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# Association of Seven Polymorphisms of the D2 Dopamine Receptor Gene with Brain Receptor-Binding Characteristics*

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Association of alleles at the *Taq*I A, *Taq*I B, intron 6, *Taq*I D, exon 7, exon 8, and promoter-141C sites of the D2 dopamine receptor gene with D2 dopamine receptor binding characteristics in the caudate nucleus of Caucasian alcoholic and nonalcoholic subjects was determined. For the *Taq*I D, exon 7, exon 8, and promoter-141C sites there were no significant allelic differences in Bmax (number of binding sites) or Kd (binding affinity) of the D2 dopamine receptors. However, subjects having the minor alleles at the *Taq*I A, *Taq*I B, and intron 6 sites had significantly lower Bmax than subjects not having them. None of these three polymorphisms had any significant effect on Kd. Highly significant linkage disequilibria were observed among the *Taq*I A, *Taq*I B, and intron 6 polymorphic sites, but linkage disequilibria between these three sites and each of the *Taq*I D, exon 7, exon 8, and promoter-141C sites were of lesser or of no significance. Taken together, these findings suggest that the *Taq*I A, *Taq*I B, and intron 6 polymorphisms, but not the *Taq*I D, exon 7, exon 8, and promoter-141C polymorphisms, are in linkage disequilibrium with a functional allelic variant that affects D2 dopamine receptor expression.

**KEY WORDS:** D2 dopamine receptor gene; D2 dopamine receptor binding; polymorphism; alcoholism; linkage disequilibrium; allelic association.

## INTRODUCTION

Emerging evidence suggests that the mesolimbic dopaminergic pathway, through the D2 dopamine receptors, is a key neural substrate for alcohol- and other drug-related reinforcement and reward behavior (1,2). We initially demonstrated an association of the minor *Taq*I A allele (A1) of the D2 dopamine receptor (DRD2) gene with severe alcoholism (3). Since that study, a large number of national and international studies have attempted to replicate this population-based study, with many affirming this finding (4–12) while others have not (13–20). However, at least eight independent meta-analyses of non-Hispanic Caucasians have demonstrated this association to be significant (9,21–27). Another meta-analysis (28) did not find a significant association; however, see Noble and Blum (29). In addition to alcoholism, the DRD2 A1 allele has been associated with a variety of other substance use disorders. These include polysubstance abuse (30–32), cocaine dependence (33), psychostimulant abuse (34), nicotine dependence (35–37), opioid dependence (38), and obesity (39–42). Two other DRD2 gene polymorphisms have also been shown to associate with alcoholism: a polymorphism in the intron 6 region (43) and the *Taq*I B polymorphism (43,44).

A neural correlate of functional significance to the DRD2 allelic association with alcoholism and other

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substance abuse was shown in an early pharmacological study (45). In that study, D2 dopamine receptor binding characteristics were measured in the caudate nucleus of deceased alcoholic and nonalcoholic subjects. The results showed a significant reduction (~30%) in the number of D2 dopamine receptor binding sites (Bmax) in both alcoholic and nonalcoholic subjects carrying the DRD2 *Taq*I A1 allele compared to subjects who did not carry this allele. However, no allelic difference was found in D2 dopamine receptor binding affinity (Kd). Subsequent investigations using autoradiography (46) and positron emission tomography (47,48) further demonstrated differences in D2 dopamine receptor density involving the *Taq*I A, the *Taq*I B, and the promoter-141C polymorphisms. Although these investigations suggest polymorphism-related alterations in D2 dopamine receptor number, the precise mechanism by which these mutations of the DRD2 gene affect receptor expression is currently not known.

The current study extends our original study (45) by examining the relationships of various DRD2 polymorphisms to D2 dopamine receptor binding parameters in the brains of deceased human subjects. The DRD2 gene polymorphic sites of interest include the following sites: *Taq*I A (located 10 kb 3′ to the final exon), intron 6, *Taq*I B (bordering the 5′ side of the second exon), *Taq*I D (in intron 2), exon 7, exon 8 (52 base pairs [bp] downstream of the stop codon), and the promoter-141C region (located in the 5′ end of the gene).

## EXPERIMENTAL PROCEDURE

*Materials.* Oligonucleotide primers were custom synthesized by Gibco BRL (Grand Island, NY, USA). *Taq* DNA polymerase was purchased from Gibco BRL (Grand Island, NY, USA). Restriction endonucleases were purchased from New England BioLabs (Beverly, MA, USA). $^{3}$H-spiperone (32.4 Ci/mM) was purchased from NEN Life Science Products, Inc. (Boston, MA, USA). S-(−) sulpiride was purchased from Sigma (St. Louis, MO, USA).

*Brain Samples.* Samples consisted of brain tissue from 20 alcoholic and 21 nonalcoholic subjects obtained from the National Neurological Research Bank at the Wadsworth Veterans Affairs Medical Center, Los Angeles, CA. The frontal gray cortex and caudate nucleus of each subject were removed from the brain at autopsy by a neuropathologist and immediately frozen at −70°C until used. The brains analyzed were the Caucasian non-Hispanic subset of those previously studied (45). The experimental protocols were approved by the UCLA institutional review committee.

*DRD2 Allelic Determinations.* Genomic DNA was extracted from frozen frontal gray cortex samples by standard procedures and subsequently used as a template for PCR (49).

The oligonucleotide primers and the method for determining the *Taq*I A DRD2 alleles have been previously described (40,50). Two alleles were obtained: the A1 allele (the uncleaved 310 bp fragment) and the A2 allele (the cleaved 180 bp and 130 bp fragments).

The oligonucleotide primers and the method for determining the *Taq*I B DRD2 alleles as described by Castiglione et al. (51) were utilized. Two alleles were obtained: the B1 allele (the uncleaved 459 bp fragment) and the B2 allele (the cleaved 267 bp and 192 bp fragments).

The oligonucleotide primers and the method for simultaneously determining the intron 6 and exon 7 DRD2 alleles were those described by Sarkar et al. (52) as modified by Comings et al. (39). For the intron 6 polymorphism, two alleles were found: the INT6-G allele (a T → G substitution, the uncleaved 339 bp fragment) and the INT6-T allele (a G → T substitution, the cleaved 305 bp and 34 bp fragments). Two alleles were also found for the exon 7 polymorphic site: the EX7-C allele (a T → C substitution, the uncleaved 339 bp fragment) and the EX7-T allele (a C → T substitution, the cleaved 234 bp and 105 bp fragments).

The oligonucleotide primers and the method for determining the *Taq*I D alleles were those described by Kidd et al. (53). Two alleles were found: the D1 allele (uncleaved 419 bp fragment) and the D2 allele (the cleaved 272 bp and 147 bp fragments).

The oligonucleotide primers and the method for determining the -141C deletion/insertion alleles in the promoter region of the DRD2 gene were those described by Arinami et al. (54). Two alleles were obtained: the insertion (Ins) allele (a cytosine insertion producing the uncleaved 304 bp fragment) and the deletion (Del) allele (a cytosine deletion producing the cleaved 160 bp and 144 bp fragments).

The oligonucleotide primers and procedure for determining the exon 8 alleles were those described by Samochoweic et al. (55) Two alleles were found: the EX8-G allele (an A → G substitution producing two fragments of 283 and 65 bp) and the EX8-A allele (a G → A substitution producing an uncleaved fragment of 348 bp).

