# $D_2$ Dopamine Receptor Gene *TaqI* A1 and B1 Restriction Fragment Length Polymorphisms: Enhanced Frequencies in Psychostimulant-Preferring Polysubstance Abusers

Antonio M. Persico, Geoffrey Bird, Frances H. Gabbay, and George R. Uhl

*Several lines of evidence suggest that presence of a $D_2$ dopamine receptor (DRD2) gene variant marked by* TaqI *restriction fragment length polymorphisms (RFLPs) might contribute to vulnerability to substance abuse. Psychostimulants display the most robust enhancement of dopamine activity in mesolimbic/mesocortical circuits important for behavioral reward. The present study tests the hypothesis that a DRD2 gene variant might be more prominent in polysubstance users who preferentially use psychostimulants than in addicts with preferential opiate use or in those with no drug preference. Polysubstance users with histories of heavy daily preferential psychostimulant use more often displayed one or two copies of the* TaqI *A1 (27/62 = 43.5% vs 33/119 = 27.7% for controls), and B1 (20/62 = 32.3% vs 23/119 = 19.8% for controls) markers at the DRD2 locus. DRD2 gene marker distributions in abusers with more prominent opiate use, or those with no history of drug preference, were similar to control genotypes. Psychostimulant-preferring drug users also reported earlier onset of psychostimulant use. Our data are consistent with the hypothesis that DRD2 gene variants marked by these polymorphisms may work, probably in concert with other genetic and environmental factors, to enhance vulnerability to psychostimulant abuse.*

**Key Words:** Dopamine $D_2$ receptor, genetic association, cocaine, amphetamine, substance abuse, alcoholism

BIOL PSYCHIATRY 1996;40:776–784

## Introduction

Genetic contributions to the etiology of substance abuse are supported by family, twin, and adoption studies (Pedersen 1984; Cadoret et al 1987; Pickens et al 1991; Luthar et al 1992; Uhl et al 1995). Although most abused drugs share the ability to activate mesolimbic/mesocortical dopaminergic circuits critical for reward and reinforcement in animals, psychostimulants appear to provide the largest effects (Di Chiara and Imperato 1988; Wise and Rompre 1989). Inherited variants of genes coding for proteins involved in dopaminergic neurotransmission could thus

From the Molecular Neurobiology Branch (AMP, GB, GRU), and Office of the Director (GRU), Intramural Research Program/Division of Intramural Research, Addiction Research Center, National Institute on Drug Abuse/National Institutes of Health; Department of Medical and Clinical Psychology (FHG), Uniformed Services University of the Health Sciences, Bethesda; and Departments of Neurology and Neuroscience (GRU), Johns Hopkins University School of Medicine, Baltimore, MD.

Address reprint requests to George R. Uhl, MD, PhD, Office of the Director, Addiction Research Center, National Institute on Drug Abuse/National Institutes of Health, Box 5180, Baltimore, MD 21224.

A.M. Persico's present address is Laboratory of Neuroscience, Libero Istituto Universitario Campus Bio-Medico, Via Longoni 83, 00155 Rome, Italy.

Received June 3, 1994; revised September 6, 1995.



0006-3223/96/$0.00
SSDI 0006-3223(95)00483-1

contribute to the genetic underpinnings of drug addiction and in particular might confer increased vulnerability to psychostimulant abuse.

Modest, but significant associations between $D_2$ dopamine receptor (DRD2) gene variants marked by the *Taq*I A1 restriction fragment length polymorphism (RFLP) and polysubstance abuse have been described in several studies that have included varying proportions of psychostimulant abusers (for review see Uhl et al 1992, 1993, 1995; Persico et al 1993; Gelernter et al 1993; Noble 1993).

In a probably-polygenic disorder with large environmental influences, such as substance abuse, the effect of a single gene might conceivably account for clinical traits most readily detected in a specific subset of patients. Smith et al (1993) found no association between DRD2 polymorphisms and psychopathy in incarcerated drug users, suggesting that possible links between DRD2 polymorphisms and addiction may not be mediated by antisocial personality, which represents a common substance abuse comorbidity. Since animal models suggest that enhanced dopaminergic release from mesolimbic/mesocortical terminals may play a more critical role in psychostimulant than in opiate self-administration (Pettit et al 1984; Vaccarino et al 1985; Kuhar et al 1991; Pulvirenti et al 1992), we wondered if DRD2 gene variants could exert more profound influences on psychostimulant abuse than on the use of abused drugs with less fully-dopamine-dependent mechanisms of action.

This study tests this hypothesis. We report that the DRD2 *Taq*I A1 and B1 markers are more frequent among Caucasian polysubstance abusers who, despite access and exposure to both psychostimulants and opiates, develop severe addiction to psychostimulants alone. These results contrast with those from opiate-preferring abusers, from addicts reporting prolonged daily use of both drugs, and from heavy users of multiple drugs including psychostimulants and opiates. The specificity of these genetic findings is further confirmed by the lack of association displayed by a variable number tandem repeat (VNTR) marker at another important dopaminergic gene locus, the dopamine transporter (DAT1) (Vandenbergh et al 1992).

