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A Family-Based Analysis of the Association of the Dopamine D2 Receptor (*DRD2*) with Alcoholism

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The possible association of the *DRD2* locus, and in particular the *TaqI*-A1 allele, with alcoholism remains controversial, in part because of differences in allele frequencies among populations. To avoid problems associated with differences in allele frequencies in different populations, we tested whether the *DRD2* locus is associated with alcohol dependence in a large family-based sample. Neither the transmission/disequilibrium test nor the Affected Family-Based Controls test provide any evidence of linkage or association between the *DRD2* locus and alcohol dependence.

Key Words: Alcoholism, Dopamine D2 Receptor, Association Study, Family Study.

SEVERAL LINES of evidence—including adoption, half-sibling, twin, and family studies—point to a genetic component to the risk for alcoholism.[1–4] Alcoholism is a complex disease, with no simple pattern of inheritance and with substantial environmental and social influences. The genes that affect the risk for alcoholism have been sought by several methods, including studies of candidate genes and searches of the whole genome.

The *DRD2* gene on chromosome 11q22-q23 encodes the dopamine D2 receptor.[5,6] The dopamine system has been considered a candidate for involvement in alcoholism, with postulated links to novelty seeking and central nervous system reward.[7,8] An association between the *Taq*I-A1 polymorphism in the *DRD2* gene and alcoholism was first reported by Blum et al.[9] Shortly thereafter, the first nonreplication was reported.[10]

This polymorphism has subsequently been examined by many groups, with differing results. There have been positive reports of the association of the *Taq*I-A1 allele with alcoholism[11–16] and polysubstance use and abuse.[13,17,18] Many of these studies analyze the so-called "prevalence" of the *Taq*I-A1 allele, defined as either the *Taq*I-A1/A1 or *Taq*I-A1/A2 genotype, rather than the *Taq*I-A1 allele frequency. This approach, implying a dominant (or nearly dominant) mode of action, might not be appropriate for alcoholism, nor for association studies in general, because the likelihood of carrying a putative disease-susceptibility allele given the associated marker is twice as high in the homozygote as in the heterozygote.[19–21] Some studies found an association with only a subset of alcoholics with "severe" disease, but different definitions of severity have been used[11,12,15,22,23] (and see Ref. 20). Neiswanger et al.[16] found a significant population association between the *Taq*I-A1 allele and alcoholism in the absence of any evidence for family-based association. Alcoholics did not differ from unscreened (population-based) control samples, but both of those groups differed from control groups screened to eliminate individuals with alcoholism and/or other psychopathologies.[16]

Many studies have reported no association of *DRD2* polymorphisms with alcoholism[10,18,19,23–32]; among these are studies that compared alcoholics with screened, nonalcoholic controls.[19,26,28,30] Various reviews and meta-analyses have not reached consensus on the question of whether the *Taq*I-A1 allele is associated with an increased risk for alcoholism or for "severe" alcoholism.[20–22,33–42] A potential confounding factor in association studies is the difference in *Taq*I-A1 allele frequencies among populations, which ranges from 0.09 to 0.80.[18,19,21,23,25,26,30,31,37,43,44] This emphasizes the need for either careful ethnic matching of alcoholics and controls or the use of family-based analyses.

Studies of possible linkage between polymorphisms in or near the *DRD2* locus and alcoholism have produced negative results. Bolos et al.[10] reported no evidence of linkage in two families. Parsian et al.[12] found no evidence for either linkage or cosegregation of the *Taq*I-A1 allele with alcoholism or severe alcoholism in 17 nuclear families drawn from 12 multigenerational pedigrees. Neiswanger et al.[16] found no evidence of linkage in 20 families of male alcoholic probands under any model tested, using both parametric and nonparametric methods. Affected family-based association (AFBAC) tests were also negative.[16] Cook et

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al.[45] reported evidence for linkage of the *DRD2* locus with heavy drinking and with alcoholism as defined by Research Diagnostic Criteria, but not with the more strictly defined alcohol dependence syndrome (which the authors describe as broadly equivalent to DSM-III-R), in a set of 11 British families. However, this reported linkage was entirely due to a single family with 10 siblings; the remaining families, and a replication sample of seven additional families, showed no evidence for linkage.[45] In the other families, the *Taq*I-A1 allele-sharing was actually <50%.

