II also provides the probabilities of allelic identity by descent (ibd), as computed by SIBPAL, for the three sib-pair classes (unaffected, discordant, and affected). As expected for genetic linkage, estimated ibd in discordant sib-pairs is <0.50 for all loci with a significant regression. Moreover, estimated ibd in concordant affected pairs is >0.50 for all but one locus with a significant regression.

Linkage to alcohol dependence is strongly suggested for *D11S1984,* which is located at chromosome 11p15.5. For *D11S1984,* the nominal regression *P* value is 0.00007. This locus also shows a strong reduction in ibd, sharing in discordant sib-pairs (estimated ibd = 0.3950, nominal $P < 0.01$) and a strong excess of ibd, sharing in concordant affected sibpairs (estimated ibd = 0.5889, nominal $P < 0.01$). From Lander and Kruglyak [1995], a nominal $P = 0.00007$ translates to a nominal lod score of 3.1 (although it does not imply that a usual pedigree linkage analysis was performed). Observations this extreme would be observed by chance in only about one in six full-genome scans on a perfect genetic map that included the X chromosome. However, this is a conservative reference point because only autosomal genes are analyzed here, and because such extreme nominal *P* values would be less frequent with a more sparse genetic map. For example, we would expect an observation at this significance level in only 1 in 33 full-genome scans using a 10-cM map.

Ten loci with nominal $P < 0.05$ are located on chromosome 4. Seven of these loci are on the short arm; the lowest nominal regression *P* value (0.0002), equivalent to a lod score of about 2.8, is observed at *D4S3242.* This locus also shows a strong reduction in ibd sharing in discordant sib-pairs (estimated ibd = 0.3997, nominal $P < 0.01$) and a significant excess of ibd sharing in both concordant unaffected sib-pairs (estimated ibd = 0.6011, nominal $P < 0.05$) and concordant affected sibpairs (estimated ibd = 0.5564, nominal $P < 0.05$). We would expect one observation this extreme by chance in about 2–3 full-genome scans with a perfectly informative map, and less frequently with a more sparse map. In addition, we observed three closely linked loci with nominal *P* values in the $0.01 < P < 0.05$ range on chromosome 4q, *D4S1647, D4S3256,* and *D4S3240.* These loci map into the type I alcohol dehydrogenase (ADH) gene cluster. ADH catalyzes the first step in the principal ethanol metabolic pathway.

The two-point linkage analyses described above were followed with the multipoint extension [Fulker et al., 1995] of the Haseman-Elston linkage test. As shown in Figure 1 for chromosomes 4 and 11, the multipoint results closely parallel the two-point linkage findings. On chromosome 4, a position for an alcoholism-linked gene is sharply localized to the short arm, at p11–p13, and nearby *D4S3242.* The multipoint evidence for the other

TABLE II. Two-Point Sib-Pair Linkage Results, P values ≤ 0.05†

Chromosome	Locus	cM from pter	ibd in sib-pairs			Nominal regression P value†
			Unaffected	Discordant	Affected	
3	D3S2403	32.0	0.5800	0.4479*	0.5418*	0.00147
3	D3S2432	54.3	0.4758	0.4454	0.5754**	0.01351
3	D3S1746	165.3	0.5226	0.4401	0.5692*	0.02144
3	D3S1763	174.1	0.6207*	0.4461	0.5548*	0.00780
3	D3S3053	181.1	0.4922	0.4259*	0.5570*	0.01232
4	D4S2632	52.3	0.4347	0.3996**	0.5035	0.03783
4	D4S2382	58.3	0.5013	0.4126*	0.5303	0.00226
4	D4S174	61.8	0.4914	0.3775**	0.4745	0.02821
4	D4S1627	66.0	0.5193	0.4212*	0.5184	0.01650
4	D4S3242	68.0	0.6011*	0.3997**	0.5564*	0.00020
4	D4S1645	79.2	0.5585	0.4211**	0.5146	0.01476
4	D4S2456	88.5	0.4610	0.4659	0.5602*	0.04485
4	D4S1647	112.1	0.5696	0.4328	0.5423	0.01853
4	D4S3256	118.4	0.5024	0.4578	0.5728*	0.02667
4	D4S3240	119.7	0.4484	0.4376	0.5385	0.03517
5	D5S2501	124.3	0.5746	0.4173**	0.4952	0.00777
7	D7S531	0.7	0.5744	0.4327*	0.5278	0.00922
9	D9S319	41.6	0.5797	0.3957*	0.5269	0.00958
10	D10S1435	6.0	0.4935	0.4500	0.5460	0.02643
11	D11S1984	0.0	0.5330	0.3950**	0.5889**	0.00007
11	D11S2368	32.7	0.5696	0.4554	0.5383	0.04560
11	D11S1392	52.9	0.6027*	0.4148*	0.5052	0.01674
11	D11S976	126.3	0.5602	0.4253*	0.5182	0.02066
13	D13S895	114.3	0.5711	0.4300	0.5417	0.02780
13	D13S285	123.4	0.4641	0.4444	0.5441	0.04769
15	D15S153	63.3	0.5372	0.3973**	0.5020	0.02492
16	GATA5H07	12.5	0.5335	0.4642	0.5387*	0.02134
17	D17S1308	0.0	0.4973	0.3825**	0.5066	0.00491
17	GATA65G11	118.3	0.4458	0.4385*	0.5693**	0.00120
18	D18S844	124.0	0.5225	0.4589	0.5923**	0.00895

