# ASSOCIATION STUDY OF DOPAMINE D2 AND D3 RECEPTOR GENE POLYMORPHISMS WITH COCAINE DEPENDENCE

Guilherme Messas [1,2], Ivanor Meira-Lima [1]; Marília Turchi [3]; Olavo Franco [3]; Adauto Castelo[3], Ronaldo Laranjeira [2] and Homero Vallada [1*]

[1]Neuroscience Laboratory (LIM-27), Department of Psychiatry, University of São Paulo School of Medicine, Brazil;

[2]UNIAD (Unit of Drug and Alcohol Research), Department of Psychiatry, Federal University of São Paulo, Brazil;

[3]Department of Medicine, Federal University of Sao Paulo, Brazil.

**Running title**: Dopamine receptor genes and Cocaine

*** Correspondence:**
H Vallada, MD, PhD
Laboratory of Neuroscience,
Department of Psychiatry,
University of São Paulo School of Medicine
Rua Dr. Ovídio Pires de Campos s/n
05403-010 São Paulo – S.P.
Brazil
Email: hvallada@usp.br
Tel./Fax.: ++ 55 11 3069 7129

**Abstract**

Genetic factors play a role in the vulnerability to cocaine dependence. The reinforcing properties of cocaine are related with the dopaminergic system, and in particular the dopamine receptors have been linked to the reward mechanisms. The present study examines the role of the variants *Taq*I A of the dopamine D2 receptor gene (DRD2) and *Bal*I of the dopamine D3 receptor gene (DRD3) in a Brazilian sample consisting of 730 cocaine dependents and 782 healthy controls. The studied polymorphisms did not show any difference in allelic frequencies or genotypic distribution between the groups. Our data does not support a role for the DRD2 *Taq*I A and DRD3 *Bal*I gene polymorphisms in the susceptibility to cocaine dependence in Brazilian subjects.

**Keywords**: Genetics, dopamine receptor, addiction, gene polymorphism

## Introduction

Family and twin studies suggest a substantial genetic component in the vulnerability of individuals to become dependent after exposed to cocaine (Merikangas et al, 1998; Bierut et al, 1998; Kendler & Prescott, 1998). The reinforcing properties of cocaine are related with the dopaminergic system, and in particular the dopamine receptors have been implicated in "reward" mechanisms (Comings & Blum, 2000; Noble EP, 2000). The effect of neurotransmitter interaction at the mesolimbic brain region induces a "reward" when dopamine is released from the neuron at the nucleus accumbens and interacts with a dopamine receptor. A dopaminergic mechanism may therefore be responsible for interindividual differences in the susceptibility to developing cocaine dependence. In the context of a molecular genetic approach to this issue, candidate genes related to this vulnerability include the dopamine receptor genes.

Considerable attention has been focused on a possible relationship of $D_2$ dopamine receptor (DRD2) gene polymorphisms with alcohol and drug dependence. Numerous studies have reported an allelic association between the A1 allele at the *TaqI* "A" restriction fragment length polymorphism at DRD2 and alcoholism, (Blum et al, 1990; Comings et al, 1991; Arinami et al, 1993, Pato et al, 1993). Cocaine dependence and alcoholism share a somewhat overlapping genetic risk (Cadoret et al 1995) and, considering a stronger involvement of dopamine in the effects of cocaine than in the alcohol, the findings of an association of DRD2 alleles with alcoholism suggests that a similar association might also be detected with cocaine dependence. Smith et al (1992) reported an increased frequency of the DRD2 alleles in polysubstance abusers compared to controls. Noble et al (1993) reported strong association between the DRD2 gene polymorphisms and cocaine-abusing subjects. Comings et al (1994) reported an association between the DRD2 *TaqI* A1 allele and drug and alcohol dependence, they also reported significant associations with measures of severity of substance

dependence. Comings et al (1996) reported that the same allele is also a risk factor for cigarette smoking. Persico et al (1996) demonstrated an association of the DRD2 *TaqI* A1 allele and (cocaine or amphetamine) psychostimulant-preferring polysubstance abusers.

The dopamine receptor sub-type 3 (DRD3), which is preferentially expressed in limbic areas, is also related to rewarding behaviour and could therefore be a plausible candidate gene for the vulnerability to develop cocaine dependence (Diaz et al, 1995). Evidence from animal models has suggested a role of this receptor in cocaine abuse. Nader & Mach (1996) have shown that the self-administration by rhesus monkeys of dopamine D3 agonist 7-OH DPAT changes if animals are priorily exposed to cocaine. Xu (1998) and Garner & Baker (1999) have observed that DRD3 modulates animal responses to psychostimulants, including cocaine, and Pilla et al. (1999) have stressed the importance of this receptor in cocaine-induced craving. Human post-mortem studies also found similar results, showing a two-fold higher density of this receptor in cocaine overdose victims (Segal et al, 1997; Mash & Staley, 1999), indicating that neuroadaptations in DRD3 receptor could be involved in the vulnerability to cocaine dependence.