## Methods

### *Subjects*

A total of 528 Caucasian individuals volunteered for this study under informed consent and confidentiality provisions. Subjects were recruited from the Baltimore/Washington area by the National Institute on Drug Abuse, Division of Intramural Research, at the Addiction Research Center (N = 450), a nearby hemodialysis unit (N = 12), an adjacent public health facility studying HIV infections (N = 52) (Baltimore, Maryland) and by the Department of Medical and Clinical Psychology, Uniformed Services University of the Health Sciences (N = 14) (Bethesda, Maryland). Subjects reporting at least 25% or more Native American or Hispanic origin were excluded. A portion of this sample is represented by subjects whose dopamine $D_2$ receptor *Taq*I RFLP and DAT1 VNTR status have been the object of previous reports (Smith et al 1992; Persico et al 1993b).

All subjects completed the Drug Use Survey (DUS), a validated interview which records the age of onset and the amount, frequency, and/or dollar cost at the time of lifetime peak use for each of 15 addictive substances or drug classes, including alcohol and nicotine (Smith et al 1992). A subgroup of 131 subjects was also administered the Diagnostic Interview Schedule (DISIII-R), as previously described (Smith et al 1992).

Peak lifetime drug use was rated based on DUS reports by raters who were blind to genotype information, as described by Smith et al (1992). Briefly, use of each substance was scored on a 4-point scale (0 = none; + = experimental, sporadic or minimal; ++ = frequent or moderate; +++ = daily or heavy), according to a set of specific criteria for each drug class (Smith et al 1992). Each subject was then assigned a "total use" index, derived by pooling scores for individual substances, with 0 indicating up to minimal use of alcohol, nicotine and marijuana, in the absence of any other drug use; 1 indicating up to frequent use of alcohol and nicotine, in the presence of up to experimental/minimal use of illicit drugs; 2 indicating heavy use of alcohol or nicotine, frequent use of marijuana and/or up to frequent use of other drugs; and 3 indicating daily/heavy use of any illicit drug. DUS rating criteria relevant to opiates and psychostimulants are listed in Table 1. Control subjects (N = 119) were then defined on the basis of a "total use" index of 0 or 1, and drug users (N = 409) had indices of 2 or 3.

Drug-use patterns were next analyzed to test whether DRD2 gene variants correlated with more prominent daily use of either psychostimulants or opiates. In order to minimize the influence of drug unavailability or other environmental factors in determining preferential substance use, only drug users who reported lifetime use of both a psychostimulant (either cocaine or amphetamines) and an opiate (either heroin or other opiates) were included in analyses. Furthermore, only drug users reporting heavy/daily lifetime peak use of psychostimulants and/or opiates were considered. Drug "preference" was therefore defined on the basis of reported peak lifetime drug use and not on subjective liking of drug effects. Using these guidelines, the original sample of 409 substance abusers was divided into 4 samples: psychostimulant-preferring

Table 1. Drug Use Survey: Criteria for Self-Reported Lifetime Peak Use of Psychostimulants and Opiates[a]

| | | |
|---|---|---|
| Cocaine | 0: | Never used cocaine. |
| | +: | <2 grams per week (≤$150 per week); typical use about 1 gram per month. |
| | ++: | 2–4 grams per week (>$150 per week but <$300 per week). |
| | +++: | >4 grams per week, usually up to 7–10 grams per week; (>$300 per week, usually much higher; daily use common in these subjects). |
| Amphetamines | 0: | Never used amphetamines. |
| | +: | <1 pill per week (<4 pills per month). |
| | ++: | 1–6 pills per week (4–24 pills per month). |
| | +++: | ≥7 pills per week (≥24 pills per month). |
| Heroin | 0: | Never used heroin. |
| | +: | Used 1 time per week or <$30.00 per week. |
| | ++: | Typical use—2–3 times per week (up to 6 times per week), spending $30 to $100 per day. |
| | +++: | Daily use, typically spending ≥$100 per day. |
| Other opiates | 0: | Never used morphine, methadone, codeine, dilaudid, or other opiate analgesics. |
| | +: | Used 1 time per week or <$30.00 per week. |
| | ++: | Typical use—2–3 times per week (up to 6 times per week), spending $30 to $100 per day. |
| | +++: | Daily use, typically spending ≥$100 per day. |

[a]Prescription drug use was coded as "0" if taken for valid medical reasons.

drug users (N = 62), opiate-preferring drug users (N = 40), substance abusers reporting daily use of both drugs (N = 47), and heavy polysubstance users with daily use of drugs in 6 or more categories, including both psychostimulants and opiates (N = 42). Drug use rating criteria for each of these samples, age of onset, and demographic information are shown in Table 2. These subjects were contrasted with 119 controls, with mean age 29 ± 9.1 years, M/F = 94/25 and total drug use scores of 0 (N = 61) or 1 (N = 58).

Samples from distinct racial and ethnic groups, assessed for potential differences in dopamine transporter VNTR allelic frequencies, include 405 Caucasian-Americans, 82 African-Americans, 348 Italians, 107 Japanese, and 203 Chinese individuals. The Caucasian-American sample, partly overlapping with smaller samples that were the object of previous assessments (Persico et al 1993b), includes all 155 polysubstance users and 71 controls from this study, as well as 179 additional drug users. African-Americans are represented by 29 controls and 53 polysubstance users. Italians include 135 normal controls, 149 schizophrenics and 64 patients with delusional disorders (Persico A.M., Macciardi F., Catalano M., *manuscripts in preparation*). Japanese subjects are from Sano et al (1993), and the Chinese sample from Li et al (1994), includes 105 schizophrenics and 98 normal controls. In all studies, patients and normal controls displayed virtually identical allelic frequencies and were therefore combined.

