# Dopamine Transporter Gene Polymorphisms Are Not Associated with Polysubstance Abuse

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## Introduction

Susceptibility to drug and alcohol abuse is presently thought to arise from a combination of biological, psychological, and social factors. Genetic contributions to substance use, abuse, and dependence are supported by family, twin, and adoption studies (Pedersen 1984; Cadoret et al 1987; Luthar et al 1992).

Most substances that are abused by humans can enhance dopamine activity in mesolimbic/mesocortical circuits important for behavioral reward and reinforcement (Di Chiara and Imperato 1988). Such neurobiological findings have stimulated interest in the possibility that interindividual differences in the structure or expression of genes of dopaminergic neurotransmission could contribute to individual differences in vulnerability to substance abuse. Interest is also spurred by evidence that variation at the dopamine $D_2$ receptor gene may help to determine such vulnerability (Blum et al 1990; Uhl et al 1992; Smith et al 1992). Although the effect of a single gene is unlikely to account for the entire genetic contribution to substance abuse vulnerability, these results lend credence to searches for possible involvement of other dopaminergic genes.

The dopamine transporter (DAT1) gene encodes a dopamine-cell–specific protein that is the direct target of cocaine action (Ritz et al 1987). DAT1 is thus a candidate gene for involvement in substance abuse vulnerability. We have recently cloned human DAT1 complementary DNAs, and identified a 5′ polymorphic restriction fragment length polymorphism (RFLP) (Vandenbergh et al 1992a) and a 3′ variable number tandem repeat (VNTR) marker for the human DAT1 gene locus (Vandenbergh et al 1992b). These advances now allow study of possible allelic association of these human DAT1 gene markers with substance abuse.

## Materials and Methods

A total of 234 subjects from the National Institute on Drug Abuse Addiction Research Center, an adjacent hemodialysis unit, and an adjacent public health facility studying HIV infections volunteered for this study under informed consent and confidentiality provisions. Subjects completed the Drug Use Survey interview, which quantifies the amount, frequency, and/or dollar cost at the time of lifetime peak use for each addictive substance, and/or the Diagnostic Interview Schedule (DIS-III-R) as described (Smith et al 1992).

Blood was obtained, DNA extracted as described (Smith et al 1992), and *Taq*I RFLP and VNTR analyses performed as described (Vandenbergh et al 1992a, Vandenbergh et al 1992b). Dopamine $D_2$ receptor *Taq*I RFLP status for these same individuals was extracted from data described for a larger number of volunteers by Smith et al (1992).

The ASSOCIATE program (Ott 1991) was used to assess Hardy-Weinberg equilibrium, to quantitate linkage disequilibrium between the RFLP and VNTR markers, and to estimate haplotype frequencies. $\chi^2$ tests with Yates' continuity corrections were used to test hypotheses of association between presence of an RFLP or a VNTR and either (1) moderate to heavy substance use assessed by the Drug Use Survey or (2) DSM-III-R lifetime diagnoses of either alcohol or drug abuse or dependence, as assessed by the DIS-III-R.

## Results

"Taq 492" reliably detects a diallelic pattern in Southern analyses of DNA fragments containing DAT1 genomic sequences from different individuals (Vandenbergh et al 1992a); polymerase chain reaction amplification of genomic DNA also produced fragments with sizes corresponding to three to 11 copies of the 40-base repetitive element in the DAT1 3′ untranslated region as previously described (Vandenbergh et al 1992b). The frequencies of the *Taq*I A1 and A2 fragments obtained here, 0.22 and 0.78 respectively, agree well with frequencies of 0.26 and 0.74 previously obtained in Caucasian population samples (Vandenbergh et al 1992a). The number and distribution of VNTR fragments also agree with prior results, with 9 and 10 copy fragment frequencies of 0.27 and 0.71 compared to 0.24 and 0.70 found by Vandenbergh et al (1992b); other copy number variants were found at much lower frequencies. The maximum likelihood estimates of haplotype frequencies display no significant departure from Hardy-Weinberg equilibrium and, in accordance with prior data (Vandenbergh et al 1992a), there was no evidence for linkage disequilibrium between the 5′ *Taq*I A RFLP and the 3′ VNTR

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Table 1. Dopamine Transporter Gene Markers in Polysubstance Abusers and Controls[a]

| | | *Drug users* | | |
|---|---|---|---|---|
| | Controls | Total | DSM-III-R | DUS |
| *Taq*I RFLP[b] | | | | |
| A1/A1 | 2.0 (1) | 3.3 (4) | 3.4 (2) | 3.2 (2) |
| A1/A2 | 43.1 (22) | 36.7 (44) | 36.2 (21) | 37.1 (23) |
| A2/A2 | 54.9 (28) | 60.0 (72) | 60.3 (35) | 59.7 (37) |
| VNTR[c] | | | | |
| 9/9 | 6.3 (3) | 7.1 (13) | 9.5 (8) | 5.1 (5) |
| 9/10 | 43.8 (21) | 42.6 (78) | 44.0 (37) | 41.4 (41) |
| 10/10 | 50.0 (24) | 50.3 (92) | 46.4 (39) | 53.5 (53) |

Values are percent (number).