The investigations of DRD3 gene variations in drug dependence are concentrated to studies using a *Bal I* (*MscI*) restriction polymorphism, that has been described by Lannfelt et al (1992), within the first exon of DRD3 gene resulting in a serine to glycine change at amino acid 9. Freimer et al. (1998) reported a non-significant trend for an increase in homozygosity frequency of this variant in cocaine dependents compared to controls; Duaux et al. (1998) could not find differences in frequencies of *BalI* DRD3 polymorphism in a population of opiate dependents. However, the authors found an excess of homozygosity in a sub-sample of the group, containing patients with a higher sensation-seeking score (Zückerman scale), indicating that the genetic vulnerability could be transmitted through this temperamental trait. Krebs et al.(1998) described an association between homozygosity of same polymorphism

and a population of schizophrenics with substance abuse. Finally, Comings et al. (1999) observed a reduction in heterozygosis frequency in cocaine dependents when compared to controls ($p<0.028$). This reduction is still more pronounced if the analysis considers sub-samples using cocaine for more than 10 or 15 years.

In the light of these findings, the DRD2 *TaqI* A and the DRD3 *BalI* (ser9gly) polymorphisms were analysed, in a population-based association study, to investigate whether these variants increase the susceptibility to cocaine dependence in a Brazilian sample.

## Methods

### Subjects

Subjects in this study came from seven distinct units dedicated to the treatment of drug dependence in São Paulo, Brazil. Six of them are inpatients units and only one of them is exclusively an outpatient unit, however only the severe cases form this outpatient unit were included in the study. All patients above 17 years old attending these units from August 1997 to October 1998 were selected, excluding those with a history of exclusive alcohol dependence or psychotic symptomatology at the moment of the interview. A written informed consent was obtained from each patient.

All patients were submitted to an interview based on a questionnaire developed and validated for the Brazilian population (Dunn & Laranjeira, 1999). A diagnostic of cocaine dependence was based on previous clinical records and on the data from the questionnaire. Seven hundred and thirty cocaine dependents were included in this study, aged between 17-46 years (mean age 26.7 years, SD ± 7.2), of which 95.6% were male and 65.3% Caucasians.

Controls

Controls were recruited from the Blood Unit of Hospital das Clinicas, from the Faculty of Medicine – University of São Paulo. Each blood donor is submitted to a questionnaire investigating contagious diseases and the use of any kind of drug. Subjects showing a past history of drug abuse or having a recent use of substance of potential abuse were excluded. During the act of donation a short interview was conducted (by GPM and DJ), excluding subjects with a history of a lifetime psychiatric inward treatment or suffering from a psychiatric condition in the moment of the interview. All these subjects agreed in taking part in the study, and signed an informed consent. Seven hundred and eighty one subjects, aged between 18-60 (mean age 31.7, SD ± 10), 69.2% male and 50.3% Caucasians, were included as healthy controls.

Genotyping

Genomic DNA was extracted through standard methods from blood samples collected in tubes containing EDTA.

DRD2 *TaqI* A polymorphism: Genotyping was carried out using PCR-based restriction analysis described by Grandy et al. (1993) with primers 5'-CCGTCGACGGCTGGCCAAGTTGCTCTA and 5'-CCGTCGACCCTTCCTGAGTGTCATCA. PCR products (310 bp) were digested using *TaqI* A with the A1 allele uncut and the A2 allele cut into 130-bp and 180-bp fragments.

DRD3 *Bal I* polymorphism (ser-9-gly): PCR was performed as described (Lannfelt et al., 1992). The PCR primer sequences used were 5'-GCTCTATCTCCAACTCTCACA and 5'-AAGTCTACTCACCCTCCAGGTTA. The 462-bp products were digested with *MscI*, an

isoschizomer of *BalI*, resulting in products of either 304-bp (allele 1 or Ser9) or 206-bp and 98-bp (allele 2 or Gly9). Constant bands of 47-bp and 11-bp were also produced.

The digested products were separated by electrophoresis in a 2% agarose gel and identified by U.V. light using ethidium bromide.