Figure 1 shows the polymorphic sites of the DRD2 gene studied herein.

*D2 Dopamine Receptor Binding Assay.* Caudate nuclei samples were ground to a fine powder in liquid nitrogen using a mortar and pestle and stored at −70°C. From this fine powder, aliquots were used for assays at different times. The samples were never allowed to thaw at any time before usage in any assay. Cell membrane suspensions from the samples were prepared, and D2 dopamine receptor binding characteristics were determined as previously described (45). Saturation binding curves were obtained using 12 duplicate increasing concentrations (10–1000 pmol/L) of $^{3}$H-spiperone (32.4 Ci/mM). Nonsaturable binding was measured by addition of S-(−) sulpiride to a final concentration of 10 μM/L. Protein content was also determined using bovine serum albumin as standard (56). The maximum number of binding sites (Bmax) and equilibrium binding constant (Kd) were estimated using the weighted, nonlinear, least squares, curve-fitting program LIGAND (57). Data were fit for both one-site and two-site models. Two-site models were accepted only if a statistically significant improvement was obtained over the one-site model.

*Statistical Analysis.* When specific alleles from two polymorphic loci in a defined population are highly associated with each other, they are said to be in linkage disequilibrium. In this study, pair-wise composite genotypic linkage disequilibria for each pair of the polymorphic loci were analyzed with the LD86 program (58). LD86, based on equations described by Weir and Cockerham (59), calculates disequilibrium coefficients at two bi-allelic loci (x and y) when double heterozygotes cannot be distinguished. Utilizing this program, the linkage disequilibria (Δxy) and chi square statistics were determined for each pair of DRD2 polymorphic loci.

The frequency distributions for Bmax and Kd were examined to determine normality. The Kd distribution was found to have skew-

ness and kurtosis values outside the limits of ±1, and values were reexpressed as a log transformation. The Bmax distribution was found to be within acceptable limits for skewness and kurtosis.

Correlations were estimated by regression analysis for Bmax with age and log Kd with age. No correlation was observed between log Kd and age. However, for Bmax, linear correlation was found with age ($r = -0.297$, $P = .0199$). Age was therefore used as a covariate to remove its effects from the measures of Bmax. No correlations were observed between Bmax or log Kd and autolysis time.

Because the brain samples used in this study were obtained from both alcoholic and nonalcoholic subjects, mean differences between groups were tested using two-factor analysis of covariance (ANCOVA) to determine the statistical significance of the main effects of allele and alcoholism, as well as the interaction between alleles and alcoholism.

For all statistical analyses, P values = .05 were considered statistically significant. Where appropriate, a more conservative level of significance, using Bonferroni correction for multiple ($n$) testing [P (corrected) = P (uncorrected) × $n$], was applied.

## RESULTS

The *Taq*I A polymorphism had a genotypic distribution of 1 A1/A1, 16 A1/A2 and 24 A2/A2 subjects. The genotypic distribution for the *Taq*I B polymorphism was 1 B1/B1, 12 B1/B2, and 28 B2/B2 subjects. For the intron 6 polymorphism the genotypic distribution was 1 INT6-T/INT6-T, 14 INT6-G/INT6-T, and 26 INT6-G/INT6-G subjects. The promoter-141C had a genotypic distribution of 1 Del/Del, 11 Ins/Del, and 29 Ins/Ins subjects. For the exon 7 polymorphism the genotypic distribution consisted of 2 EX7-T/EX7-T, 20 EX7-C/EX7-T, and 19 EX7-C/EX7-C subjects. The exon 8 polymorphism had a genotypic distribution of 3 EX8-G/EX8-G, 20 EX8-A/EX8-G, and 18 EX8-A/Ex8-A subjects. Because for each of these six polymorphic loci only a very small number of subjects were homozygous for the minor allele, subjects homozygous and heterozygous for the minor allele were, as in previous studies (10,38,45), combined and compared with subjects homozygous for the major allele. These polymorphic loci in the DRD2 gene were thus classified and assessed as follows: *Taq*I A ($A1^+$ [A1/A1 and A1/A2 genotypes] versus $A1^-$ [A2/A2 genotype]), *Taq*I B ($B1^+$ [B1/B1 and B1/B2 genotypes] versus $B1^-$ [B2/B2 genotype]), intron 6 ($INT6\text{-}T^+$ [INT6-T/INT6-T and INT6-G/INT6-T genotypes] versus $INT6\text{-}T^-$ [INT6-G/INT6-G genotype]), promoter-141C ($Del^+$ [Del/Del and Ins/Del genotypes] versus $Del^-$ [Ins/Ins genotype]), exon 7 ($EX7\text{-}T^+$ [EX7-T/EX7-T and EX7-C/EX7-T genotypes] versus $EX7\text{-}T^-$ [EX7-C/EX7-C genotype]), and exon 8 ($EX8\text{-}G^+$ [EX8-G/EX8-G and EX8-A/EX8-G genotypes] versus $EX8\text{-}G^-$ [Ex8-A/EX8-A genotype]).

For the *Taq*I D polymorphism the genotypic distribution consisted of 14 D1/D1, 19 D1/D2, and 8 D2/D2 subjects. The age-adjusted Bmax values ± SEM for these three genotypes were 78.8 ± 8.8, 65.2 ± 7.9, and 63.9 ± 11.7 fmol/mg protein, respectively. The log Kd values ± SEM for these three genotypes were 1.88 ± 0.04, 1.94 ± 0.06, and 1.94 ± 0.06, respectively. Because both the mean log Kd and mean Bmax values for subjects homozygous and heterozygous for the minor D2 allele were virtually identical, these subjects were combined and compared with subjects homozygous for the D1 allele. The *Taq*I D polymorphism was thus classified and assessed as follows: $D2^+$ [D2/D2 and D1/D2 genotypes] versus $D2^-$ [D1/D1 genotype].

Analysis of saturation curves, using $^3$H-spiperone as the binding ligand and S-(−) sulpiride to measure nonsaturable binding, revealed a single-site binding model for each of the 41 caudate nuclei studied. Table I shows the log Kd and age-adjusted Bmax values in caudate nuclei for the allelic groups of the seven polymorphic loci of the DRD2 gene examined in this investigation. The ranges of Kd and Bmax value estimates shown in Table I were consistent with reported results of D2 dopamine receptor binding characteristics in human caudate nucleus tissue (60–62).

No consistent significant differences were observed between alcoholic and nonalcoholic subjects for the log Kd and age-adjusted Bmax binding parameters using two-factor ANCOVA to determine the main effects of allele and alcoholism for the seven DRD2 polymorphic loci studied (data not shown). In addition,

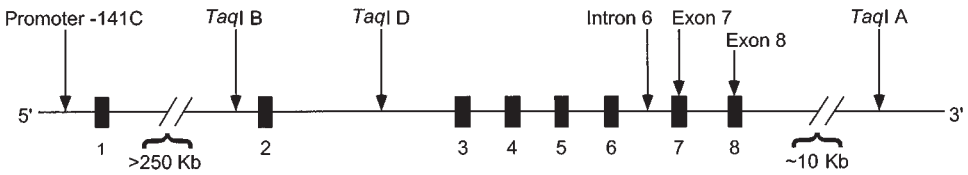


**Fig. 1.** D2 dopamine receptor gene polymorphic sites. Solid boxes represent the exons of the D2 dopamine receptor gene; the horizontal line represents the introns and flanking regions. The seven polymorphic sites studied are shown by arrows.