A large study examined *DRD2* alleles for differences that would affect the amino acid sequence of the encoded protein.[46] This study included individuals whose *Taq*I-A polymorphisms had been examined in several earlier studies, some of which had reported the association. Structural differences were rare, and none showed any association with alcoholism.[46]

To clarify the relationship between the *DRD2* receptor locus and alcoholism, this study presents data from a large, systematically ascertained sample of families containing multiple alcoholics. This sample, collected by the Collaborative Study on the Genetics of Alcoholism (COGA)[47] allows examination of the possible association of *DRD2* alleles with alcoholism using family-based methods. Families with multiple alcoholic members are likely to carry an increased load of genetic risk factors. To avoid false positives due to population stratification, the transmission/disequilibrium test (TDT)[48–51] and the AFBAC test[52] were used. A preliminary analysis of data on a simple tandem repeat polymorphism (STRP) in intron 2 of the *DRD2* gene in a subset of the probands was presented in abstract form.[53] The present study presents a detailed analysis of the *Taq*I-A polymorphism, as well as the STRP, using a larger dataset.

SUBJECTS AND METHODS

Ascertainment and Assessment of Subjects

Probands meeting both DSM-III-R criteria for alcohol dependence[54] and the Feighner criteria for definite alcoholism[55] were systematically recruited from both inpatient and outpatient alcoholism treatment facilities.[47] This combination of DSM-III-R and Feighner criteria (called "COGA criteria") identifies individuals who are clearly alcohol dependent, and allows comparability with earlier studies. Probands were excluded if they were intravenous drug users, or if they had AIDS, a severe head injury, or a life-threatening illness that was not alcohol related. Alcoholic cirrhosis or other medical conditions were not grounds for exclusion. Individuals were interviewed using the Semi-Structured Assessment for the Genetics of Alcoholism (SSAGA),[56,57] a polydiagnostic instrument that allows extraction of data compatible with multiple criteria sets.[58] Families were accepted into the study if they included a sibship of at least three and parents were available (or larger sibships if parents were not available). All available family members were interviewed for assessment of adult lifetime psychiatric status using the SSAGA[56] and for evaluation of major psychiatric disorders, including substance use disorders, in their relatives using a structured family history interview.[59] The study was approved by the appropriate institutional review boards of participating institutions, and informed consent was obtained.

Pedigrees were systematically extended through affected first-degree relatives, or to a secondary branch if at least two relatives in that part of the pedigree were affected, based on family history data. Families were accepted into the genetic study if the proband and at least two first-degree relatives were alcohol-dependent as determined by personal interview, and at least one parent was unaffected. A subset of these individuals was chosen for genotyping based on the pedigree structure. Parents were included when DNA was available to allow determination of identity by descent (IBD). Where parental DNA was not available, additional family members were genotyped to allow inference of IBD.

Genotyping was conducted on 987 individuals selected from 105 pedigrees. Of these, 764 individuals in 86 families were non-Hispanic Caucasians. The genotyped individuals in the total dataset (and the non-Hispanic Caucasian subset, in parentheses) included 433 (347) individuals diagnosed as alcohol-dependent by COGA criteria, 369 (295) by DSM-IV criteria,[60] and 267 (214) by ICD-10 criteria.[61] There were 74 (61) genotyped individuals who drank but had no symptoms of alcohol dependence or alcohol abuse, 286 (232) nonalcoholics with four or fewer sporadic symptoms and no diagnosis of alcohol dependence or abuse, and 401 (326) nonalcoholics with 8 or fewer symptoms and no diagnosis of alcohol dependence or abuse.

