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D_2 Dopamine Receptor Gene (DRD2) Allele and Haplotype Frequencies in Alcohol Dependent and Control Subjects: No Association with Phenotype or Severity of Phenotype

J. Gelernter, M.D., and H. Kranzler, M.D.

Possible association between polymorphisms at the D_2 dopamine receptor gene (DRD2) and alcohol dependence has been controversial since first proposed in 1990. The most studied polymorphisms to date are the TaqI "A" and "B" systems; they are unlikely to convey a physiological effect directly, and have not been demonstrated to be in linkage disequilibrium with any common polymorphism more likely to convey such an effect, in populations of European ancestry. A recently-described polymorphism in the promoter region of the DRD2 gene with possible effects on gene regulation is the first functional polymorphism described at this locus frequent enough in European-Americans (EAs) to have the potential to explain the positive findings. The goals of this study were to determine if we could replicate any previously reported associations, particularly with the "A" and "B" systems and alcohol dependence or severity of alcohol dependence, using a screened control group design. We also studied the promoter system, "D" system, and 3 locus haplotypes. To test the hypothesis of an association rigorously, we studied four DRD2 polymorphic systems in 160 EA alcohol dependent subjects and 136 screened EA control subjects. To increase our potential to detect association with other polymorphisms at the locus, we also constructed 3 locus haplotypes including the DRD2 "A," "D," and promoter systems in both samples. There were no significant differences in allele frequencies between alcohol dependent and screened control subjects for any of the four systems studied. There were also no differences in 3-locus haplotype frequencies between these groups. Analysis based on severity of alcohol dependence also yielded no significant association. The screened control allele frequencies did not differ from allele frequencies we reported previously in unscreened controls. Thus, we replicated previous findings of no association between DRD2 alleles and alcohol dependence. These results can now be extended to include haplotypes containing the possibly-functional promoter system polymorphism. Explanations previously offered to explain lack of association (regarding alcohol dependence severity, and use of screened vs. unscreened controls) were not validated. These results are consistent with no effect of DRD2 polymorphisms on behavioral phenotypes related to alcohol dependence. ***[Neuropsychopharmacology 20:640–649, 1999]*** *© 1999 American College of Neuropsychopharmacology. Published by Elsevier Science Inc.*

From the Yale University School of Medicine, Department of Psychiatry, Division of Molecular Psychiatry (JG), New Haven, CT; and VA Connecticut Healthcare System (JG), West Haven Campus, West Haven, CT; and the Department of Psychiatry, University of Connecticut School of Medicine (HK), Farmington, CT.

Address correspondence to: J. Gelernter, M.D., Psychiatry 116A2, VA CT Healthcare System, West Haven, 950 Campbell Avenue, West Haven, CT 06516.

Received May 20, 1998; accepted August 25, 1998.

NEUROPSYCHOPHARMACOLOGY 1999–VOL. 20, NO. 6

Published by Elsevier Science Inc.
655 Avenue of the Americas, New York, NY 10010

0893-133X/99/$–see front matter
PII S0893-133X(98)00110-9

KEY WORDS: *DRD2; TaqI "A" system; Haplotypes; Promoter; Candidate genes; Alcoholism; Substance dependence; Genetic association*

Since 1990, many studies have addressed possible association of D_2 dopamine receptor gene (DRD2) alleles with alcoholism (Blum et al. 1990), and with a range of other behavioral phenotypes. The meaning of positive findings that have emerged remains controversial. They have been explained in terms of a DRD2 mediated "reward deficiency" syndrome (Blum et al. 1997); however, they could also be explained in terms of population stratification, or through other mechanisms well known in population genetics (Gelernter et al. 1993a), some of which could account for a replicable finding of association that nonetheless has no pathophysiological significance. An example of such an association, due to population stratification, would be observation of increased frequency of a variant of a gene controlling skin pigmentation in subjects with sickle cell anemia (who would be mostly African American), compared to a random sample of American subjects (who would be mostly European American (EA)).