**Table I.** D2 Dopamine Receptor Binding Characteristics For DRD2 Polymorphic Sites[a]

| *Taq*I A Alleles | $A1^+$ (n = 17) | $A1^-$ (n = 24) | P-value |
|---|---|---|---|
| Log Kd | 1.84 ± 0.05 | 1.95 ± 0.04 | ns |
| Bmax (age adjusted) | 49.2 ± 8.2 | 83.4 ± 6.4 | 0.002 |
| *Taq*I B Alleles | $B1^+$ (n = 13) | $B1^-$ (n = 28) | P-value |
| Log Kd | 1.84 ± 0.06 | 1.93 ± 0.04 | ns |
| Bmax (age adjusted) | 48.6 ± 10.0 | 75.5 ± 6.0 | 0.033 |
| Intron 6 Alleles | INT6-$T^+$ (n = 15) | INT6-$T^-$ (n = 26) | P-value |
| Log Kd | 1.87 ± 0.05 | 1.92 ± 0.04 | ns |
| Bmax (age adjusted) | 52.2 ± 8.9 | 76.8 ± 6.3 | 0.031 |
| Promoter-141C Alleles | $Del^+$ (n = 12) | $Del^-$ (n = 29) | P-value |
| Log Kd | 1.95 ± 0.05 | 1.91 ± 0.04 | ns |
| Bmax (age adjusted) | 70.0 ± 9.6 | 70.7 ± 6.0 | ns |
| *Taq*I D Alleles | $D2^+$ (n = 27) | $D2^-$ (n = 14) | P-value |
| Log Kd | 1.93 ± 0.05 | 1.89 ± 0.05 | ns |
| Bmax (age adjusted) | 64.7 ± 6.1 | 78.9 ± 8.6 | ns |
| Exon 7 Alleles | EX7-$T^+$ (n = 22) | EX7-$T^-$ (n = 19) | P-value |
| Log Kd | 1.97 ± 0.04 | 1.86 ± 0.04 | ns |
| Bmax (age adjusted) | 70.5 ± 7.1 | 68.6 ± 7.6 | ns |
| Exon 8 Alleles | EX8-$G^+$ (n = 23) | EX8-$G^-$ (n = 18) | P-value |
| Log Kd | 1.96 ± 0.04 | 1.86 ± 0.04 | ns |
| Bmax (age adjusted) | 68.9 ± 7.0 | 70.5 ± 8.0 | ns |

[a] The classification and assessment of the DRD2 polymorphic sites are described in the Results section. For each polymorphic site, mean differences in Kd and Bmax binding parameters between groups were determined using two-factor ANCOVA to determine the main effects of allele and alcoholism. Only the main effect of allele is listed above. Values are presented as the mean ± SEM. Units for Kd are pmol/L. Units for Bmax are fmol/mg of protein. ns = not significant.

no significant interactions were observed between the main effects of allele and alcoholism for any of the seven DRD2 polymorphic loci studied.

There was a significant gene effect with the age-adjusted mean Bmax of the *Taq*I A alleles with the $A1^-$ allelic group being significantly higher than the $A1^+$ allelic group (see Table I). Similar results were found with the *Taq*I B alleles with the mean for the $B1^-$ allelic group being significantly higher than for the $B1^+$ allelic group. The age-adjusted mean Bmax for the intron 6 INT6-$T^-$ allelic group was also similarly significantly higher than the mean for the INT6-$T^+$ allelic group. However, there were no significant differences in mean log Kd between the *Taq*I A, intron 6, or the *Taq*I B allelic groups (see Table I).

For the -141C polymorphic locus of the DRD2 promoter region, no significant differences were found between the $Del^+$ and $Del^-$ alleles for age-adjusted mean Bmax values and log transformed mean Kd values as shown in Table I. No significant differences were observed between the *Taq*I D $D2^+$ and $D2^-$ alleles for either age-adjusted mean Bmax values or log transformed mean Kd values (see Table I). Likewise, there were no significant differences between the exon 7 EX7-$T^+$ and EX7-$T^-$ alleles for age-adjusted Bmax and log Kd values. Finally, no significant differences were found for age-adjusted Bmax and log Kd values between the EX8-$G^+$ and EX8-$G^-$ exon 8 alleles (see Table I).

Table II shows the composite genotypic disequilibria for each pair of DRD2 allelic sites. For each pair, bi-allelic coefficients ($\Delta xy$) and chi square values were obtained. The results showed a pattern of significant and nonsignificant coefficients depending on which sites were compared. The strongest linkage disequilibria were between 3′-untranslated/intron, intron/intron, and exon/exon sites (*Taq*I A and *Taq*I B, *Taq*I A and intron 6, *Taq*I B and intron 6, and exon 7 and exon 8 sites). Each of these four biallelic assessments had P-values less than $10^{-7}$. These four assessments remained statistically highly significant ($P < 10^{-5}$) after Bonferroni correction for multiple testing.

Linkage disequilibrium coefficients at a second intermediate level of statistical significance ($10^{-5} < P < 10^{-3}$) were observed with pairings of the intronic *Taq*I D site with most of the other DRD2 polymorphic sites (*Taq*I B, intron 6, exon 7, and exon 8 sites). These comparisons also remained statistically significant after Bonferroni correction for multiple testing. The linkage disequilibria between the *Taq*I A and *Taq*I D, *Taq*I A and exon 8, *Taq*I B and promoter-141C, intron 6 and promoter-141C and between promoter-141C and *Taq*I D sites were also significant, but lost significance when Bonferroni corrected. No linkage disequilibria of statistical significance were observed between the *Taq*I A and promoter-141C, *Taq*I A and exon 7, *Taq*I B and exon 7, *Taq*I B and exon 8, intron 6 and exon 7, intron 6 and exon 8, promoter-141C and exon 7, and promoter-141C and exon 8 sites.

**Table II.** Composite Genotypic Disequilibria at DRD2 Polymorphic Sites

| Sites | Δxy[a] | $\chi^2$ | p |
|---|---|---|---|
| *Taq*I A and intron 6 | 0.122 | 28.7 | $8.68 \times 10^{-8}$[b] |
| *Taq*I A and *Taq*I B | 0.120 | 29.3 | $6.24 \times 10^{-8}$[b] |
| *Taq*I A and promoter-141C | 0.040 | 3.38 | 0.066 |
| *Taq*I A and *Taq*I D | −0.081 | 7.06 | 0.008 |
| *Taq*I A and exon 7 | 0.043 | 3.04 | 0.081 |
| *Taq*I A and exon 8 | 0.054 | 4.26 | 0.039 |
| *Taq*I B and intron 6 | 0.129 | 34.5 | $4.36 \times 10^{-9}$[b] |
| *Taq*I B and promoter-141C | 0.043 | 4.26 | 0.039 |
| *Taq*I B and *Taq*I D | −0.110 | 14.17 | $1.67 \times 10^{-4}$[b] |
| *Taq*I B and exon 7 | 0.027 | 1.26 | 0.262 |
| *Taq*I B and exon 8 | 0.035 | 1.95 | 0.163 |
| Intron 6 and promoter-141C | 0.048 | 4.94 | 0.026 |
| Intron 6 and *Taq*I D | −0.102 | 11.49 | $7.00 \times 10^{-4}$[b] |
| Intron 6 and exon 7 | 0.029 | 1.27 | 0.260 |
| Intron 6 and exon 8 | 0.038 | 2.23 | 0.135 |
| Promoter-141C and *Taq*I D | −0.072 | 6.22 | 0.013 |
| Promoter-141C and exon 7 | −0.029 | 1.54 | 0.215 |
| Promoter-141C and exon 8 | −0.021 | 1.50 | 0.221 |
| *Taq*I D and exon 7 | −0.128 | 15.38 | $8.79 \times 10^{-5}$[b] |
| *Taq*I D and exon 8 | 0.120 | 12.03 | $5.24 \times 10^{-4}$[b] |
| Exon 7 and exon 8 | 0.168 | 35.98 | $2.00 \times 10^{-9}$[b] |

[a] Δxy represents pairwise linkage disequilibria analyzed according to the procedure of Weir (1996).