### *Laboratory Methods*

Blood was obtained, DNA extracted, and DRD2 *Taq*I RFLP and DAT1 VNTR analyses were performed as previously described (Smith et al 1992; Vandenbergh et al 1992; Persico et al 1993b). Briefly, 15 ml of blood were drawn into EDTA-containing tubes, DNA extractions were performed using standard methods (Sambrook et al 1989) on isolated nuclei for samples stored at 4°C and on sedimented white blood cells for samples frozen at −70°C. DRD2 *Taq*I RFLPs were analyzed using 5–10 μg of DNA digested with *Taq*I, electrophoresed using 0.8% agarose gels, transferred to nylon membranes, UV-crosslinked, pre-hybridized, and hybridized as described (Smith et al 1992), washed for 20 minutes in 2× SSC at

Table 2. Demographic Characteristics, Age of Onset and Lifetime Peak Drug Use Patterns in Substance Users Categorized According to Drug Preference and Extent of Polysubstance Abuse[a]

| | Psychostimulant preference | Opiate preference | No preference | Heavy poly-drug users |
|---|---|---|---|---|
| Age (yrs) | 31.9 ± 5.9 | 37.7 ± 6.9 | 33.9 ± 7.9 | 37.5 ± 7.2 |
| Sex (M/F) | 56/6 | 33/7 | 37/10 | 39/3 |
| Peak cocaine or amphetamine use | +++ (N = 62) | + (N = 17) ++ (N = 23) | +++ (N = 47) | +++ (N = 42) |
| Peak heroin or other opiate use | + (N = 28) ++ (N = 34) | +++ (N = 40) | +++ (N = 47) | +++ (N = 42) |
| Number of +++ drug classes | Any | Any | <6 | ≥6 |
| Age of onset of psychostimulant use | 18.8 ± 4.8 | 22.5 ± 6.9 | 21.4 ± 6.9 | 17.9 ± 4.2 |
| Age of onset of opiate use | 23.5 ± 5.6 | 19.5 ± 5.7 | 21.6 ± 6.2 | 17.6 ± 4.0 |
| Psychostimulant use/opiate use age of onset ratio | 0.83 ± 0.26 | 1.17 ± 0.27 | 1.03 ± 0.38 | 1.05 ± 0.30 |
| Age of onset of any drug use | 12.4 ± 2.7 | 13.0 ± 2.7 | 12.6 ± 2.6 | 12.0 ± 2.8 |

[a]Data are expressed as mean ± standard deviation.

room temperature followed by two 30-minute washes in 0.4× SSC/0.5% SDS at 55°C and exposed to film (Kodak XAR) for 1–6 days with an intensifying screen at −70°C or to a phosphorimager (Molecular Dynamics).

DRD2 *Taq*I A RFLP patterns were detected using the phD2-9 probe, subcloned from the λhD2G1 human genomic clone (obtained from O. Civelli) as previously described (Smith et al 1992), containing a 1.7-kilobase (kb) *Bam*HI fragment which detects each of the four currently-identified *Taq*I A patterns (Persico et al 1993c). Rare A3 and A4 polymorphisms of the DRD2 *Taq*I A system have been recoded A2 and A1, respectively, according to Persico et al (1993c). *Taq*I B polymorphisms were ascertained using the λhD2G2 clone (obtained from O. Civelli), as described (Smith et al 1992). *Taq*I D patterns were assessed using the phD2-244 probe (obtained from A. Parsian), containing a 3.8 kb *Xba*I fragment spanning the entire exon 3 and portions of introns 2 and 3 of the human DRD2 gene (Parsian et al 1991).

Dopamine transporter gene markers were assessed using polymerase chain reaction (PCR) (Vandenbergh et al 1992). Briefly, a 40-base pairs (bp) variable number tandem repeat (VNTR) located in the 3′ untranslated region of the DAT1 cDNA was amplified from 40 ng of genomic DNA using 1.25u of AmpliTaq DNA polymerase through 35 cycles as follows: denaturing for 1 min at 93°C and annealing/extension for 1 min at 72°C, in buffer supplied by the manufacturer (Perkin-Elmer) and in the presence of primers 5′-TGTGGTGTAGGGAACGGCCT-GAG-3′ and 5′-CTTCCTGGAGGTCACGGCTCAA-GG-3′ at $5 \times 10^{-7}$M. Amplification products were electrophoresed using 5% polyacrylamide gels and product sizes were determined by comparison with molecular weight standards (BRL).