Genotyping and Data Checking

Genotyping of the *Taq*I-A polymorphism in *DRD2* was performed by a polymerase chain reaction (PCR) based method slightly modified from Grandy et al.[62] The final 15 μl reactions contain 50 mM KCl; 10 mM Tris (pH 8.3); 1.5 mM $MgCl_2$; 0.01% gelatin; 0.2 mM each dATP, dCTP, dGTP, and dTTP; 6 pmol of each primer; 96 ng of DNA; and 1.0 unit of Taq polymerase (Perkin Elmer/Cetus). The PCRs were conducted on the Perkin Elmer/Cetus GeneAmp PCR System 9600 thermocycler. Cycling conditions were 5 min at 93°, followed by 35 cycles of 93° (30 sec), 58° (15 sec), 72° (50 sec), and then 5 min at 72°. After amplification, 5 μl of the product was digested overnight at 65° with 12 units of *Taq*I restriction endonuclease (Boehringer/Mannheim, Indianapolis, IN) in a total of 15 μl, in the buffer supplied by the manufacturer. Aliquots were electrophoresed on 1.5% agarose gels containing 0.5 μg/ml ethidium bromide, and visualized by ultraviolet illumination. Data were independently entered by two individuals. The results were compared, and discrepancies were reexamined. Only alleles without remaining discrepancies were forwarded to the database. These readings were done without using pedigree information.

Genotyping of the STRP in the second intron of *DRD2*[63,64] and flanking STRPs was done by semiautomated methods using fluorescent tags on the forward primer. Five-microliter reactions contained 100 ng of template DNA; 3.5 pmol of end-labeled (fluorescent) forward primer and unlabeled reverse primer; 0.2 mM each dATP, dCTP, dGTP, and dTTP; 1 unit of Taq DNA polymerase (Perkin Elmer/Cetus); 1.5 mM $MgCl_2$; 50 mM KCl; and 10 mM Tris · HCl (pH 8.3). Cycling conditions were 4 min at 96°, followed by 26 cycles of 94° (60 sec), 58° (60 sec), 72° (45 sec), and 5 min at 72°. The PCR products were pooled with those from other markers for electrophoretic analysis. Allele sizes were determined by comparison with internal standards in each lane using the ABI Model 373 instrument (PE Applied Biosystems) with Genescan and Genotyper software.

A specially constructed database, GeneMaster, was used to check for alleles in an individual not present in his/her parents; any such discrepancies were re-examined. Markers were further tested for non-Mendelian inheritance first using CRI-MAP[65] and then using the USERM13 option of the MENDEL suite of programs.[66] Each family with an identified noninheritance was reviewed. If data remained incompatible after review, the genotypic data from one or more individuals incompatible with the rest of the family were removed. The individuals determining allele sizes and reviewing potential discrepancies were blind to diagnostic data.

Statistical Methods

*Taq*I-A allele frequency estimates were obtained from the data using the maximum likelihood methods developed by Boehnke[66] and imple-

Table 1. ETDT on Multiple-Affected Individuals Per Family

Allele	COGA		DSM-IV		ICD-10	
	TRANS*	NOT*	TRANS	NOT	TRANS	NOT
A. *DRD2 Taq*I-A Polymorphism						
1	57	70	56	64	37	49
2	70	57	64	56	49	37
Total	127	127	120	120	86	86
	p value = 0.25		p value = 0.47		p value = 0.19	
B. *DRD2* STRP Polymorphism						
76	2	0	3	0	2	0
78	43	45	36	39	25	31
80	58	52	49	47	34	30
82	102	85	90	76	64	50
84	45	65	45	55	28	40
86	14	17	13	19	9	11
Total	264	264	236	236	162	162
	p value† = 0.20		p value† = 0.24		p value† = 0.25	

Allele sizes are in base pairs.

* TRANS, transmitted to offspring; NOT, not transmitted.

† p value was calculated by the allele-wise test.[51]

mented in the program USERM13. The families were stratified by racial group. In several large families, the racial group of one or two individuals differed from that of the others; those families were assigned based on their predominant racial group. Allele frequencies in the non-Hispanic Caucasian and African-American families were calculated separately.