[b] Significant difference ($P < .05$) remains after Bonferroni correction for multiple (21) testing.

## DISCUSSION

In our initial investigation (45) on D2 dopamine receptor binding in the caudate nucleus from deceased subjects of both Caucasian and African-American race, we observed significantly lower bInding (Bmax) in subjects carrying the $A1^+$ allele (A1/A1 and A1/A2 genotypes) of the *Taq*I A polymorphism than those having the $A1^-$ allele (A2/A2 genotype). Thus, in the current study, it is not surprising that binding characteristics of the D2 dopamine receptors in the caudate nucleus of a subset of subjects from our initial study consisting of 41 deceased Caucasian subjects revealed that those carrying the $A1^+$ allele had significantly and more than 40% fewer receptors than those who had the $A1^-$ allele. However, we have extended the results of the first investigation by ascertaining D2 dopamine receptor binding characteristics of alleles of six other DRD2 polymorphisms. Two of these polymorphisms, the *Taq*I B and the intron 6, showed allelic Bmax values very similar to those shown by the *Taq*I A alleles. The Bmax of subjects with the $B1^+$ allele was significantly and more than 35% lower than the Bmax of subjects with the $B1^-$ allele. Furthermore, subjects with the INT6-$T^+$ allele had significantly and more than 30% fewer D2 dopamine receptors than those with the INT6-$T^-$ allele. It should be noted that for each of these three DRD2 polymorphisms, it is the minor allelic variant that shows significantly reduced D2 dopamine receptor density. However, no differences were observed in D2 dopamine receptor binding affinity (Kd) between the allelic groups for each of these three polymorphisms, an observation also found in our initial binding study with the *Taq*I A alleles (45).

There have been several other investigations regarding the effects of the above polymorphisms on D2 dopamine receptor binding properties that further substantiate the results of the present study. Thompson et al. (46), in the United Kingdom, measured the binding of the specific D2 dopamine receptor ligand $^3$H-raclopride to brain samples by autoradiography. They found a significant 30–40% reduction in receptor density in the striatum of individuals with the *Taq*I A $A1^+$ allele compared with those with the $A1^-$ allele. In addition, a Finnish study (47) using $^3$H-raclopride and positron emission tomography (PET) found a significant reduction in D2 dopamine receptor availability, reflecting a decrease in receptor density, in healthy $A1^+$ volunteers compared with $A1^-$ allelic subjects. However, there was no difference in apparent Kd between the two groups. Furthermore, Jönsson et al. (48), also using PET with $^3$H-raclopride in healthy Swedish subjects, found the presence of both the *Taq*I A $A1^+$ allele and the *Taq*I B $B1^+$ allele was associated with measures of low D2 dopamine receptor density. The results of our current study, along with those mentioned above, however, are at variance with a study using single positron emission computerized tomography (SPECT) with $^{123}$I-IBZM where no association was found between either *Taq*I A or *Taq*I B allelic status and D2 dopamine receptor density (63). One possible explanation that has been given for this discrepancy (48) concerns the different methodology used wherein the relatively low resolution of SPECT coupled with the weaker signal of $^{123}$I-IBZM results in lower resolution and reduced reliability in comparison with the two PET studies. Moreover, an editorial (64) indicates that Laruelle et al. (63) combined the results of both schizophrenics and controls. Because schizophrenics showed a trend in the opposite direction than controls, the results on D2 dopamine receptor binding potential and allelic association may have been confounded in these subjects by prior neuroleptic treatment. Indeed, a recent report (65) did find increased D2 dopamine receptor binding in schizophrenics after treatment with antipsychotics.

The D2 dopamine receptor binding characteristics associated with the alleles of the promoter-141C, exon 7, exon 8, and the *Taq*I D polymorphisms in the current study were found to differ from those with the alleles of the *Taq*I A, *Taq*I B, and intron 6 polymorphisms. No significant allelic differences for any of these four polymorphisms were observed for either the Bmax or the Kd. For the promoter-141C site, this observation is substantiated by a PET study using $^{3}$H-raclopride in which no difference in receptor binding was observed between subjects carrying the Del allele and those having the Ins/Ins genotype (66). However, another PET study, by Jönsson et al. (48), also using $^{3}$H-raclopride, found significant association between the Del allele and high striatal D2 dopamine receptor density. The current study found the D2 dopamine receptor Bmax for those subjects carrying the $Del^{+}$ allele to be virtually identical with those having the $Del^{-}$ allele. Although those subjects carrying the $D2^{+}$ allele of the *Taq*I D site had a mean Bmax lower than those having the $D2^{-}$ allele, this difference, was not statistically significant. Further, the Bmax values were essentially the same for the EX7-$T^{+}$ and EX7-$T^{-}$ alleles of the exon 7 polymorphism as well as for the EX8-$G^{+}$ and EX8-$G^{-}$ alleles of the exon 8 polymorphism. To our knowledge, this is the first time binding data for the D2 dopamine receptor *Taq*I D, exon 7, and exon 8 alleles have been published.

The results in Table I underscore the significant allelic effects on D2 dopamine receptor density with the *Taq*I A, *Taq*I B, and intron 6 polymorphisms. However, it should not be surprising that all three of these polymorphisms are associated with such a profound effect on D2 dopamine receptor density. There exits a strong and highly significant ($P < 10^{-7}$) bi-allelic linkage disequilibrium among the *Taq*I A, *Taq*I B, and intron 6 polymorphic sites, as demonstrated in this investigation (see Table II), as well as in an earlier report (43), which used a larger Caucasian population that included the subjects used in the present study. This is reflected in very similar higher Bmax values for the more commonly found $A1^{-}$, $B1^{-}$, and INT6-$T^{-}$ alleles, as well as a similar and significantly reduced receptor density observed with the minor $A1^{+}$, $B1^{+}$, and INT6-$T^{+}$ alleles of these three polymorphisms (see Table I). This contrasts with what is observed with the exon 7 and exon 8 polymorphisms. A highly significant bi-allelic linkage disequilibrium ($P < 10^{-8}$) exists between these two sites, an observation also found by Samochowiec et al. (55). However, the D2 dopamine receptor Bmax values for the two alleles of each of these two DRD2 exonic polymorphisms are virtually identical (see Table I), suggesting that these alleles have little effect on D2 dopamine receptor density.