Previous results lend support to the existence of an association between substance abuse and DRD2 *Taq*I A1 and B1 polymorphisms (for review see Uhl et al 1992; Uhl et al 1993; Persico et al 1993a; Noble 1993; Uhl et al 1995). In one report, the *Taq*I A1 marker was also associated with a moderate decrease in brain DRD2 $B_{max}$, while alcohol intake appeared to have an impact only on DRD2 Kd (Noble et al 1991). In Caucasians, these polymorphisms have been found to be in linkage disequilibrium with the *Taq*I D2 marker (Persico et al *manuscript in preparation*). Furthermore, enhanced dopaminergic release from mesolimbic/mesocortical terminals may play a critical role only in animal models of psychostimulant and a less critical role in opiate self-administration (Pettit et al 1984; Vaccarino et al 1985; Kuhar et al 1991; Pulvirenti et al 1992). Therefore, one-tail $\chi^2$ tests were performed on genotype distributions, to test the hypothesis of an association between enhanced presence of the DRD2 *Taq*I A1, B1, and D2 polymorphisms and preferential abuse of psychostimulants. Few subjects displayed homozygote A1/A1, and B1/B1 genotypes; data from these individuals were combined with those from heterozygotes and $\chi^2$ tests with Yates' continuity correction performed on the resulting contingency tables. The same statistical procedures were performed on DAT1 VNTR genotype distributions, under the hypothesis of no association, in accordance with earlier data (Persico et al 1993b). Age of onset of psychostimulant use, opiate use and any drug use were analyzed using one-way analysis of variance (ANOVA) and Student's $t$ tests. Results are reported as mean ± SD and statistical significance is set at $p < .05$.

## Results

The DRD2 *Taq*I A, B, and D probes identified fragments of the expected sizes (Grandy et al 1989; Hauge et al 1991; Parsian et al 1991). PCR amplification of genomic DNA also produced fragments with sizes corresponding to 3–10 copies of the 40 base repetitive element in the DAT1 3′ untranslated region, as previously described (Vandenbergh et al 1992), with 450 of 454 alleles displaying either 9 or 10 copy VNTR polymorphisms. Both DRD2 RFLP and DAT1 VNTR genotypes were assigned by two independent investigators with 100% agreement.

Dopamine D2 receptor genotype data for polysubstance user samples and for controls are reported in Table 3. More of the psychostimulant-preferring polysubstance users display either one or two copies of the *Taq*I A1 and B1 markers, as compared with control subjects, whereas *Taq*I D2 markers show a non-significant trend in this direction. Distributions of dopamine transporter (DAT) VNTR alleles do not distinguish any substance user or control sample in this study (Table 4a). Differences in DAT allelic frequencies, however, are evident among distinct racial and ethnic groups, as summarized in Table 4b (for alleles 9 and 10, $\chi^2 = 175.07$, 4 df, $p < .0001$).

Polysubstance user samples differ from control populations in age of onset of psychostimulant use (one-way ANOVA: $F = 5.43$ [3 162], two-tail $p = .0014$), age of onset of opiate use ($F = 8.99$ [3 159], $p < .0001$), and ratio between age of first psychostimulant use and age of first opiate use ($F = 8.81$ [3 158], $p < .0001$) (Table 2). No differences in the age of onset for use of any drug was found, however ($F = 0.85$ [3 158], $p = .4667$) (Table 2). Psychostimulant-preferring and opiate-preferring drug users display earlier onset of psychostimulant ($t = 2.96$, 86 df, two-tail $p = .004$) and of opiate ($t = 2.98$, 83 df, $p = .004$) use, respectively. These findings are reflected in a statistically-significant difference in the age of onset ratio, obtained by dividing the age of first psychostimulant use by the age of first opiate use ($t = 5.7$, 82 df, $p <$

Table 3. Dopamine $D_2$ Receptor Genotypes in Drug Users with Different Patterns of Drug Preference and Controls

| | Polysubstance Users | | | | |
|---|---|---|---|---|---|
| DRD2 *TaqI* Genotypes | Psychostimulant preference | Opiate preference | No preference | Heavy polysubstance use | Controls |
| A1/A1 | 2 (3.2%) | 2 (5.0%) | 2 (4.3%) | 0 | 4 (3.4%) |
| A1/A2 | 25 (40.3%) | 10 (25.0%) | 12 (25.5%) | 13 (31.0%) | 29 (24.3%) |
| A2/A2 | 35 (56.5%) | 28 (70.0%) | 33 (70.2%) | 29 (69.0%) | 86 (72.3%) |
| $\chi^2$ | 3.92 (1 df) | 0.01 (1 df) | 0.01 (1 df) | 0.04 (1 df) | |
| *p* | 0.024 | 0.471 | 0.471 | 0.422 | |
| B1/B1 | 1 (1.6%) | 1 (2.5%) | 1 (2.1%) | 0 | 4 (3.4%) |
| B1/B2 | 19 (30.6%) | 9 (22.5%) | 9 (19.1%) | 12 (28.6%) | 19 (16.4%) |
| B2/B2 | 42 (67.8%) | 30 (75.0%) | 37 (78.8%) | 30 (71.4%) | 93 (80.2%) |
| $\chi^2$ | 2.76 (1 df) | 0.22 (1 df) | 0.00 (1 df) | 0.91 (1 df) | |
| *p* | 0.048 | 0.321 | 0.500 | 0.170 | |
| D1/D1 | 13 (24.5%) | 15 (42.9%) | 15 (38.5%) | 16 (40.0%) | 34 (33.7%) |
| D1/D2 | 32 (60.4%) | 16 (45.7%) | 17 (43.6%) | 20 (50.0%) | 45 (44.5%) |
| D2/D2 | 8 (15.1%) | 4 (11.4%) | 7 (17.9%) | 4 (10.0%) | 22 (21.8%) |
| $\chi^2$ | 3.49 (2 df) | 2.08 (2 df) | 0.39 (1 df) | 2.67 (1 df) | |
| *p* | 0.087 | 0.177 | 0.412 | 0.132 | |

Each group of drug users and control sample genotypes are compared using one-tail $\chi^2$ tests; continuity correction is applied whenever df = 1.