A large, family-based sample was used to test for association between the *DRD2* polymorphisms and alcohol dependence. This avoids the problem of false-positive results due to population stratification by using nontransmitted alleles from the same parents as controls. These alleles are exactly matched ethnically to the alleles transmitted to the affected offspring, because they come from the same parents. Genotypes of some individuals for whom data were missing were inferred, when possible, using the program UNKNOWN, part of the LINKAGE package.[67] Data presented in the tables are from non-Hispanic Caucasians, because African, Asian, and New World populations have different patterns of haplotype frequencies.[21]

We first used the TDT,[48–50] extended to markers with multiple alleles by Sham and Curtis[51] in their extended TDT (ETDT). The TDT and ETDT test for unequal transmission of alleles from heterozygous parents to their affected offspring. Under the null hypothesis (lack of linkage), data from related individuals in a pedigree are independent, so the TDT can be applied to each affected individual as a test for linkage.[50] When a single affected individual from each pedigree is used, along with his/her parents, the TDT is a valid test of linkage disequilibrium. In the ETDT generalization to multiallelic markers, the allele-wise analysis tests the hypothesis that different alleles may vary in their preferential transmission. The allele-wise test remains unbiased and not prone to false-positives even when pedigree data are used.[51] We chose the logistic-regression model of Sham and Curtis[51] to accommodate nonindependence of alleles when examining the multiallelic STRP marker. In addition to testing affected individuals under the COGA definition of alcohol dependence, we tested the subsets of alcoholics defined by even more stringent criteria (DSM-IV and ICD-10[58]) to be sure we were not missing an effect only evident among more severe alcoholics. These markers were also tested in the sample of unaffected individuals in these families.

For valid tests of association in these families, a reduced dataset consisting of a single affected individual from each family plus his or her parents was produced. To increase power, the selection of the single affected offspring in each pedigree was based on heterozygosity of the parental genotypes for the *Taq*I-A locus: offspring with two heterozygous parents were preferred, followed by those with a single heterozygous parent, and finally those with two homozygous parents. If more than one individual in a family met the most stringent guidelines, the proband was preferentially selected as the affected offspring when possible. A reduced dataset of one unaffected individual from each family was also constructed.

As results from the TDT and ETDT were uniformly negative, we decided to use the AFBAC test[52] for association between alcohol dependence and the *Taq*I-A polymorphism to be sure we were not missing an effect. The AFBAC test uses data from all parents of affected offspring, not only heterozygotes, counting the two alleles passed as "transmitted" and the other two as "nontransmitted." The resulting statistic is sensitive to both excess and deficits of a particular allele's transmission. For these tests, a single affected offspring per family was included to provide a valid test of association in an unstructured population.[52]

RESULTS

TDT

We used a large, family-based sample of carefully characterized alcoholics to test whether the *DRD2* locus was linked or associated with alcohol dependence. Alcohol dependence was defined for our primary analyses by the COGA definition, which requires the individual to meet both DSM-III-R criteria for alcohol dependence[54] and Feighner criteria for definite alcoholism,[55] because that definition was used in the systematic recruitment and extension of families. There was no evidence for linkage to either the *Taq*I-A locus or the STRP in intron 2 (Table 1).

The polydiagnostic nature of the SSAGA instrument[56–58] allowed classification of individuals based on criteria from DSM-IV[60] and ICD-10.[61] As a means of restricting the phenotype to a more severe form, we also examined persons classified as alcohol-dependent by these other criteria. There was no evidence for linkage of either marker at the *DRD2* locus with alcohol dependence defined by any of these criteria (Table 1). If anything, the

Table 2. ETDT on Multiple Unaffected Individuals Per Family

Allele	0 Sx*		≤4 Sx		≤8 Sx	
	TRANS†	NOT†	TRANS	NOT	TRANS	NOT
A. *DRD2 Taq*I-A Polymorphism						
1	3	3	14	15	32	33
2	3	3	15	14	33	32
Total	6	6	29	29	65	65
	p value = 1.0		p value = 0.85		p value = 0.90	
B. *DRD2* STRP Polymorphism						
76	0	0	0	0	2	3
78	3	4	15	16	30	22
80	2	5	13	26	25	41
82	6	5	32	21	48	45
84	7	3	24	18	39	32
86	0	1	1	4	5	6
Total	18	18	85	85	149	149
	p value ‡ = 0.52		p value‡ = 0.12		p value‡ = 0.39	