One might expect mutations in the promoter region of the DRD2 gene to be good candidates for association with alterations in D2 dopamine receptor density. However, in this investigation, no difference in Bmax was observed between the $Del^{+}$ and $Del^{-}$ alleles of the promoter-141C polymorphism. Moreover, much weaker bi-allelic linkage disequilibria were observed between the promoter-141C site and each of the *Taq*I A, *Taq*I B, and intron 6 sites when compared with the strong, highly significant linkage disequilibrium coefficients observed among the latter three sites with each other. This may support, in part, the negligible allelic effect of the promoter-141C polymorphism on D2 dopamine receptor density in contrast with the significant allelic differences observed with the the *Taq*I A, *Taq*I B, and intron 6 polymorphisms. Similar weaker linkage disequilibria were observed for the *Taq*I A and promoter-141C, *Taq*I B and promoter-141C, and intron 6 and promoter-141C comparisons in an earlier study conducted by us that used a larger subject population that included those of the current investigation (43). Others (48,55,67–69) similarly found either relatively weaker or no significant linkage disequilibrium between the promoter-141C and *Taq*I A polymorphisms.

The *Taq*I D polymorphism is interesting in that it had a significant bi-allelic linkage disequilibrium with all of the other polymorphic sites examined in this investigation. Furthermore, the disequilibrium coefficients of the *Taq*I D site with the *Taq*I B, intron 6, exon 7, and exon 8 sites remained significant even after correction for multiple testing. Kidd et al. (70), in European Caucasians, obtained significant linkage disequilibria between the *Taq*I A and *Taq*I D sites, and the *Taq*I B and *Taq*I D sites. Moreover, they also found, as observed in the current study, these linkage disequilibrium constants to be of much lesser significance than the linkage disequilibrium constant observed between the *Taq*I A and *Taq*I B sites. This is interesting in that while a difference in D2 dopamine receptor Bmax was observed between the *Taq*I D $D2^{+}$ and $D2^{-}$ alleles in the present study, this difference, in contrast with what was found for the *Taq*I A, *Taq*I B, and intron 6 sites, was not statistically significant. Further studies with a larger Caucasian population may be necessary to draw a more definitive conclusion regarding the *Taq*I D polymorphism and its possible relationship with D2 dopamine receptor density.

Of primary interest in the current investigation is the significant allelic effects the DRD2 gene *Taq*I A, *Taq*I B, and intron 6 polymorphisms have on D2 dopamine receptor density. Studies in human sub-

jects using PET and $^{11}$C-raclopride to measure D2 dopamine receptors have shown reduced receptor levels in the brains of alcoholics when compared with nonalcoholics (71,72). Moreover, Thanos et al. (73) demonstrated that overexpression of DRD2 reduces alcohol self-administration in rats, suggesting that high D2 dopamine receptor levels may be protective against alcohol abuse. There are a substantial number of reports demonstrating a significant association of the minor *Taq*I A A1 and *Taq*I B B1 alleles with alcoholism and other substance use disorders (for review see [25]). The identification of a D2 dopamine receptor deficit in subjects carrying the A1$^+$, B1$^+$, and INT6-T$^+$ alleles further strengthens the concept that these variants have a biological significance with regard to substance use disorders such as alcoholism. Based primarily on work with the *Taq*I A polymorphism, it has been postulated (25,74) that subjects having the A1$^+$ allele may compensate for the deficiency of their dopaminergic system by the use of alcohol and other substances, agents known to enhance the dopaminergic system by increasing brain dopamine levels. The resultant stimulation by dopamine of the A1$^+$ allelic subjects' fewer D2 dopamine receptors would then enhance feelings of reward and pleasure. Eventually the continued substance usage could then lead to dependence. This hypothesis may now be extended to include the current evidence differentiating dopaminergic function based on allelic status of the intron 6 and *Taq*I B polymorphisms.

The question remains as to how these mutations in the noncoding regions of the DRD2 gene affect receptor expression. A possible explanation is that these three polymorphisms are in very strong linkage disequilibrium with a functional allelic variant that affects receptor expression (6,31). This variant could be located in various genomic regions of this large 270-kb gene, of which exons represent only a small fraction of the total length of the gene. It could be located in the noncoding exon 1, a region separated from the *Taq*I A, *Taq*I B, and intron 6 sites by a large 250-kb intron in the DRD2 gene (75,76). However, that seems unlikely because exon 1 is in close proximity with the promoter-141C mutation, which was shown to have no effect on D2 dopamine receptor binding and which is in only relatively weak linkage disequilibria, if at all, with the *Taq*I A, intron 6, and *Taq*I B mutations. It could be located in the large 250-kb intron that separates exon 1 and exon 2, or in other controlling (enhancer) sequences of the DRD2 gene. In other systems, 3′- and 5′-untranslated regions have been shown to affect gene expression (77–79). Furthermore, physiologically important mutations can be up to several kilobases from the gene they affect (80,81). Thus it cannot be ruled out that an allelic variant in a controlling region in close proximity to the 3′-untranslated region where the *Taq*I A mutation occurs may be responsible for the *Taq*I A, intron 6, and *Taq*I B allelic effects on D2 dopamine receptor binding. Indeed, Samochowiec et al. (55), studying polymorphisms that span the entire DRD2 gene, suggest a predominant influence of the 3′-region of the gene on various neuropsychiatric traits in German alcoholics. Thus it would seem that the controlling regions of the DRD2 gene remain fruitful areas for investigating the underlying mechanisms contributing to the observed gene effects on D2 dopamine receptor binding and ultimately to alcohol addiction and other substance use disorders.

In conclusion, the results of the current study demonstrate a significantly diminished density of D2 dopamine receptors in subjects having the minor alleles at the *Taq*I A, *Taq*I B, and intron 6 DRD2 polymorphic sites. No allelic differences in D2 dopamine receptor density were observed with the DRD2 *Taq*I D, exon 7, exon 8, and promoter-141C polymorphic sites. Furthermore, based on linkage disequilibrium determinations, the *Taq*I A, *Taq*I B, and intron 6 DRD2 polymorphisms are tightly linked with each other, but not with the other four DRD2 polymorphic sites studied in this investigation. Taken together, these findings suggest that the *Taq*I A, *Taq*I B, and intron 6 DRD2 polymorphisms, but not the *Taq*I D, exon 7, exon 8, and promoter-141C polymorphisms, are in linkage disequilibrium with a functional allelic variant that affects D2 dopamine receptor expression. Moreover, the strong association of the minor alleles of the *Taq*I A, *Taq*I B, and intron 6 DRD2 polymorphisms with alcoholism (44) and abuse of other substances (25) suggests that the expression of the D2 dopamine receptor gene may make a significant contribution to the development of alcoholism and other substance abuse disorders.