.0001). No difference in this ratio is evident in non-preferring users of both drugs or in heavy polysubstance users (t = 0.27, 73 df, $p$ = .79), although the latter display significantly earlier onsets of opiate (t = 3.35, 73 df, $p$ = .001) and psychostimulant use (t = 2.64, 73 df, $p$ = .01).

## Discussion

This study addresses the hypothesis that DRD2 gene variants marked by the *TaqI* A1, B1, and D2 polymorphisms may modulate a specific feature found in some polysubstance abusers, preferential use of psychostimulants over opiate drugs that may display less dopamine-specific mechanisms of action. In order to test this hypothesis, we have selected from a larger sample of polysubstance abusers, individuals who have been exposed to both psychostimulants and opiates, and have become severely addicted to drugs in at least one of these two categories. We have thus attempted to reduce the impact of environmental factors, such as the availability of abused substances in general, which may prevent a polysubstance abuser from ever using certain drugs, regardless

Table 4. Distribution of Dopamine Transporter (DAT) VNTR Allele Frequencies in: (A) Drug Users with Different Patterns of Drug Preference and Controls; (B) Distinct Racial and Ethnic Groups

| | Dopamine Transporter VNTR Alleles | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| A | 3 | 5 | 6 | 7 | 8 | 9 | 10 | 11 |
| Psychostimulant preference (96 chr) | — | — | — | — | — | 0.3333 (32) | 0.6667 (64) | — |
| Opiate preference (64 chr) | — | — | — | — | — | 0.3438 (22) | 0.6562 (42) | — |
| No preference (78 chr) | 0.0256 (2) | — | — | — | 0.0256 (2) | 0.2821 (22) | 0.6667 (52) | — |
| Heavy polysubstance use (78 chr) | — | — | — | — | — | 0.2821 (22) | 0.7179 (56) | — |
| Controls (142 chr) | — | — | — | — | — | 0.2817 (40) | 0.7183 (102) | — |
| B | | | | | | | | |
| Caucasian-Americans (810 chr) | 0.0037 (3) | 0.0025 (2) | — | 0.0037 (3) | 0.0062 (5) | 0.2889 (234) | 0.6901 (559) | 0.0049 (4) |
| African-Americans (164 chr) | 0.0122 (2) | 0.0122 (2) | — | 0.0183 (3) | 0.0305 (5) | 0.2195 (36) | 0.7012 (115) | 0.0061 (1) |
| Italians (696 chr) | — | 0.0029 (2) | 0.0029 (2) | 0.0029 (2) | 0.0014 (1) | 0.3520 (245) | 0.6322 (440) | 0.0057 (4) |
| Japanese[a] (214 chr) | — | — | — | 0.0093 (2) | — | 0.0421 (9) | 0.9299 (199) | 0.0187 (4) |
| Chinese[b] (406 chr) | — | — | — | — | 0.0099 (4) | 0.0591 (24) | 0.9088 (369) | 0.0222 (9) |

[a]From Sano et al 1993.
[b]From Li et al 1994.

of a potentially predisposing genetic background. Using these guidelines, summarized in Table 2, we have identified samples of: a) "psychostimulant-preferring" polysubstance users, who reported a history of daily, severe psychostimulant abuse and less-frequent peak lifetime use of opiate compounds; and b) "opiate-preferring" addicts, who conversely reported prolonged daily use of opiates and sporadic use of psychostimulants. We also identified c) a "no preference" group of individuals, with equally severe use of both drugs, and d) a group of "heavy" polysubstance abusers with a lifetime history of severe, daily use of most or all drugs they had ever tried, including psychostimulants and opiates. For each of these samples, our data also allow appraisal of the relationship between age-of-first-use of specific drugs and their preferential use later in life.

Our results suggest that population variants in some dopaminergic genes, but not in others, and environmental factors that include age-of-first-contact with specific drugs, may contribute to the development of differential patterns of drug use by polysubstance abusers.

The specific association between preferential psychostimulant use and the DRD2 *Taq*I A1 and B1 markers provides evidence that DRD2 allelic differences may play detectable roles in conferring vulnerability to specific patterns of drug use, rather than equally conferring general vulnerability toward all addictions. The impact exerted by DRD2 gene variants might thus be described as a "modifying" influence, with more significance in specific subgroups of patients. The magnitude of this effect, however, is less profound than previously suggested by initial findings in alcoholics (Blum et al 1990). Conceivably, it could contribute most of the influences noted when mixed populations of polysubstance abusers are examined (e.g. Smith et al 1992). In the current sample, comparisons of psychostimulant-preferring drug users display odds ratios of 2.01 and 1.96 for DRD2 A1 and B1 markers, respectively, when compared to controls. The effect sizes for the association between psychostimulant preference and DRD2 markers, calculated according to the method of Cohen (1977), are 0.249 and 0.215, respectively. These effect sizes are comparable to the greater prevalence of substance abuse diagnoses found in individuals co-diagnosed with anxiety disorders (Regier et al 1990). Such moderate but statistically significant contributions are consistent with the complex polygenic mode of transmission and important environmental influences suggested by classical genetic studies (Uhl et al 1995).