Allele sizes are in base pairs.
* Sx, number of symptoms.
† TRANS, transmitted to offspring; NOT, not transmitted.
‡ p value was calculated by the allele-wise test.[51]

*Taq*I-A1 allele was transmitted less frequently to alcoholic offspring than was the A2 allele, although in no case was the deviation from the null hypothesis significant.

Because there have been reports of differences between control groups without any alcohol dependence and general population controls,[16] the "unaffected" phenotype was also analyzed. In these families that contain a high density of alcoholics, those who remain unaffected by the disease might carry protective alleles. The number of genotyped people who drank alcohol, but had no symptoms at all of alcohol dependence, abuse, or "harmful use" and had heterozygous parents, was too low for a robust test. Therefore, the definition of "unaffected" was broadened to encompass individuals who had up to either 4 or 8 sporadic symptoms, but who did not meet *any* of the DSM-III-R, DSM-IV, or ICD-10 definitions of alcohol dependence or alcohol abuse. As shown in Table 2, there was no preferential transmission of either *Taq*I-A allele or of any STRP allele to the unaffected individuals in these families.

The ETDT tests were repeated on a reduced dataset consisting of only one affected individual from each family, so that the offspring were all independent (see "Subjects and Methods"). There was no evidence for linkage or association of either the *Taq*I-A locus or the STRP with an alcohol dependence susceptibility gene, under any of the definitions (Table 3). In no case was the *Taq*I-A1 allele transmitted more frequently to the alcoholic offspring (Table 3). Analyses of one unaffected individual per family showed no significant association with the *Taq*I-A1 allele under any of the three models, nor with the STRP allele using either 0 symptoms or ≤8 symptoms; the analysis with ≤4 symptoms gave a nominal p value of 0.02 (uncorrected for the multiple testing).

AFBAC Tests

The AFBAC test uses only one affected offspring per family to provide a test for association that is not affected by population stratification. Because it uses data from both parents of the affected offspring, not just heterozygotes as in the TDT, the number of alleles tested is increased. AFBAC tests were performed on one parent/offspring trio per pedigree, using all three definitions of alcohol dependence (COGA, DSM-IV, and ICD-10). In no case was there significant evidence for association of the *Taq*I-A1 allele with alcohol dependence (Table 4). Once again, the *Taq*I-A1 allele was transmitted less frequently to the alcohol-dependent offspring than was the A2 allele, but the differences were not significant. There was also no evidence for association of either *Taq*I-A allele with the unaffected phenotypes (Table 4).

Allele Frequencies

The *Taq*I-A allele frequency differs among populations. The frequency of the *Taq*I-A1 allele in the non-Hispanic Caucasian population from which most of our families were drawn is estimated [by maximum likelihood methods[66]] to be 0.19 (95% confidence interval 0.17–0.21). The allele frequency in the African-American population from which some of our families were drawn is estimated to be 0.31 (95% confidence interval 0.26–0.36). The difference between ethnic groups was significant ($p < 0.0001$).

DISCUSSION

Data presented herein, gathered from a large sample of families selected for the presence of multiple alcoholics, do not show evidence for linkage or association of the *DRD2*

Table 3. ETDT on a Single Affected Individual Per Family

Allele	COGA		DSM-IV		ICD-10	
	TRANS*	NOT*	TRANS	NOT	TRANS	NOT
A. *DRD2 Taq*I-A Polymorphism						
1	17	26	20	23	17	20
2	26	17	23	20	20	17
Total	43	43	43	43	37	37
	p value = 0.17		*p* value = 0.65		*p* value = 0.62	
B. *DRD2* STRP Polymorphism						
76	2	0	2	0	2	0
78	18	14	16	15	14	16
80	22	18	19	19	20	15
82	31	33	31	29	28	28
84	16	24	19	22	16	22
86	4	4	3	5	4	3
Total	93	93	90	90	84	84
	p value† = 0.45		*p* value† = 0.60		*p* value† = 0.50	