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## REFERENCES

1. Koob, Y. and LeMoal, M. 1997. Drug abuse: Hedonic homeostatic dysregulation. Science 278:52–58.
2. Tanda, G., Pontieri, F. E., and Di Chiara, G. 1997. Cannabinoid and heroin activation of mesolimbic dopamine transmission by

a common μl opioid receptor mechanism. Science 276:2048–2050.
3. Blum, K., Noble, E. P., Sheridan, P. J., Montgomery, A., Ritchie, T., Jagadeeswaren, P., Nogami, H., Briggs, A. H., and Cohn, J. B. 1990. Allelic association of the human dopamine $D_2$ receptor gene in alcoholism. J. Am. Med. Assoc. 263:2055–2060.
4. Amadéo, S., Abbar, M., Fourcade, M. L., Waksman, G., Leroux, M. G., Medec, A., Selin, M., Champiat, J.-C., Brethome, A., Leclair, Y., Castelnau, D., Venisse, J.-L., and Mallet, J. 1993. D2 dopamine receptor gene and alcoholism. J. Psychiatr. Res. 27:173–179.
5. Blum, K., Noble, E. P., Sheridan, P. J., Finley, O., Montgomery, A., Ritchie, T., Ozkaragoz, T., Fitch, R. J., Sadlack, F., Sheffield, D., Dahlmann, T., Halbardier, S., and Nogami, H. 1991. Association of the A1 allele of the human D2 dopamine receptor gene with severe alcoholism. Alcohol 8:409–416.
6. Comings, D. E., Comings, B. G., Muhleman, D., Dietz, G., Shahbahrami, B., Tast, D., Knell, E., Kocsis, P., Baumgarten, R., Kovacs, B. W., Levy, D. L., Smith, M., Borison, R. L., Evans, D. D., Klein, D. N., MacMurray, J., Tosk, J. F., Sverd, J., Gysin, R., and Flanagan, S. D. 1991. The dopamine $D_2$ receptor locus as a modifying gene in neuropsychiatric disorders. J. Am. Med. Assoc. 266:1793–1800.
7. Hietala, J., Pohjalainen, T., Heikkila-Kallio, U., West, C., Salaspuro, M., and Syvälahti, E. 1997. Allelic association between D2 but not D1 dopamine receptor gene and alcoholism in Finland. Psychiat. Genet. 7:19–25.
8. Lawford, B. R., Young, R. M., Rowell, J. A., Gibson, J. N., Feeney, G. F. X., Ritchie, T. L., Syndulko, K., and Noble, E. P. 1997. Association of the D2 dopamine receptor A1 allele with alcoholism: Medical severity of alcoholism and type of controls. Biol. Psychiatry 41:386–393.
9. Neiswanger, K., Hill, S. Y., and Kaplan, B. B. 1995. Association and linkage studies of the TaqI A allele of the dopamine D2 receptor gene in samples of female and male alcoholics. Am. J. Med. Genet. 60:267–271.
10. Noble, E. P., Syndulko, K., Fitch, R. J., Ritchie, T., Bohlman, M. C., Guth, P., Sheridan, P. J., Montegomery, A., Heinzmann, C., Sparkes, R. S., and Blum, K. 1994. D2 dopamine receptor TaqI A alleles in medically ill alcoholic and nonalcoholic patients. Alcohol Alcohol. 29:729–744.
11. Ovchinnikov, I. V., Druzina, E., Ovchinnikova, O., Zagorovskaya, T., Nebrakova, T., Anokhina, I. P. 1999. Polymorphism of dopamine D2 and D4 receptor genes and Slavic-surnamed alcoholic patients. Addiction Biol. 4:399–404.
12. Parsian, A., Todd, R. D., Devor, E. J., O'Malley, K. L., Suarez, B. K., Reich, T., and Cloninger, C. R. 1991. Alcoholism and alleles of the human D2 receptor locus: Studies of association and linkage. Arch. Gen. Psychiatry 48:655–663.
13. Bolos, A. M., Dean, M., Lucase-Derse, S., Ramsburg, M., Brown, G. L., Goldman, D. 1990. Population and pedigree studies reveal a lack of association between dopamine D2 receptor gene and alcoholism. JAMA 264:3156–3160.
14. Cook, B. L., Wang, Z. W., Crowe, R. R., Hauser, R., and Freimer, M. 1992. Alcoholism and the D2 receptor gene. Alcohol. Clin. Exp. Res. 16:806–809.
15. Geijer, T., Neiman, J., Rydberg, U., Gyllander, A., Jöhnson, E., Sedvall, G., Valverius, P., and Terenius, L. 1994. Dopamine D2 receptor gene polymorphisms in Scandinavian chronic alcoholics. Eur. Arch. Psychiatry Clin. Neurosci. 244:26–32.
16. Gelernter, J., O'Malley, S., Risch, N., Kranzler, H. R., Krystal, J., Merikangas, K., Kennedy, J. F., and Kidd, K. K. 1991. No association between an allele of the D2 dopamine receptor gene (DRD2) and alcoholism. JAMA 266:1801–1807.
17. Goldman, D., Dean, M., Brown, G. L., Bolos, A. N., Tokola, R., Virkkunen, M., and Linnoila, M. 1992. D2 dopamine receptor genotype and cerebral spinal fluid homovanillic acid, 5-hydroxyindoleacetic acid and 3-methoxy-4-hydroxyphenylglycol in alcoholics in Finland and the United States. Acta Psychiatr. Scand. 86:351–357.
18. Heintz, A., Sander, T., Harms, H., Finckh, U., Kuhn, S., Dufeu, P., Dettling, M., Gräf, K., Rolfs, A., Rommelspacher, H., and Schmidt, L. G. 1996. Lack of allelic association of dopamine D1 and D2 (TaqI A) receptor gene polymorphisms with reduced dopaminergic sensitivity in alcoholism. Alcohol. Clin. Exp. Res. 20:1109–1113.
19. Sander, T., Murat, L., Samochowiec, J., Finckh, U., Romelspacher, H., and Schmidt, L. G. 1999. Lack of an allelic association between polymorphisms of the dopamine D2 receptor gene and alcohol dependence in the German population. Alcohol. Clin. Exp. Res. 23:578–581.
20. Suarez, B. K., Parsian, A., Hampe, C. L., Todd, R. D., Reich, T., and Cloninger, C. R. 1994. Linkage disequilibria and the D2 dopamine receptor locus (DRD2) in alcoholics and controls. Genomics 19:12–20.
21. Blum, K., Sheridan, P. J., Wood, R. C., Braverman, E. R., Chen, I. J. H., and Comings, D. E. 1995. Dopamine D2 receptor gene variants: Association and linkage studies in impulsive-addictive-compulsive behavior. Pharmacogenetics 5:121–141.
22. Cloninger, C. R. 1991. D2 dopamine receptor gene is associated but not linked with alcoholism. JAMA 266:1833–1834.
23. Cook, C. C. H. and Gurling, H. M. D. 1994. The D2 dopamine receptor gene and alcoholism: A genetic effect on the liability for alcoholism. J. R. Soc. Med. 87:400–402.
24. Gorwood, P., Ades, J., and Feingold, J. 1994. Are genes coding for dopamine receptors implicated in alcoholism? Eur. Psychiatry 9:63–69.
25. Noble, E. P. 2000. The DRD2 gene in psychiatric and neurological disorders and its phenotypes. Pharmacogenomics 1:309–333.
26. Pato, C. N., Macciardi, F., Pato, M. T., Verga, M., and Kennedy, H. 1993. Review of the putative association of dopamine D2 receptor and alcoholism: A meta-analysis. Am. J. Med. Genet. 48:78–82.
27. Uhl, G., Blum, K., Noble, E., and Smith, S. 1993. Substance abuse vulnerability and D2 receptor gene. Trends Neurosci. 16: 83–88.
28. Gelernter, J., Goldman, D., and Risch, N. 1993. The A1 allele of the D2 dopamine receptor gene and alcoholism. JAMA 268: 1673–1677.
29. Noble, E. P. and Blum, K. 1993. Alcoholism and the D2 receptor gene [letter]. JAMA 270:1547.
30. Smith, S. S., O'Hara, B. F., Persico, A. M., Gorelick, D. A., Newlin, D. B., Vlahov, D., Solomon, L., Pickens, R., and Uhl, G. R. 1992. Genetic vulnerability to drug abuse: The $D_2$ dopamine receptor TaqI B1 restriction fragment length polymorphism appears more frequently in polysubstance abusers. Arch. Gen. Psychiatry 49:723–727.
31. O'Hara, B. F., Smith, S. S., Bird, G., Persico, A. M., Suarez, B. K., Cutting, G. R., and Uhl, G. R. 1993. Dopamine $D_2$ receptor RFLPs, haplotypes and their association with substance use in black and caucasian research volunteers. Hum. Hered. 43: 209–218.
32. Comings, D. E., Muhleman, D., Ahn, C., Gysin, R., and Flanagan, S. D. 1994. The dopamine $D_2$ receptor gene: A genetic risk factor in substance abuse. Drug Alcohol Depend. 34:175–180.
33. Noble, E. P., Blum, K., Khalsa, M. E., Ritchie, T., Montgomery, A., Wood, R. D., Fitch, R. J., Ozkaragoz, T., Sheridan, P. J., Anglin, M. D., Parades, A., Treiman, L. J., and Sparkes, R. S. 1993. Allelic association of the $D_2$ dopamine receptor gene with cocaine dependence. Drug Alcohol Depend. 33:271–285.
34. Persico, A. M., Bird, G., Gabbay, F. H., and Uhl, G. R. 1996. $D_2$ dopamine receptor gene TaqI A1 and B1 restriction fragment length polymorphism: Enhanced frequencies in psychostimulant-preferring polysubstance abusers. Biol. Psychiatry 40:776–784.
35. Noble, E. P., St Jeor, S. T., Ritchie, T., Syndulco, K., St Jeor, S. C., Fitch, R. J., Brunner, R. L., and Sparkes, R. S. 1994. $D_2$