Data obtained here suggest that DRD2 allelic variants may modulate a specific feature of polysubstance abuse, psychostimulant preference, rather than enhancing general vulnerability to compulsive drug use. These observations fit well with neurobiological data suggesting that molecular variants potentially implicated in mesolimbic/mesocortical dopaminergic function may indeed be more likely to modulate psychostimulant, rather than opiate use. In fact, lesions or pharmacological blockade of mesolimbic dopaminergic terminals in the rat nucleus accumbens selectively block psychostimulant, but not opiate self-administration (Pettit et al 1984; Vaccarino et al 1985; Pulvirenti et al 1992; for review see Kuhar et al 1991).

The DRD2 gene contains several polymorphic RFLPs, including *Taq*I A, located approximately 10 kb 5′ of the last exon, *Taq*I B, in the first intron near the junction with exon 2, and *Taq*I D, found in intron 2 near the junction with exon 3 (Grandy et al 1989; Hauge et al 1991; Parsian et al 1991). Different variants or alleles of the DRD2 gene may be present in the general population. In Caucasians, the *Taq*I A1, B1, and, to a lesser extent, D2 RFLPs appear to "mark" an allele of the DRD2 gene conferring vulnerability to preferential psychostimulant abuse in this study. Conceivably, this allele may be characterized by the presence of a putative mutation in linkage disequilibrium with the RFLP markers employed in our study (Persico et al 1993a). The stronger association found here with markers located in flanking regions of the DRD2 gene, the reported lack of mutations in exons of "A1-marked" DRD2 alleles (Gejman et al 1993), and the possible decreased DRD2 $B_{max}$ reported with presence of the A1 marker in the absence of $K_D$ changes (Noble et al 1991), each suggest that putative mutations located in 3′ or 5′ non-coding regions of the gene that were maintained in linkage disequilibrium with the polymorphic markers used here could plausibly influence DRD2 transcription and/or mRNA stability and thus affect DRD2 receptor number. The lack of association with *Taq*I D2 also fits with population genetic analyses demonstrating that, whereas *Taq*I A and B are in strong linkage disequilibrium with each other, the A1 3′ flanking marker displays less disequilibrium with *Taq*I D, whose weak association with psychostimulant preference might have therefore been anticipated (Persico et al 1993a; Suarez et al 1994).

Our data extend previous results, indicating that in several studies DRD2 *Taq*I A1 and B1 RFLPs were more commonly found in groups of drug abusers that were often selected on the basis of psychostimulant addiction (most significant results reported by Smith et al 1992; Commings et al 1993; Noble et al 1993, trend toward similar effect reported by Gelernter et al 1993b). Indeed, if the strength of the association between DRD2 and polysubstance abuse in different samples is in part dependent upon their clinical composition, some of the sample-to-sample variability noted in the literature would be anticipated.

Case-control association studies can potentially be undermined by other confounding variables, such as stratifi-

cation. Distinct racial and ethnic groups display significant differences in DRD2 allelic distributions (Barr and Kidd 1993; Gelernter et al 1993a; Goldman et al 1993; O'Hara et al 1993). Spurious false-positive or false-negative results may therefore derive from unequal racial and ethnic composition of the affected and control samples. To reduce possible contributions of stratification bias, we selected only Americans of largely European descent, because African-Americans differ significantly from Caucasian-Americans in allelic and genotype frequencies, as well as in haplotype frequencies and linkage disequilibrium at this locus (O'Hara et al 1993; Persico et al 1993a; Goldman et al 1993). We then excluded subjects reporting significant Hispanic or American Indian origin, because allelic and genotype frequencies in these groups have also been described as differing significantly from those found in Caucasians (Barr and Kidd 1993; Gelernter et al 1993a; Goldman et al 1993). No strong evidence for significant differences between American-Caucasians of distinct European origin at this locus currently exists, to our knowledge. Further, markers at two other gene loci that may exhibit such stratification fail to display associations with substance abuse vulnerability. Although preliminary assessments suggest that marker frequencies at the vesicular transporter locus (Surratt et al 1993) in Caucasian-Americans originating from different European countries show substantial heterogeneity, no association with substance abuse is evident. VNTR polymorphisms at the dopamine transporter (DAT1) gene locus differ significantly even between individuals belonging to distinct Caucasian ethnic groups (Table 4b). For alleles 9 and 10, Caucasian-Americans differ from Italians, ($\chi^2$ = 6.29, 1 df with Yates' correction, $p < 0.05$). Yet, this DAT1 gene marker shows no association with polysubstance abuse (Persico et al 1993b) or with preferential use of psychostimulants (present study). These results fail to provide evidence that stratification totally explains the observed association that we describe at the DRD2 locus.