†Nominal P-values (last column) are from the Haseman-Elston sib-pair regression, which combines information over all three affected/unaffected classes of sib-pairs. Probabilities of allelic identity by descent (ibd), as computed by SIBPAL, are given individually for the three sib-pair classes.
*$P < 0.05$.
**$P < 0.01$.

linkage on chromosome 4, near *ADH,* is enigmatic because the evidence for linkage shown at the individual loci diminishes when they are analyzed in concert. Multipoint analysis does little to enhance the significant finding on chromosome 11. This is unsurprising because no flanking loci are distal to *D11S1984.*

DISCUSSION

Table II demonstrates that all three sib-pair categories (unaffected, discordant, and concordant) contributed to the significant linkage findings. In fact, discordant sib-pairs are likely the most informative sib-pair category, which is expected given the high base rate of alcohol dependence in this population. This underscores the value of using the regression method of Haseman and Elston [1972], which combines information from all three categories. Methods which rely solely on affected sib-pairs are less powerful [Davis and Weeks, 1997] and potentially compromise linkage analysis of common heritable disorders.

Several interesting candidate genes related to neurotransmission are located in the vicinity of *D11S1984* at the distal short arm of chromosome 11. Most prominent is the *DRD4* dopamine receptor gene, which has recently been associated with novelty-seeking in Israelis and Euroamericans [Ebstein et al., 1996; Benjamin et al., 1996] (but see Malhotra et al. [1996]). There are functional repeat polymorphisms at *DRD4* that can be directly assessed. However, allele frequencies are different between American Indian and European populations [Chang et al., 1996], and this can influence the power to detect linkage, linkage disequilibrium, and phenotypic associations [Thompson et al., 1988]. The tyrosine hydroxylase gene is also on chromosome 11 [O'Malley and Rotwein, 1988], near *D11S1984*. Tyrosine hydroxylase is the rate-limiting enzyme in dopamine biosynthesis. Therefore, variability in its coding or regulatory sequences may be related to individual differences in alcoholism vulnerability. Finally, it should be mentioned that the tryptophan hydroxylase gene, which encodes the rate-limiting enzyme in serotonin biosynthesis, is also located on chromosome 11p [Nielsen et al., 1992]. However, this gene is approximately 30 cM from *D11S1984* towards the centromere.

The strongest chromosome 4 candidate locus is the β*1 GABA* receptor gene (*GABARB1*), which is close to D4S3242 [Dean et al., 1991]. GABA (γ-aminobutyric acid) is an inhibitory neurotransmitter, and GABA receptors are allosterically modulated by benzodiazepines, barbiturates, and ethanol. While no functional alleles are known at *GABARB1,* it will be worthwhile to screen for them. The positive *ADH* findings cannot be dismissed. There are well-known alleles at genes in the *ADH* gene family that enhance catalytic activity,

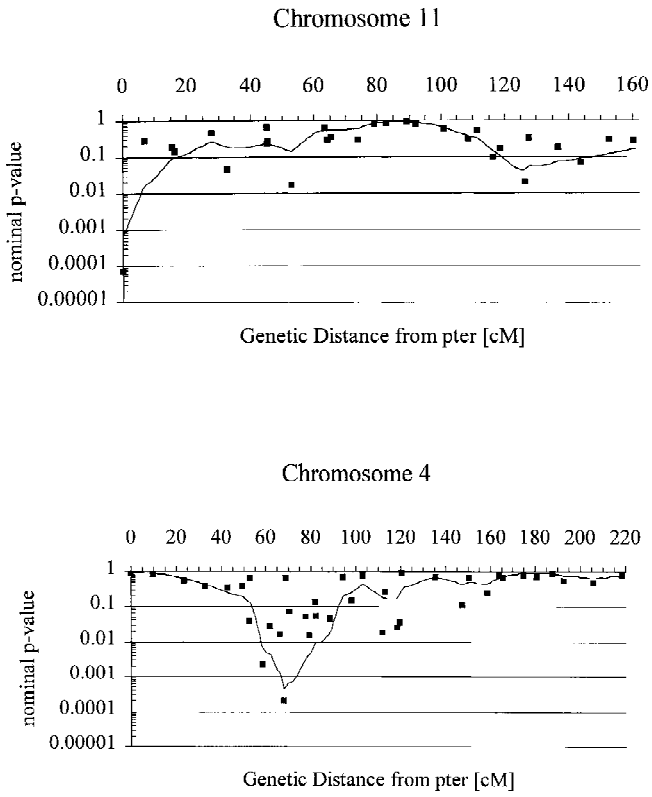

Fig. 1. Log_{10} *P* values for two-point and multipoint linkage analyses for alcohol dependence vs. microsatellite loci. Squares represent two-point results for alcohol dependence and specific microsatellite marker loci. Lines represent the multipoint extension of Fulker et al. [1995] of the method of Haseman-Elston [1972]. Results are shown only for chromosomes 4 and 11.

e.g., *ADH2*2* and *ADH3*1* [Thomasson et al., 1995]. Since high ADH activity can cause flushing, and thereby lower tolerance to alcohol, carriers of these alleles are less likely to be alcoholic [Chen et al., 1996]. Thus, variant alleles at *ADH* loci exert a protective effect, where they influence alcoholism vulnerability. Interestingly, *ADH2*2* is absent in North American Indians, while *ADH3*1* is often frequent [Rex et al., 1985].

In summary, these suggestive linkage findings provide interesting foci for future investigations. Populations, such as this American Indian tribe, with a very high prevalence of alcoholism may afford unique opportunities for finding protective factors, in addition to those seen with *ADH*. After further confirmation, it will be of interest to determine whether or not these suggestive linkage findings generalize to other American Indian or non-American Indian populations.

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