Published DRD2 association studies can be grouped according to population studied, and according to polymorphisms studied. Almost all of the positive findings occurred in European, or EA, populations (e.g. Blum et al. 1990; Parsian et al. 1991); studies in other populations have generally been negative (e.g. Chen et al. 1996). Studies with specific, well-defined (i.e. less admixed) European populations have been negative more often than studies in more admixed populations. Also, numerous polymorphisms at the DRD2 locus have been described. These can be grouped as follows: nonfunctional polymorphisms with multiple positive findings (e.g., the TaqI "A" [DRD2*A] and "B" [DRD2*B] systems); functional polymorphisms (e.g., $Ser^{311} \rightarrow Cys^{311}$ (Gejman et al. 1994; Cravchik et al. 1996); −141C*Ins/Del* (Arinami et al. 1997); and nonfunctional polymorphisms with either single positive findings or negative findings. Functional polymorphisms are obviously more likely than nonfunctional ones to be responsible for an association by a well-understood physiological mechanism; however, the polymorphism affecting protein sequence with highest frequency in European populations, $Ser^{311} \rightarrow Cys^{311}$ (or "Ser311Cys"), has frequency in those populations of only about 0.03 (Gejman et al. 1994). −141C*Ins/Del*, located in the DRD2 promoter region, was described only recently (alleles referred to here as P*Del*; deletion variant; P*Ins*, insertion variant). This polymorphism was reported to have an effect on efficiency at directing luciferase synthesis in reporter constructs, with the P*Del* allele having lower efficiency, and also reported to be associated with schizophrenia in a Japanese population (Arinami et al. 1997). (In this study, Japanese schizophrenic subjects had a significantly lower frequency of the P*Del* allele than Japanese controls). We demonstrated previously that this polymorphic system is in, at most, weak linkage disequilibrium (LD) with the most studied DRD2*A system in EAs, and therefore could not be responsible for the positive studies (Gelernter et al. 1998). (However, the DRD2*A and −141C*Ins/Del* systems were in significant LD in African-Americans). Although the DRD2*A system is itself located about 10 kb 3′ to the coding region of the gene and is therefore very likely to be nonfunctional itself, limited data exist supporting association to functional measures such as postmortem D_2 receptor numbers (Noble et al. 1991); data supporting no association with D_2 binding potential exist also (Laruelle et al. 1998).

Some proponents of a pathophysiologically significant association have addressed the differences between their results and the negative results of other research groups. The arguments repeated most frequently concern (a) measures of affection in the "ill" groups, and (b) issues about the composition of control groups. In the first category, some authors have argued that association between the DRD2*A1 allele and alcohol dependence increases in strength with increasing severity of the phenotype measured; it has also been argued that studies with negative results derived from comparisons using groups of alcohol dependent subjects less severely affected than studies with positive results (Noble, Syndulko et al. 1994). In the second category, some authors have argued that observation of behavioral phenotype in the control group is critically important, and that whether or not control groups are screened to exclude psychiatric illness, and most particularly alcohol dependence, has a major bearing on ability to detect a genetic association. We have demonstrated previously that use of a random control group, which includes alcohol dependent subjects at their expected population prevalence, should not materially affect predicted differences in allele frequencies in most cases, and specifically in the case of DRD2 and alcoholism, in a model using observed allele frequencies at the DRD2 locus (Gelernter et al. 1991). We are not aware of this argument having been refuted directly, however many authors have stated that composition of the control group, especially its status with respect to screening, is very important. One research group, in reporting DRD2*A system allele frequencies differing between alcohol dependent subjects and screened controls in their sample, suggested that the difference "while not appearing to be artifactual, is not specific to the alcoholism phenotype per se" (Neiswanger et al., 1995; p. 267), and they attributed this observation to the screening of their control group (rather than, for example, population stratification or sampling error). Consistent findings were reported by Lawford et al. (1997). We had demonstrated some years earlier that the difference between positive and negative reports to that point could be substantially