In disorders with multiple genetic and environmental influences, single genes may be expected to exert modest impacts on diagnosis. Detection of single-gene contributions in polygenic disorders may therefore require assessments performed in patient subgroups, defined through hypothesis-driven selection criteria, and thresholds for statistical significance that do not discard small to moderate effect sizes. Sole attention, in such circumstances, to avoiding false-positive findings may substantially increase the risk of accepting false-negative results. The hypothesis-driven approach that we have adopted here suggested the single primary statistical comparison between psychostimulant-preferring drug users and controls that was employed in this work. The validity of this comparison appears to have been confirmed by the results of additional secondary comparisons among different subgroups of drug users and controls. However these results, as those of any single study, should be interpreted with caution as a body of work derived from independent clinical samples and well-chosen controls is completed. Other approaches, such as haplotype relative risk (Falk and Rubinstein 1987; Knapp et al 1993) and intrafamilial association (George and Elston 1987; Swift et al 1990; Hodge 1993) can complement the current approach, and provide additional controls for stratification. Limitations of haplotype relative risk, including unreliability in "control" gene marker frequencies in the presence of assortative mating or reduced reproductive rates and the power limitations that may make sample-size adjustments and precise targeting of clinical subpopulations even more critical than in classical case-control studies, should also be kept in mind (Knapp et al 1993).

Earlier onset of use of the opiate or psychostimulant drug preferentially used later in life by the same individual is evident from our data. Interestingly, age of first use of "any drugs", namely alcohol, tobacco or marijuana, displays no correlation with the extent of later polysubstance abuse. Early concomitant exposure to both psychostimulants and opiates occurs in most individuals destined to become heavy polysubstance users. Differences in drug availability are unlikely to be sufficiently widespread and/or to persist long enough to account entirely for these findings. Conceivably, craving-inducing stimuli might precipitate use of drugs which have previously "primed" the individual.

The specificity of the association between the DRD2 *Taq*I A1 RFLP and preferential psychostimulant use, the lack of similar association with DAT1 VNTR markers, and the compelling evidence for prominent interactions between psychostimulants and brain dopamine systems, all argue that dopamine $D_2$ receptor gene variants marked by specific polymorphisms may play a role in interindividual differences in vulnerability to psychostimulant abuse. These data also underscore the critical importance of phenotype definition in case-control studies of genetic influences on likely-polygenic neuropsychiatric disorders with large environmental influences.

This work was supported by the Intramural Research Program of the National Institute on Drug Abuse and by NIDA grant DA07110 to F.H.G.

The authors thank Mark Kinski and Anne Miretsky for technical assistance, Olivier Civelli for providing the λhD2G1 and λhD2G2 clones, Abbas Parsian for providing the phD2-244 clone, Jim Anthony and Angelo Serio for assistance with statistical analyses, and Luigi Pulvirenti for helpful discussions.

## References


Barr CL, Kidd KK (1993): Population frequencies of the A1 allele at the dopamine $D_2$ receptor locus. *Biol Psychiatry* 34:204–209.

Blum K, Noble EP, Sheridan PJ, et al (1990): Allelic association of human dopamine $D_2$ receptor gene in alcoholism. *JAMA* 263:2055–2060.

Cadoret RJ, O'Gorman T, Troughton E, et al (1987): An adoption study of genetic and environmental factors in drug abuse. *Arch Gen Psychiatry* 43:1131–1136.

Cohen J (1977): *Statistical Power Analysis for the Behavioral Sciences,* rev. ed., New York, NY: Academic Press.

Commings DE, MacMurray J, Johnson JP, et al (1993): The dopamine D2 receptor gene: A genetic risk factor in substance abuse. *Drug Alc Depend* 34:175–180.

Di Chiara G, Imperato A (1988): Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proc Natl Acad Sci USA* 85:5274–5278.

Falk CT, Rubinstein P (1987): Haplotype relative risk: An easy reliable way to construct a proper control sample for risk calculations. *Ann Hum Genet* 51:227–233.

Gejman PV, Ram A, Gelernter J, et al (1993): No structural mutation in the dopamine $D_2$ receptor gene in alcoholism or schizophrenia. *JAMA* 271:204–208.

Gelernter J, Goldman D, Risch N (1993a): The A1 allele at the $D_2$ dopamine receptor gene and alcoholism. *JAMA* 269:1673–1677.

Gelernter J, Kranzler H, Satel S (1993b): No association between DRD2 alleles and cocaine abuse. *College on Problems of Drug Dependence,* Toronto, Canada, annual meeting, June 16.

George VT, Elston RC (1987): Testing the association between polymorphic markers and quantitative traits in pedigrees. *Genet Epidemiol* 4:193–201.

Goldman D, Dean M, Brown GL, et al (1993): DRD2 dopamine receptor genotype, linkage disequilibrium and alcoholism in American Indians and other populations. *Alcohol Clin Exp Ther* 17:199–204.

Grandy DK, Litt M, Allen L, et al (1989): The human dopamine $D_2$ receptor gene is located on chromosome 11 at q22-q23 and identifies a *Taq*I RFLP. *Am J Hum Genet* 45:778–785.

Hauge XY, Grandy DK, Eubanks JH, et al (1991): Detection and characterization of additional DNA polymorphisms in the dopamine $D_2$ receptor gene. *Genomics* 10:527–530.

Hodge SE (1993): Linkage analysis versus association analysis: Distinguishing between two models that explain disease-marker associations. *Am J Hum Genet* 53:367–384.

Knapp M, Seuchter SA, Baur MP (1993): The Haplotype-Relative-Risk (HRR) method for analysis of association in nuclear families. *Am J Hum Genet* 52:1085–1093.

Kuhar MJ, Ritz MC, Boja JW (1991): The dopamine hypothesis of the reinforcing properties of cocaine. *Trends Neurosci* 14:299–302.

Li T, Yang L, Wiese C, et al (1994): No association between alleles or genotypes at the dopamine transporter gene and schizophrenia. *Psychiatry Res* 52:17–23.