Statistics

Allelic and genotypic distribution of the variants studied among patients and control groups were analysed with Pearson's Chi-square test and a multivariate logistic regression implemented in the statistical package SPSS for windows version 10.0. A test for deviations from the Hardy-Weinberg equilibrium was performed using the HWE program (Ott J, 1999). For all statistic tests the level of significance adopted was $\alpha <. 5$, or 5%.

**Results**

Genotypic distribution of the DRD2 *TaqI* A and of the DRD3 *BalI* polymorphism were consistent with a Hardy-Weinberg equilibrium. The allelic frequencies and genotypic distribution of DRD2 and DRD3 gene polymorphism in Cocaine dependents and controls subjects are shown in table 1.

There was no significant difference observed in the frequency of allele and genotype distribution between patients and control groups for DRD2 gene (genotype: $X^2$=0.08, 2DF, p=0.9; and allele:$X^2$=0.01, 1df, p=0.9) and for DRD3 gene (genotype:$X^2$=0.4, 2df, p=0.8; and allele: $X^2$=0.0, 1df; p=0.9).

Logistic regression models were created considering a dominant and a recessive mechanism of action for the studied polymorphism. We also investigated a model comparing the frequency of heterozygotes between dependents vs. controls. All these models were

controlled for age, sex and ethinicity. However, there was no association between Cocaine dependents and the studied genes polymorphisms.

## Discussion

Dopamine is one of the most important neurotransmitters involved in the rewards pathways in the brain, which is thought to be crucial for reinforcing properties of cocaine and other substances of abuse (Comings & Blum, 2000). We thus, investigated the role of polymorphisms of the D2 and D3 receptors genes in cocaine dependence. Our data do not support an allelic association between DRD2 *TaqI* A or DRD3 *BalI* alleles and cocaine dependence, in Brazilian subjects.

However, if the drug availability within a population is facilitated, it may increase the number of drug abuser or dependents, and therefore include the ones with a less pronounced genetic vulnerability, diluting a possible genetic effects. This population heterogeneity could even be responsible for the lack of consistency among the association studies found in the literature (Noble et al, 1993; Comings et al, 1999). Nevertheless, our data is consistent with previous studies which have shown no effect of DRD2 and DRD3 alleles on phenotype cocaine-dependence (O'Hara et al, 1993; Berrettini et al, 1996; Freimer et al, 1998, Gelernter et al, 1999).

Population stratification could also be a potential explanation for the present negative findings; indeed, there is a large variation of DRD2 allele frequencies depending on the population group sampled (Goldman et al, 1993; O'Hara et al, 1993; Castiglione et al, 1995). However, we addressed this issue by adjusting for age, gender, and ethnicity in our logistic regression analysis. In addition, we analysed only a sub group of our sample consisting of male and Caucasian subjects and we also failed to demonstrate an association (data not show).

We concluded that the studied genetic variant in the D2 and D3 receptor do not increase the vulnerability to develop cocaine dependence in our sample. However, it is still possible that a sub-group of the cocaine dependents presenting one specific temperamental trait could be associated with this polymorphism. A better definition of phenotypic categories linked to genetic functional variations is needed for such scientific studies.

**Acknowledgements**

This work was supported by FAPESP (Fundação para o Amparo à Pesquisa do Estado de São Paulo), Grant number 99/04678-2.

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**Table 1**. Genotypic and allelic frequencies for the DRD2 *TaqI* A and DRD3 *BalI* polymorphisms in Cocaine dependents and Controls subjects

| Variable | Cocaine dependents n = 730 | Subjects Controls n = 781 |
|---|---|---|
| **DRD2 TaqI Alleles*** | | |
| A1 | 414 (0.28) | 441 (0.28) |
| A2 | 1046 (0.72) | 1121 (0.72) |
| Genotype[t] | | |
| A1/A1 | 60 (0.08) | 66 (0.08) |
| A1/A2 | 294 (0.40) | 309 (0.40) |
| A2/A2 | 376 (0.52) | 406 (0.52) |
| **DRD3 BalI Alleles[#]** | | |
| 1 | 774 (0.53) | 828 (053.) |
| 2 | 686 (0.47) | 734 (047.) |
| Genotypes[§] | | |
| 1–1 | 212 (0.29) | 233 (0.30) |
| 1–2 | 350 (0.48) | 362 (0.46) |
| 2–2 | 168 (0.23) | 186 (0.24) |

*$X^2$ = 0.01, 1df, p = 0.9
[t]$X^2$ = 0.08, 2df, p=0.9
[#]$X^2$=0.0, 1df, p=0.9
[§]$X^2$=0.4, 2df, p=0.8