dopamine receptor gene and cigarette smoking: A reward gene? Med. Hypotheses 42:257–260.
36. Comings, D. E., Ferry, L., Bradshaw-Robinson, S., Burghette, R., Chiu, C., and Muhleman, D. 1996. The dopamine $D_2$ receptor (DRD2) gene: A genetic risk factor in smoking. Pharmacogenetics 6:73–79.
37. Spitz, M. R., Shi, H., Yang, F., Hudmon, K. S., Jiang, H., Chamberlain, R. M., Amos, C. I., and Wan, Y. 1998. Case-control study of the $D_2$ dopamine receptor gene and smoking status in lung cancer patients. J. Natl. Cancer Inst. 90:358–363.
38. Lawford, B. R., Young, R. McD., Noble, E. P., Sargent, J., Rowell, J., Shadforth, S., Zhang, X., and Ritchie, T. 2000. The $D_2$ dopamine receptor $A_1$ allele and opioid dependence: Association with heroin use and response to methadone treatment. Am. J. Med. Genet. (Neuropsychiat. Genet.) 96:592–598.
39. Comings, D. E., Flanagan, S. D., Dietz, G., Muhleman, D., Knell, E., and Gysin, R. 1993. The dopamine $D_2$ receptor (DRD2) as a major gene in obesity and weight. Biochem. Med. Metabol. Biol. 50:176–185.
40. Noble, E. P., Noble, R. E., Ritchie, T., Syndulko, K., Bohlman, M. C., Noble, L. A., Zhang, Y., Sparkes, R. S., and Grandy, D. K. 1994. Allelic association of the human $D_2$ dopamine receptor gene and obesity. Int. J. Eat. Disord. 15:205–217.
41. Comings, D. E., Gade, R., MacMurray, J. P., Muhleman, D., and Peters, W. R. 1996. Genetic variants of the human obesity (OB) gene: Association with body mass index in young women, psychiatric symptoms, and interaction with the dopamine D2 receptor (DRD2) gene. Molec. Psychiatry 1:325–335.
42. Spitz, M. R., Detry, M. A., Pillow, P., Hu, Y. H., Amos, C. I., Hong, W. K., and Wu, X. F. 2000. Variant alleles of the D2 dopamine receptor gene and obesity. Nutri. Res. 20:371–380.
43. Noble, E. P., Zhang, X., Ritchie, T. L., and Sparkes, R. S. 2000. Haplotypes at the DRD2 locus and severe alcoholism. Am. J. Med. Genet. (Neuropsychiat. Genet.) 96:622–631.
44. Blum, K., Noble, E. P., Sheridan, P. J., Montgomery, A., Ritchie, T., Ozkaragoz, T., Fitch, R. J., Wood, R., Finley, O., and Sadlack, F. 1993. Genetic predisposition in alcoholism: Association of the $D_2$ dopamine receptor TaqI B RFLP in severe alcoholics. Alcohol 10:59–67.
45. Noble, E. P., Blum, K., Ritchie, T., Montgomery, A., and Sheridan, P. J. 1991. Allelic association of the $D_2$ dopamine receptor gene with receptor-binding characteristics in alcoholism. Arch. Gen. Psychiatry 48:648–654.
46. Thompson, J., Thomas, N., Singleton, A., Piggott, M., Lloyd, S., Perry, E. K., Morris, C. M., Perry, R. H., Ferrier, I. N., and Court, J. A. 1997. $D_2$ dopamine receptor gene (DRD2) TaqI A polymorphism: Reduced dopamine $D_2$ receptor binding in the human striatum associated with the A1 allele. Pharmacogenetics 7:479–484.
47. Pohjalainen, T., Rinne, J. O., Nåagren, K., Lehikoinen, P., Anttila, K., Syvälahti, E. K. G., and Hietala, J. 1998. The A1 allele of the human $D_2$ dopamine receptor gene predicts low $D_2$ receptor availability in healthy volunteers. Molec. Psychiatry 3:256–260.
48. Jönsson, E. G., Nöthen, M. M., Grünhage, F., Farde, L., Nakashima, Y., Propping, P., and Sedvall, G. C. 1999. Polymorphisms in the dopamine D2 receptor gene and their relationships to striatal dopamine receptor density of healthy volunteers. Molec. Psychiatry 4:290–296.
49. Saiki, R. K., Gelfand, D. H., Stoffel, S., Scharf, S. J., Higuchi, R., Horn, G. T., Mullins, K. B., and Erlich, H. A. 1988. Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491.
50. Grandy, D. K., Zhang, Y., and Civelli, O. 1993. PCR detection of the TaqI A RFLP at the DRD2 locus. Hum. Mol. Genet. 2: 2197.
51. Castiglione, C. M., Deinard, A. S., Speed, W. C., Sirugo, G., Rosenbaum, H. C., Zhang, Y., Grandy, D. K., Grigorenko, E. L., Bonne-Tamir, B., Pakstis, A. J., Kidd, J. R., and Kidd, K. K. 1995. Evolution of haplotypes at the DRD2 locus. Am. J. Hum. Genet. 57:1445–1456.
52. Sarkar, G. and Sommer, S. S. 1991. Haplotyping by double PCR amplification of specific alleles. Biotechniques 10:436–440.
53. Kidd, K. K., Pakstis, A. J., Castiglione, C. M., Kid, J. R., Speed, W. C., Goldman, D., Knowler, W. C., Lu, R.-B., and Bonne-Tamir, B. 1996. DRD2 haplotypes containing the TaqI A1 allele: Implications for alcoholism research. Alcohol. Clin. Exp. Res. 20:697–705.
54. Arinami, T., Gao, M., Hamaguchi, H., and Toru, M. 1997. A functional polymorphism in the promoter region of the dopamine D2 receptor gene is associated with schizophrenia. Hum. Mol. Genetic. 6:577–582.
55. Samochowiec, J., Ladehoff, M., Pelz, J., Smolka, M., Schmidt, L. G., Rommelspacher, H., and Finckh, U. 2000. Predominant influence of the 3′-region of dopamine D2 receptor gene (DRD2) on the clinical phenotype in German alcoholics. Pharmacogenetics 10:471–475.
56. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193:265–275.
57. Munson, P. J. and Rodbard, D. 1980. LIGAND: A versatile computerized approach for characterization for ligand-binding systems. Anal. Biochem. 107:220–239.
58. Weir, B. S. 1996. Genetic data analysis II: Methods for discrete population genetic data. Sinauer Associates, Sunderland, Massachusetts.
59. Weir, B. S. and Cockerham, C. C. 1989. Complete characterization of disequilibrium at two loci. Pages 86–110, in Feldman, M. E. (ed.), Mathematical Evolutionary Theory, Princeton University Press, New Jersey.
60. Seeman, P., Ulpian, C., Bergeron, C., Riederer, P., Jellinger, K., Gabriel, E., Reynolds, G. P., and Tourtellotte, W. W. 1984. Bimodal distribution of dopamine receptor densities in brains of schizophrenics. Science 225:728–730.
61. De Keyser, J., Walraevens, H., De Backer, J.-P., Ebinger, G., and Vauquelin, G. 1989. $D_2$ dopamine receptors in the human brain: Heterogeneity based on differences in guanine nucleotide effect on agonist binding, and their presence on corticostriatal nerve terminals. Brain Res. 484:36–42.
62. Severson, J. A., Marcusson, J., Winblad, B., and Finch, C. E. 1982. Age-correlated loss of dopaminergic binding sites in human basal ganglia. J. Neurochem. 39:1623–1631.
63. Laruelle, M., Gelernter, J., and Innis, R. B. 1998. $D_2$ receptors binding potential is not affected by TaqI polymorphism at the $D_2$ receptor gene. Mol. Psychiatry 3:261–265.
64. Hitzemann, R. J. 1998. The regulation of $D_2$ dopamine receptor expression. Mol. Psychiatry 3:198–203.
65. Silvestri, S., Seeman, M. V., Negrete, J.-C., Houle, S., Shammi, C. M., Remington, G. J., Kupar, S., Zipursky, R. B., Wilson, A. A., Christensen, B. K., and Seeman, P. 2000. Increased dopamine D2 receptor binding after long-term treatment antipsychotics in humans: A clinical PET study. Psychopharmacology 152:174–180.
66. Pohjalainen, T., Nåagren, K., Syvälahti, E. K., and Hietala, J. 1999. The dopamine D2 receptor 5′-flanking variant, -141C Ins/Del, is not associated with reduced dopamine D2 receptor density in vivo. Pharmacogenetics 9:505–509.
67. Gelernter, J., Kranzler, H., Cubells, J. F., Ichimose, H., and Nagatsu, T. 1998. DRD2 allele frequencies and linkage disequilibria, including the -141C Ins/Del promoter polymorphism, in European-American, African-American and Japanese subjects. Genomics 51:21–26.
68. Ishiguro, H., Arinami, T., Saito, T., Akazawa, S., Enomoto, M., Mitushio, H., Fujishiro, H., Tada, K., Akimoto, Y., Mifune, H., Shioduka, S., Hamaguchi, H., Toru, M., and Shibuya, H. 1998. Association study between the -141C Ins/Del and TaqI A polymorphisms of the doamine $D_2$ receptor gene and alcoholism. Alcoholism Clin. Exp. Res. 22:845–848.