Luthar SS, Anton SF, Merikangas KR, et al (1992): Vulnerability to substance abuse and psychopathology among siblings of opioid abusers. *J Nerv Ment Dis* 180:153–161.

Noble EP (1993): The D2 dopamine receptor gene: A review of association studies in alcoholism. *Behav Genet* 23:119–129.

Noble EP, Blum K, Khalsa ME, et al (1993): Allelic association of the $D_2$ dopamine receptor gene with cocaine dependence. *Drug Alcohol Depend* 33:271–285.

Noble EP, Blum K, Ritchie T, et al (1991): Allelic association of the $D_2$ dopamine receptor gene with receptor-binding characteristics in alcoholism. *Arch Gen Psychiatry* 48:648–654.

O'Hara BF, Smith SS, Bird G, et al (1993): Dopamine $D_2$ receptor RFLPs, haplotypes and their association with substance use in black and caucasian research volunteers. *Hum Hered* 43:209–218.

Parsian A, Fisher L, O'Malley KL, et al (1991): A new *Taq*I RFLP within intron 2 of human dopamine $D_2$ receptor gene (DRD2). *Nucleic Acids Res* 19:6977.

Pedersen NL (1984): Multivariate analysis of familial and non-familial influences for commonality in drug use. *Drug Alcohol Depend* 14:67–74.

Persico AM, Smith SS, Uhl GR (1993a): $D_2$ receptor gene variants and substance abuse liability. *Seminars Neurosci* 5:377–382.

Persico AM, Vandenbergh DJ, Smith SS, et al (1993b): Dopamine transporter gene markers are not associated with polysubstance abuse. *Biol Psychiatry* 34:265–267.

Persico AM, O'Hara BF, Farmer S, et al (1993c): Dopamine $D_2$ receptor gene *Taq*I "A" locus map including "A4" variant: relevance for alcoholism and drug abuse. *Drug Alcohol Depend* 31:229–234.

Pettit HO, Ettenberg A, Bloom FE, et al (1984): Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. *Psychopharmacology* 84:167–173.

Pickens RW, Svikis DS, McGue M, et al (1991): Heterogeneity in the inheritance of alcoholism: A study of male and female twins. *Arch Gen Psychiatry* 48:19–28.

Pulvirenti L, Maldonado R, Koob GF (1992): NMDA-receptors in the nucleus accumbens modulate intravenous cocaine but not heroin self-administration in the rat. *Brain Res* 594:327–330.

Regier DA, Farmer ME, Rae DS, et al (1990): Comorbidity of mental disorders with alcohol and other drug abuse. *JAMA* 264:2511–2518.

Sambrook J, Fritsch EF, Maniatis T (1989): *Molecular Cloning: A Laboratory Manual,* 2nd ed., Cold Spring Harbor, NY: Cold Spring Harbor Laboratory Press.

Sano A, Kondoh K, Kakimoto Y, Kondo I (1993): A 40-nucleotide repeat polymorphism in the human dopamine transporter gene. *Hum Genet* 91:405–406.

Smith SS, O'Hara BF, Persico AM, et al (1992): Genetic vulnerability to drug abuse: The dopamine $D_2$ receptor *Taq*I B1 RFLP appears more frequently in polysubstance abusers. *Arch Gen Psychiatry* 49:723–727.

Smith SS, Newman JP, Evans A, et al (1993): Comorbid

psychopathy is not associated with increased $D_2$ dopamine receptor *Taq*I A or B gene marker frequencies in incarcerated substance abusers. *Biol Psychiatry* 33:845–848.

Suarez BK, Parsian A, Hampe CL, et al (1994): Linkage disequilibria at the $D_2$ dopamine receptor locus (DRD2) in alcoholics and controls. *Genomics* 19:12–20.

Surratt CK, Persico AM, Yang XD, et al (1993): A human synaptic vesicle monoamine transporter cDNA predicts post-translational modifications, reveals chromosome 10 gene location and identifies *Taq*I RFLPs. *FEBS Lett* 318:325–330.

Swift M, Kupper LL, Chase CL (1990): Effective testing of gene-disease associations. *Am J Hum Genet* 47:266–274.

Uhl GR, Persico AM, Smith SS (1992): Current excitement with $D_2$ receptor gene alleles in substance abuse. *Arch Gen Psychiatry* 49:157–160.

Uhl GR, Blum K, Noble E, et al (1993): Substance abuse vulnerability and $D_2$ receptor genes. *Trends Neurosci* 16:83–88.

Uhl GR, Elmer G, Labuda M, Pickens RW (1995): Genetic influences in drug abuse. In Bloom F.E., and Kupfer F. (eds): *Psychopharmacology: The Fourth Generation of Progress,* New York, NY: Raven Press.

Vaccarino FJ, Bloom FE, Koob GF (1985): Blockage of nucleus accumbens opiate receptors attenuates intravenous heroin reward in the rat. *Psychopharmacology* 85:37–42.

Vandenbergh DJ, Persico AM, Hawkins AL, et al (1992): Human dopamine transporter gene maps to chromosome 5p15.3 and displays a VNTR. *Genomics* 14:1104–1106.

Wise RA, Rompre PP (1989): Brain dopamine and reward. *Annu Rev Psychol* 40:191–225.