Allele sizes are in base pairs.
* TRANS, transmitted to offspring; NOT, not transmitted.
† *p* value was calculated by the allele-wise test.[51]

Table 4. AFBAC Tests

Allele	COGA		DSM-IV		ICD-10	
	TRANS*	NOT*	TRANS	NOT	TRANS	NOT
A. *DRD2 Taq*I-A Polymorphism: Affected Individuals						
1	17	26	20	23	17	20
2	51	42	48	45	41	38
Total	68	68	68	68	58	58
	p value = 0.097		*p* value = 0.58		*p* value = 0.55	
	0 Sx†		≤4 Sx		≤8 Sx	
Allele	TRANS	NOT	TRANS	NOT	TRANS	NOT
B. *DRD2 Taq*I-A Polymorphism: Unaffected Individuals						
1	3	3	8	10	19	16
2	9	9	24	22	37	40
Total	12	12	32	32	56	56
	p value = 0.68		*p* value = 0.58		*p* value = 0.54	

* TRANS, transmitted to offspring; NOT, not transmitted.
† Sx, number of symptoms.

*Taq*I-A1 allele with alcohol dependence. Given the controversy in this area, when our initial test provided no evidence for linkage or association, we conducted additional tests using multiple definitions of alcohol dependence to address issues of severity, and used several tests to minimize the possibility of a type II error. None of the comparisons provided evidence for linkage of either the *DRD2 Taq*I-A or the intron 2 STRP polymorphisms with alcohol dependence. In contrast to the previous studies in which the *DRD2 Taq*I-A1 allele was associated with alcoholism[11–16] and polysubstance use and abuse[13,17,18], the *Taq*I-A1 allele was transmitted to the alcoholics within our dataset somewhat less frequently than the *Taq*I-A2 allele, although in no case was the difference significant. Two important advantages of this study over previous ones are its large size and use of family-based controls. The TDT[48,50,51] and AFBAC test[52] avoid problems of population stratification, an advantage over simple population studies.

The previously reported family studies were based on significantly smaller samples than the present one. Three of these showed no evidence for linkage of the *DRD2* locus with alcohol dependence in 2, 17, and 20 families, respectively.[10,12,16] Cook et al.[45] found no evidence for linkage with the alcohol dependence syndrome (comparable with DSM-III-R) in a set of 11 British families. They did, however, find evidence for linkage with heavy drinking and with a less stringent diagnosis of alcoholism (Research Diagnostic Criteria). This potential linkage was entirely due to a

single family with 10 siblings, analyzed as all 45 possible sibling pairs (with no correction for nonindependence); neither the remaining families nor their replication sample gave any evidence for linkage.[45] In their other families, the *Taq*I-A1 allele-sharing was actually <50%. Their result could represent a type I error, or an effect in only a very small fraction of families.

It has been argued that the *Taq*I-A1 allele is associated with particularly "severe" alcoholism,[11,15,22,40,42] with the definition of "severe" often related to physical sequelae of alcoholism rather than alcohol dependence itself (but note that several negative reports included alcoholics from the most severe end of the spectrum[10,26–28,30,31,35,37]). The emphasis on physical sequelae, rather than alcohol dependence, weakens any a priori relationship to the actions of the dopamine system.[38] These sequelae may in part reflect the outcomes of prolonged excessive drinking, rather than any measure of the strength of the alcohol dependence itself.[31] In light of the argument that excluding medically ill individuals might bias a study against finding the *DRD2* association,[11,15,22,42] if that association is with a form of alcoholism that leads to physical problems, subjects in the present study were not excluded on the basis of any alcohol-related medical condition. The alcoholics in the COGA study met strict definitions of alcohol dependence (DSM-III-R + Feighner Definite Alcoholism). All probands had sought treatment for this disease. To further narrow the phenotype, we derived diagnoses by DSM-IV[60] and ICD-10[61] criteria.[58] Data from the 1992 National Longitudinal Alcohol Epidemiologic Survey, a large national probability sample, show that DSM-IV and ICD-10 identify progressively smaller but largely overlapping sets of alcohol-dependent individuals.[68] Results from the COGA study show the same trend[58]; data not shown). There was no evidence for association of the *Taq*I-A1 allele with either of the narrowed definition of severe alcohol dependence. The present results are consistent with several earlier studies that reported no association of the *DRD2* locus with any distinct subgroup of alcoholics defined by several measures of severity or typology.[10,24,26–28,30,31,37]