accounted for by reported observations of lower DRD2*A1 allele frequencies in screened control groups than in unscreened groups; our conclusion was, however, that this was likely to represent artifact rather than the logical alternative, namely that the DRD2*A2 allele was protective against psychiatric illness in a general sense (Gelernter et al. 1993b). It is hard to describe other satisfactory explanations, because removal of large numbers of DRD2*A1 alleles from control groups through population screening would require (a) the removal by screening of many subjects, and (b) a large concentration of DRD2*A1 alleles in those subjects who are removed. Given known population prevalence for alcohol dependence (about 14% lifetime prevalence; DSM-IV, American Psychiatric Association, 1987a, 1987b) and reported DRD2*A1 allele frequencies for those populations in positive studies (e.g., 0.25; Blum et al. 1991), compared to observed screened control allele frequencies (e.g., 0.18 in the present study), a large difference in allele frequency between screened and unscreened control populations cannot be accounted for. For example, with the allele frequencies and population prevalence quoted above, the predicted DRD2*A1 allele frequency in an unscreened population rises to only 0.19. This reflects adding in 14% of subjects (the population prevalence) with a DRD2*A1 allele frequency of 0.25, rather than 0.18, which is described by the equation (allele frequency observed in alcohol dependent subjects) $\times$ (prevalence of the phenotype) + (allele frequency observed in screened controls)*(1-prevalence of phenotype) = (frequency in unscreened controls). Note that this calculation assumes an observable association with the phenotype, which is not established. If a higher allele frequency in affected subjects is assumed because of greater severity (also not established), a greater impact of screening cannot be expected, because of a corresponding decrease in population prevalence of the severe phenotype. Additionally, we have argued elsewhere that, if a polymorphism really affects psychiatric phenotype, the screening process itself may be expected to introduce measurable artifact (Gelernter et al. in press (a)).

Perhaps the most telling recent study is that by Goldman et al. (1997), including Ser311Cys genotype data. The Ser311Cys variant, the rare (Cys311) allele of which has a frequency in EAs of about 0.03 (Gejman et al. 1994), is much less effective at inhibiting cAMP synthesis than the common allele (Cravchik et al. 1996). However, the Cys311 allele has frequency 0.16 in a Southwestern American Indian tribe (Goldman et al. 1997), which provided the opportunity to study the effect on phenotype of a DRD2 variant with an *in vitro* functional effect. Goldman et al. (1997) observed fifteen Cys311 homozygotes in their study population of 459 subjects. There was neither linkage nor association between alleles at this polymorphic system (or the DRD2*A system) and alcohol dependence in this sample. This demonstrated that actual functional variation at the DRD2 locus is unlikely to modulate risk for alcohol dependence, albeit in a population different from that in which the association was reported.

Of the numerous described polymorphic systems at the DRD2 locus, the DRD2*A system (Grandy et al. 1989), is the most studied by far; this site is located about 10 kb 3′ to exon 8, the last DRD2 exon. The DRD2*B system (Hauge et al. 1991), which maps 5′ to exon 2, has also been examined in numerous association studies, and this system is in strong LD with the "A" system (Hauge et al. 1991). The DRD2*D system (Parsian et al. 1991; Kidd et al. 1996) maps between exons 2 and 3 (Parsian et al. 1991; Suarez et al. 1994) (Figure 1). We reported frequencies of haplotypes including these three systems in control and cocaine dependent populations (Gelernter et al. in press (b)); there were no significant differences between these groups, nor were there differences by severity. Similarly, we reported no difference in haplotype frequencies between the same control group and a group of subjects with PTSD, many of whom had comorbid alcohol dependence (Gelernter et al. in press (a)). The control group used for these studies was partly unscreened, i.e., for some subjects it was not known whether they had psychiatric illness.

DRD2 exon 1 lies approximately 250 kb upstream from exons 2 through 8 (Eubanks et al. 1992). We previously studied LD and frequencies of haplotypes containing the DRD2*A, DRD2*D, and −141C*Ins/Del* systems in European-Americans, African-Americans, and Japanese subjects. Although we found evidence for LD across the 250 kb first intron in both American populations, we did not find significant LD between the DRD2*A system and the −141C*Ins/Del* system in a sample of 83 EA subjects ($D = -0.0087$; $D' = -0.482$) (Gelernter et al. 1998).

For the present study, we sought to provide data to address several relevant hypotheses regarding DRD2 and alcohol dependence that have not previously been addressed in large EA samples. These include the possibility that the DRD2*A1 allele might be showing association with alcohol dependence in some samples due to LD with a functional variant; the possibility that the newly described promoter variant might be associated with alcohol dependence; and the possibility that studying haplotypes of a set of markers might provide insight into potential mechanisms for positive association findings. We also directly address the question of potential for significant differences in DRD2*A1 allele frequencies between screened and unscreened control populations. Our findings do not support a physiologically significant association, and suggest, as have previous results from our group and many others, that positive results might be attributable to effects of population stratification.