69. Parsian, A., Cloninger, C. R., and Zhang, Z. H. 2000. Functional variant in the DRD2 receptor promoter region and subtypes of alcoholism. Am. J. Med. Genet. 96:407–411.
70. Kidd, K. K., Bharati, M., Castiglione, C. M., Zhao, H., Pakstis, A. J., Speed, W. C., Bonne-Tamir, B., Lu, R.-B., Goldman, D., Lee, C., Nam, Y. S., Grandy, D. K., Jenkins, T., and Kidd, J. R. 1998. A global survey of haplotype frequencies and linkage disequilibrium at the DRD2 locus. Hum. Genet. 103:211–227.
71. Hietala, J., West, C., Syvälahti, E., Någren, K., Lehikoinen, P., Sonninen, P., and Ruotsalainen, U. 1994. Striatal D2 dopamine receptor binding characteristics in vivo in patients with alcohol dependence. Psychopharmacology 116:285–290.
72. Volkow, N. D., Wang, G.-J., Fowler, J. S., Logan, J., Hitzemann, R., Ding, Y.-S., Pappas, N., Shea, C., and Piscani, K. 1996. Decreases in dopamine receptors but not in dopamine transporters in alcoholics. Alcohol. Clin. Exp. Res. 20:1594–1598.
73. Thanos, P. K., Volkow, N. D., Freimuth, P., Umegaki, H., Ikari, H., Roth, G., Ingram, D. K., and Hitzemann, R. 2001. Overexpression of dopamine D2 receptors reduces alcohol self-administration. J. Neurochem. 78:1094–1103.
74. Noble, E. P. 1996. The gene that rewards alcoholism. Sci. Am. Sci. Med. 3:52–61.
75. Gandelman, K. Y., Harmon, S., Todd, R. D., and O'Malley, K. L. 1991. Analysis of the structure and expression of the human dopamine $D_2$A receptor gene. J. Neurochem. 56:1024–1029.
76. Eubanks, J., Djabali, M., Selleri, L., Grandy, D. K., Civelli, O., McElligott, D. L., and Evans, G. A. 1992. Structure and linkage of the $D_2$ dopamine receptor and neural cell adhesion molecule genes on human chromosome 11q23. Genomics 14:1010–1018.
77. Carswell, S. and Alwine, J. C. 1989. Efficiency of utilization of the simian virus 40 late polyadenylation site: Effects of upstream sequences. Mol. Cell. Biol. 9:4248–4258.
78. Rotondaro, L., Mele, A., and Rovera, G. 1996. Efficacy of different viral promoters in directing gene expression in mammalian cells: Effect of 3′-untranslated sequences. Gene 168: 195–198.
79. Mammès, O., Betoulle, D., Aubert, R., Giraud, V., Tuset, S., Petiet, A., Colas-Linhart, N., and Fumeron, F. 1998. Novel polymorphisms in the 5′ region of the LEP gene: Association with leptin levels and response to low-calorie diet in human obesity. Diabetes 47:487–489.
80. Cooper, D. N. 1992. Regulatory mutations and human genetic disease. Ann. Med. 24:427–437.
81. Bedell, M. A., Jenkins, N. A., and Copeland, N. G. 1996. Good genes in bad neighbourhoods. Nat. Genet. 12:229–232.