[a]DUS, Drug Use Survey; RFLP, restriction fragment length polymorphism; VNTR, variable number tandem repeat.

[b]Fifty-one controls and 120 drug users.

[c]Forty-eight controls and 183 drug users.

9 and 10 copy markers ($D_{A1/10}$ = 0.0176; $\chi^2$ = 0.82, df = 1, $p$ = 0.36; $D_{A1/10}/D_{max}$ = 9.8%).

Neither VNTR nor RFLP marker frequencies were different between substance abusers and controls ($\chi^2$ = 0.02 and 0.32 respectively, df = 2). No trend toward association was evident in comparisons of subjects clinically characterized with either substantial peak lifetime substance use ($\chi^2$ = 0.04 and 0.20 respectively, df = 2) or with lifetime DSM-III-R substance abuse or dependence diagnoses ($\chi^2$ = 0.13 and 0.25, df = 2) when compared to control individuals with minimal lifetime substance use (Table 1).

## Discussion

This work tests the hypothesis that individual differences in substance abuse may, in part, be due to different alleles of the DAT1 gene. This hypothesis arises from robust evidence for interactions between abused drugs and brain dopamine systems. Furthermore, psychiatric genetic work using classic methods suggests that substance use, abuse, and dependence may show significant genetic determinants (Pedersen 1984; Cadoret et al 1987; Luthar et al 1992).

The polymorphic markers employed in this association analysis and the associated genomic segments of the DAT1 gene do not appear to provide major genetic determinants for polysubstance abuse vulnerability in our sample. Though our results exclude the involvement of these parts of the DAT1 locus, the lack of linkage disequilibrium between these 5′ RFLP and 3′ VNTR markers implies that significant areas of the gene could conceivably contribute to polydrug addiction vulnerability but escape detection with the markers employed here. Additional markers could exclude additional regions of the gene with enhanced confidence.

We have previously reported data concerning *Taq*I A1 and B1 markers of the dopamine $D_2$ receptor gene in each of the control individuals and in each of the substance abusers described in this report; the dopamine $D_2$ receptor data also reflect genotype determinations in approximately 50 additional substance abusers and five control individuals not studied in the current dopamine transporter sample (Smith et al 1992). Analyses of the dopamine $D_2$ receptor *Taq*I A1 and B1 allelic frequency data from the subset of individuals also genotyped for DAT markers reveals significant dopamine $D_2$ receptor marker frequency differences between control and substance abusers not found for the dopamine transporter polymorphisms (0.23 and 0.18 for drug users and 0.13 and 0.09 for controls; $\chi^2$ = 4.79, df = 2, $p < 0.05$; $\chi^2$ = 3.18, df = 1, $p < 0.05$, respectively). Thus, although the dopamine $D_2$ receptor polymorphism associations in substance abuse remain controversial, and current data concerning dopamine transporter associations are negative, population variants in specific dopaminergic genes could still conceivably contribute to individual differences in susceptibility to substance abuse (Uhl et al 1992, Uhl et al 1993, Gelernter et al 1993).

## References

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.

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 U S A* 85:5274–5278.

Gelernter J, Goldman D, Risch N (1993): The A1 allele at the D2 dopamine receptor gene and alcoholism—a reappraisal. *JAMA* 269:1673–1677.

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

Ott J (1991): *Analysis of Human Genetic Linkage*, revised. Baltimore, MD: Johns Hopkins University Press.

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

Ritz MC, Lamb RJ, Goldberg SR, et al (1987): Cocaine receptors on dopamine transporters are related to self-administration of cocaine. *Science* 237:1219–1223.

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.

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, Smith SS (1993): Substance abuse vulnerability and $D_2$ receptor genes. *Trends Neurosci* 16:83–88.

Vandenbergh DJ, Persico AM, Uhl GR (1992a): A human dopamine transporter cDNA predicts reduced glycosylation, displays a novel repetitive element and provides racially-dimorphic *TaqI* RFLPs. *Mol Brain Res* 15:161–166.

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