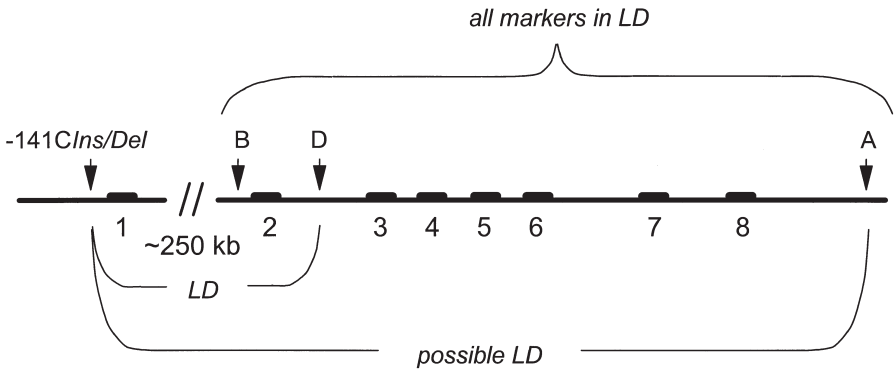

Figure 1. Schematic diagram of the DRD2 locus.

METHODS AND MATERIALS

Clinical Methods

Alcohol Dependent Subjects. All subjects studied were EA, and were recruited at the University of Connecticut (CT), School of Medicine (Farmington, CT). This sample is completely independent from that used for our previous report (Gelernter et al. 1991). Alcohol-dependent subjects were recruited from among individuals seeking treatment for substance abuse. Severity of alcohol dependence was defined on the basis of number of lifetime DSM-III-R criteria met (American Psychiatric Association, 1987a, 1987b). Subjects who met three (the minimum required for diagnosis) to six criteria were allocated to the "less severely affected" group, and those who met seven to nine criteria were considered "severely affected."

Screened Control Subjects. All subjects were screened to exclude major Axis I disorders, including substance use, psychotic, anxiety, and mood disorders. One hundred and thirty-six EA screened control subjects were collected at the VA CT Healthcare System, West Haven Campus, and at the University of CT. Of these subjects, 69 were screened with the Structured Clinical Interview for DSM-III-R (SCID; Spitzer et al. 1992), seven were screened with the Schedule for Affective Disorders and Schizophrenia, Lifetime Version (SADS-L) (Spitzer and Endicott 1975), and 12 were screened with the computerized Diagnostic Interview Schedule for DSM-III-R (C-DIS-R; Blouin et al. 1988). The remainder were screened by non-structured interview with a psychiatrist. Some (less than half) of these subjects overlap with our previously reported control groups (Gelernter et al. 1998; in press (a), (b)); up to 87 EA control subjects were described for those studies; however, 21 of those subjects were unscreened and were therefore excluded from the present study.

Laboratory Methods. DNA was extracted from whole blood by standard methods. Genotypes were obtained for four DRD2 polymorphic systems (DRD2*A, DRD2*B, DRD2*D, and −141C*Ins/Del*) as described elsewhere (Gelernter et al. 1998).

Data Analysis. For comparison of DRD2*A, DRD2*B, and −141C*Ins/Del* allele frequencies between alcohol dependent and control populations, and between "severe" and "less severe" alcohol dependent subjects, 2×2 χ^2 was used. Because there were six tests, the Bonferroni-corrected level of significance required for these comparisons was nominal $p < .008$ (to correspond to $p < .05$ for a single observation). Comparisons for the DRD2*D system were not made because of the lack of a specific prior hypothesis favoring an association with this system (in order to minimize the required Bonferroni correction for number of comparisons, and therefore maximize the possibility of detecting a significant relationship with one of the other systems). Conformance to Hardy Weinberg equilibrium (HWE) expectations for genotype distribution was tested using the program HWsim (Cubells et al. 1997).

We also compared DRD2*A allele frequency between the present screened EA control group, and a nonoverlapping unscreened EA control group reported previously (Gelernter et al. 1991).