Neiswanger et al.[16] showed that the largest difference in *Taq*I-A1 allele frequencies is between the normal population and controls screened to avoid alcoholism and other psychopathologies, rather than between alcoholics and population (unscreened) controls. Therefore, we also tested for a possible association of the *Taq*I-A alleles in unaffected individuals within these families. There was no evidence of linkage or association of the *Taq*I-A allele with the unaffected phenotype defined in three different ways. Several previous studies using screened controls also did not find any association.[19,26,28,30] Analyses of the unaffected phenotypes with the STRP also did not show significant results, with the sole exception of a nominal p value of 0.02 for the class of individuals with ≤4 symptoms tested using the ETDT with one individual per family. Because neither the 0 symptom nor ≤8 symptom analyses were significant by any of the three tests (ETDT on multiple or single unaffected individuals and AFBAC) and the ≤4 symptom class was not significant by two of the three tests, we regard this as an artifact of multiple testing.

If the *Taq*I-A1 allele were associated with increased risk for alcohol dependence, one would expect the allele frequency in these families, chosen for their high density of alcoholic members, to be elevated. However, the frequency of the *Taq*I-A1 allele was 0.19 in the non-Hispanic Caucasians studied herein. This is very similar to the allele frequency of 0.20 reported in a recent large study of control Caucasians from Britain, screened to eliminate alcoholics, heavy drinkers, and people with a family history of alcohol problems, affective disorder, or schizophrenia.[69] It is also similar to reported population frequencies among people of European descent.[10,24–26,28–30,37,70,71] The *Taq*I-A1 allele frequency of 0.31 in the African-American families was comparable with previous reports in that population.[18,19] This again suggests the lack of an association of the *DRD2* locus with alcohol dependence.

Data presented in Tables 1 to 4 are from the non-Hispanic Caucasian families in our study. This subset was analyzed because African, Asian, and New World populations have different patterns of haplotype frequencies,[21] so a given allele could be associated with a different ancestral chromosome. Analyses of the entire dataset yielded similar results: no significant association between alcoholism (defined in any of the three ways) or the unaffected phenotype (defined in three ways) and either the *Taq*I-A alleles or the STRP alleles in *DRD2*.

Families selected on the basis of a high density of alcohol dependence (at least three first degree relatives), such as those in the COGA study, would be expected to carry genetic risk factors. Yet there was no evidence that the *DRD2* locus harbors such a risk factor. Some of these genotypic data were also analyzed as part of a genome-wide screen for genes affecting the risk for alcohol dependence (Reich et al., personal communication). Both single and multipoint nonparametric-affected sibling pair linkage analysis did not provide any evidence for linkage to this region of chromosome 11q. Regression analyses, which incorporate information from unaffected and discordant sibling pairs, as well as affected sibling pairs, also failed to provide any evidence for linkage (Reich et al., personal communication). In addition, MOD-score analyses, a quasiparametric 2-point approach that maximizes the LOD score with respect to the recombination fraction, penetrances, and disease allele frequency, also did not provide any evidence of linkage (Reich et al., personal communication).

In summary, we applied family-based association tests to determine whether the *DRD2* gene is associated with alcoholism in a large and carefully ascertained and assessed group of alcoholics. These tests avoid potential problems of differences in allele frequencies in different populations. Despite extensive analyses of two different polymorphisms

in this gene, we found no evidence for association or linkage of the *DRD2* gene with alcoholism.

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