Haplotype analysis was accomplished using the 3LOCUS program (Long et al. 1995), which computes estimated haplotype frequencies for up to three loci using an expectation maximization (E-M) algorithm. This program also computes pairwise disequilibria (D). Significance of G statistics corresponding to 2-locus disequilibria between the −141C*Ins/Del* and DRD2*A systems were computed using 10,000 replications of the relevant distributions (for the full control and alcohol dependent samples, taken separately) (Long et al. 1995). Standardized disequilibria (D′) were computed as described by Lewontin (1988). Whereas D is a measure of divergence from gametic equilibrium (Spiess, 1989; p. 137) and D′ the proportion of the maximum value of D (Lewontin 1988), the test statistic, G, is proportional to the negative log likelihood ratio of the restricted model and the general model (Long et al. 1995) and thus pro-

vides a measure of the statistical significance of disequilibria.

Haplotype frequencies were used to estimate number of observations for each haplotype. These were then compared using the CLUMP program (Sham and Curtis 1995). A standard 2×7 χ^2 ("T1") was computed, and its statistical significance determined empirically using 1000 simulations, for comparisons between screened controls and all alcohol dependent subjects, and between "severely" and "less severely" affected alcohol dependent subjects. For the "less severe" alcohol dependent subjects only, the two haplotype classes with the fewest observations were combined for this analysis. Haplotype frequency was then compared between screened controls and "severe" alcohol dependent subjects; a standard 2×6 χ^2 was computed and its significance determined as above. Since three comparisons were made, the appropriate significance level was determined to be $p < .017$.

Comparisons here consider 3 locus haplotypes including the DRD2*A, DRD2*D, and "−141C*Ins/Del*" systems. The DRD2*B system is in very strong LD with the DRD2*A system (e.g., Hauge et al. 1991) and, in EAs, adds relatively little information to haplotypes, in contrast to the DRD2*D system.

RESULTS

Comparisons of Allele Frequencies

Allele frequencies for all four systems are summarized in Table 1. For the DRD2*A system, controls vs. all alcohol dependent subjects, $\chi^2 = .001$ ($p = .97$); high vs. low severity alcohol dependence, $\chi^2 = .06$ ($p = .80$). For the DRD2*B system, corresponding values are $\chi^2 = .02$ ($p = .89$) and $\chi^2 = .02$ ($p = .90$); and for the −141C*Ins/Del* system, $\chi^2 = 4.87$ ($p = .027$) and 0.14 ($p = .71$). The comparison for −141C*Ins/Del* system allele frequencies between control and alcohol dependent samples reaches only nominal significance, and does not survive a Bonferroni correction.

For our previously reported unscreened EA control group ($n = 68$), we observed 27 A1 and 109 A2 alleles (f(DRD2*A1) = 0.20) (Gelernter et al. 1991). There was no significant difference in DRD2*A allele frequency between that group and the present screened control group ($\chi^2 = 0.17$; $p = .68$).

Comparisons of Haplotype Frequencies

Estimated haplotype frequencies (Table 2) were compared between alcohol dependent and screened control groups (T1 $\chi^2 = 9.06$; $p \sim .15$), between "severe" and "less severe" alcohol dependent subjects (T1 $\chi^2 = 12.84$; $p \sim .032$), and between screened control and "severe" alcohol dependent subjects (T1 $\chi^2 = 9.68$; $p \sim .09$). Controls and "severe" alcohol dependent subjects were compared in this case because of the observation of a nominally significant "p" value for the comparison with all alcohol dependent subjects.

Although there were small (albeit nonsignificant, considering the necessary Bonferroni correction) differences in haplotype frequencies between some of the groups compared, these are best understood in the context of the much larger differences observed between other populations (Figure 2). Large differences in allele frequency for each of these polymorphic systems are observed between different populations, indicating that population stratification is a serious and relevant possibility for studies of these systems. By way of comparison, the difference between our previously reported (both partially unscreened) African American and EA control populations for this same set of haplotyped markers showed $\chi^2 = 73.9$; $p < .0001$ (Gelernter et al. 1998). Also, the difference between "severe" and "less severe" alcohol dependence appears to be driven by the A2-D1-P*Del* haplotype (with estimated frequency of 0.044 in "severe" alcoholics and 0 in "less severe" alcoholics) (Figure 2); that is, this difference has no direct relationship to DRD2*A1 alleles.

Measures of Linkage Disequilibrium

The "G" statistics for "A" system and −141C*Ins/Del* system disequilibrium were 2.68 (for the control sample)

Table 1. Allele Frequencies for Screened Control and Alcohol Dependent Samples, and for the Alcohol Dependent Group Subdivided into "Severe" and "Less Severe." (n for this and all other tables is number of individuals)

	Control	Alcohol Dependent		
		All	"Severe"	"Less Severe"
n	136	160	65	95
A1	0.176	0.172	0.162	0.179
D1	0.625	0.572	0.546	0.590
P1 (Del)	0.063	0.119	0.131	0.111
B1	0.154	0.147	0.146	0.147

Table 2. Estimated Haplotype Frequencies

	Control	Alcohol Dependent		
		All	"Severe"	"Less Severe"
n	136	160	65	95
A1-D1-PDel	0.000	0.003	0.000	0.007
A1-D1-PIns	0.018	0.032	0.021	0.036
A1-D2-PDel	0.000	0.001	0.000	0.005
A1-D2-PIns	0.158	0.136	0.140	0.131
A2-D1-PDel	0.014	0.017	0.044	0.000
A2-D1-PIns	0.593	0.520	0.481	0.546
A2-D2-PDel	0.049	0.098	0.087	0.099
A2-D2-PIns	0.168	0.194	0.227	0.176

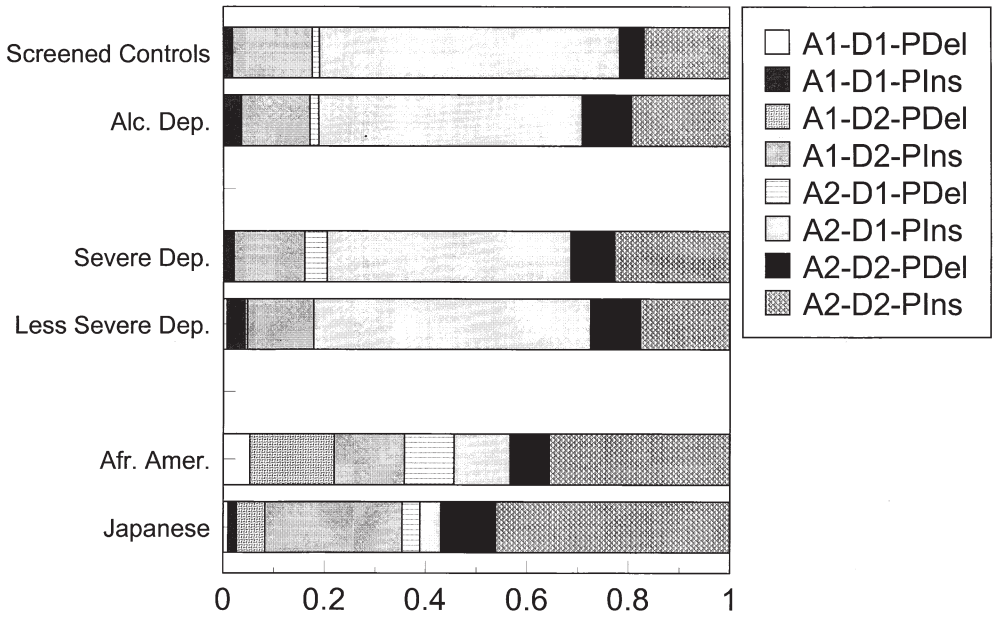

Figure 2. Estimated haplotype frequencies. Data for African American and Japanese control samples are from Gelernter et al. (1998).

($p \sim .110$) and 3.84 (for the alcohol dependent sample) ($p \sim .057$). That is, there was nearly-significant LD between these systems in the alcohol dependent sample only. Values of D and D′ are provided in Table 3.

Genotype Observations

Number of observations of each single-locus genotype are summarized in Table 4. There were no deviations from HWE in any group.

DISCUSSION

We replicated previous findings of no association between DRD2 alleles and alcohol dependence (e.g. Bolos et al. 1990; Gelernter et al. 1991). These results can now be tentatively extended to include the possibly-functional promoter system polymorphism, and to the three-locus haplotype constructed from the DRD2*A, DRD2*D, and −141C*Ins/Del* systems. Furthermore, we were unable to replicate previous findings of association with alcohol dependence, severity of alcohol dependence, or differences in allele frequency between unscreened and screened control groups. Although these results are consistent with no effect of DRD2 polymorphisms on risk for alcohol dependence or severity of alcohol dependence, the nominally significant difference in allele frequency for the −141C*Ins/Del* system between alcohol dependent and control subjects suggests that this marker should be studied further.

The allele frequencies observed here for the DRD2*A system are very similar to those we reported in our earlier study in a nonoverlapping sample (Gelernter et al. 1991). Then, we observed f(A1) of 0.20 in 68 unscreened controls, and 0.23 in 44 alcohol dependent subjects. Corresponding results for the present study are 0.18 (for 136 screened controls) and 0.17 (for 160 alcohol dependent subjects). It is of note that we found no difference in DRD2*A1 allele frequency between an unscreened control group and a group screened to exclude major psychiatric illness. Our present results are also consistent with those we found by reviewing all studies published up to 1993 (Gelernter et al. 1993a), excluding the Blum et al. (1990) hypothesis-generating study; those values were f(A1) of 0.20 in 467 alcoholics and 0.17 in 458 controls. In that review, we also determined that, considering all literature subsequent to the initial report up to 1993, there was no significant difference in allele frequency between "severe" and "not-severe" alcohol dependent subjects.

Table 3. Linkage Disequilibrium Between A, D, and −141 C*Ins/Del* Systems

	Control		Alcohol Dependent	
	D	*D′*	*D*	*D′*
A1-D1	−0.0923	−0.839	−0.0630	−0.640
A1-P*Del*	−0.0110	−0.992	−0.0164	−0.801
D1-P*Del*	−0.0253	−0.643	−0.0477	−0.701

Table 4. Genotype Counts for Each of the Four Polymorphic Systems Studied

	A1A1	A1A2	A2A2	B1B1	B1B2	B2B2
Control (n = 136)	3	42	91	2	38	96
Alcohol Dependent (n = 160)	7	41	112	5	37	118
	D1D1	D1D2	D2D2	PIns/PIns	PIns/PDel	PDel/PDel
Control (n = 136)	50	70	16	119	17	0
Alcohol Dependent (n = 160)	54	75	31	123	36	1

On the other hand, other authors have obtained quite different results in their reviews of published literature; Noble, for example, recently concluded that "there is now strong and growing evidence to implicate the DRD2 gene in alcoholism and other substance use disorders" (Noble 1997; p. 347). The present data do not support that conclusion. Population stratification and publication bias are among many possible explanations for positive reports and positive meta-analyses. Publication bias has been demonstrated to decrease the probability that negative results will be published, and increase the time to publication (Stern and Simes 1997); the effect is more marked for laboratory-based studies than for clinical trials (Easterbrook et al. 1991). Another possible explanation is that the positive results reported by other groups are correct, and the present report represents a false negative due to, for example, the effects of population stratification or random variation.

For both alcohol dependent and control samples, D′ shows that disequilibrium between the DRD2*A and −141C*Ins/Del* systems approaches maximum theoretical values given the allele frequencies observed for this sample (while still of low magnitude, as reflected by D values). Whereas the LD in the screened control group is clearly not significant, the result in the alcohol dependent sample approaches significance ($p \sim .057$). It is possible that the result was weaker in the control group because the frequency of the P*Del* allele was lower in that sample, with a corresponding reduction in ability to detect LD between that rare allele and the common allele of the other system—that is, negative disequilibrium between the respective rare alleles of the two systems. However, this observation does mirror one we made for LD at the serotonin transporter protein (SLC6A4) locus, where LD between two polymorphisms was significant in a sample of alcohol dependent subjects, but not in a sample of control subjects (Gelernter et al. 1997) (both samples overlapping those described here, the alcohol dependent sample to a greater extent than the control sample). This could reflect differences in admixture between the populations.

The observed DRD2*A/−141C*Ins/Del* LD occurs in the opposite direction from what would be required to easily reconcile an effect of the −141C*Ins/Del* system, detected through LD with the DRD2*A system, on alcohol dependence: the DRD2*A1 allele tends to predict the presence of the P*Ins* allele; that is, most P*Del* (rare) alleles were found on DRD2*A2 chromosomes. Due to the low observed frequency of the P*Del* allele in EA populations, it would be difficult to observe the absence of A1-P*Del* chromosomes in any given population, and even if they were not in LD these would be expected to account for (0.172) × (0.119) = 2% of all chromosomes (taking observed values for f(DRD2*A1) and f(P*Del*) from our alcohol dependent sample), as opposed to the <1% actually observed (see Table 2). Clearly this effect could not account for observations of association between alcohol dependence, or any other phenotype, and DRD2*A1, because it could maximally account for such a small number of chromosome observations.

Laruelle et al. (1998) did not observe a relationship between DRD2*A or DRD2*B allele frequencies and D_2 receptor binding potential, as measured by SPECT. Other authors have, however, detected association between the DRD2*A system and measures related to binding potential or receptor number (Noble et al. 1991; Thompson et al. 1997; Pohjalainen et al. 1998) or P300 amplitude or latency (Noble, Berman et al. 1994; Hill et al. 1998). The reports showing a relationship of DRD2*A1 to phenotypic measures still lack any plausible physiological explanation at this point; they could be explained eventually if another polymorphism that has an effect on function is identified, if that polymorphism is a common one in European and EA populations, and if that polymorphism is shown to be in LD with the DRD2*A system. This is certainly possible, especially the latter point, given the LD measurable between exons 2–8 and the promoter region in both EA and AA subjects (Gelernter et al. 1998), but this possibility must be viewed in the context of the many years of intensive study of this gene on the molecular level, especially since the first report by Blum et al. (1990), with no reports of polymorphisms with these properties. Still, it is a possibility that can never be excluded completely given the current state of available technology.

The appropriateness of using the number of diagnostic criteria as a measure of severity of dependence has been shown for a variety of substances, including alcohol (Feingold and Rounsaville 1995). Medical illness (used in some studies quantify severity) is not a generally accepted measure of severity. The Diagnostic and Statistical Manual (beginning with DSM-III-R; Ameri-

can Psychiatric Association 1987a, 1987b) bases the diagnosis of alcohol dependence on the alcohol dependence syndrome construct (Edwards and Gross 1976). This construct explicitly views medical problems and other alcohol-related disabilities as orthogonal to dependence *per se.* Feingold and Rounsaville (1995) found empirical support for the distinction between dependence on a variety of substances (including alcohol) and disabilities (including medical problems) related to substance use.

Use of family-based association approaches represents another powerful test of the validity of association between DRD2 markers and alcohol dependence. Several published studies include family-based tests, and results have been negative (Parsian et al. 1991; Neiswanger et al. 1995). By far the largest such study is that from the COGA group (Edenberg et al. 1998), which included genotypes from 987 individuals representing 105 families. Results from the transmission-disequilibrium (TDT) (Spielman et al. 1993) test (including 127 transmissions of DRD2*A1) were consistent with no linkage and no association between DRD2*A1 and alcohol dependence. The TDT is a family-controlled test of association and linkage disequilibrium not susceptible to population stratification artifact (Spielman et al. 1993).

We found no association of DRD2 alleles to alcohol dependence, even when less severe and more severe subtypes of that disorder were examined. There was no association between DRD2 alleles at any of four polymorphic systems, or extended DRD2 haplotypes, and either alcohol dependence or severity of alcohol dependence. Considered together with many past reports, but most particularly the reports of Goldman et al. (1997) (who showed that a DRD2 polymorphism with clear functional effect does not influence risk for alcohol dependence in a Southwestern Indian population) and Edenberg et al. (1998) (who showed no linkage or association between DRD2*A1 and alcohol dependence with use of a study design eliminating risk of population stratification), these results suggest that the DRD2 gene is unlikely to affect risk for alcohol dependence. Considered in the context of many previous reports demonstrating a wide range of DRD2 allele and haplotype frequencies depending on the population assessed, these results suggest population stratification as a possible explanation for positive results from association studies considering these markers.

ACKNOWLEDGMENTS

R. Capper, A. Wantroba, and P. Fall provided excellent technical assistance. We appreciate having received DNA samples of healthy subjects from Yale's Neurochemical Brain Imaging Program. This work was supported in part by funds from the U.S. Department of Veterans Affairs (the VA Medical Research Program, and the VA-Yale Alcoholism Research Center), NIMH grants K02-MH01387 and P30-MH30929, NIAAA grants R01-AA11330, K02-AA00239, and P50-AA03510, and NCRR grant M01-RR06192 (General Clinical Research Center).

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