Association study of dopamine D2 and D3 receptor gene polymorphisms with cocaine dependence

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Genetic factors play a role in the vulnerability to cocaine dependence. The reinforcing properties of cocaine are related to 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 and *Bal*I of the dopamine D3 receptor gene 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 do not support a role for the dopamine D2 receptor gene *Taq*I A and dopamine D3 receptor gene *Bal*I gene polymorphisms in the susceptibility to cocaine dependence in a Brazilian sample.
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Introduction

Family and twin studies suggest a substantial genetic component in the vulnerability of individuals to become dependent after being exposed to cocaine (Bierut *et al.*, 1998; Kendler and Prescott, 1998; Merikangas *et al.*, 1998). The reinforcing properties of cocaine are related to the dopaminergic system, and, in particular, the dopamine receptors have been implicated in reward mechanisms (Comings and Blum, 2000; Noble, 2000, 2003). 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 D2 dopamine receptor (DRD2) gene polymorphisms with alcohol and drug dependence. Numerous studies have reported an allelic association between the A1 allele at the *Taq*I '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 alcohol, the findings of an association of DRD2 alleles with alcoholism suggest that a similar association might also be detected with cocaine dependence. Smith *et al.* (1992) reported an increased frequency of DRD2 alleles in polysubstance abusers compared with controls. Noble *et al.* (1993) reported a strong association between the DRD2 gene polymorphisms and cocaine-abusing study participants. Comings *et al.* (1994) reported an association between the DRD2 *Taq*I 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 *Taq*I 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 and Mach (1996) have shown that the self-administration by rhesus monkeys of dopamine D3 agonist 7-OH DPAT changes if animals are previously exposed to cocaine. Xu (1998) and Garner and Baker (1999) have observed that DRD3 modulates animal responses to psychostimulants, including cocaine, and Pilla *et al.* (1999) have stressed the importance of this

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receptor in cocaine-induced craving. Human post-mortem studies also found similar results, showing a twofold higher density of this receptor in cocaine overdose victims (Segal *et al.*, 1997; Mash and Staley, 1999), indicating that neuroadaptations in the DRD3 receptor could be involved in the vulnerability to cocaine dependence.

The investigations of DRD3 gene variations in drug dependence have concentrated on studies using a *Bal*I (*Msc*I) restriction polymorphism, which has been described by Lannfelt *et al.* (1992), within the first exon of the 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 with controls. Duaux *et al.* (1998) could not find differences in frequencies of the *Bal*I DRD3 polymorphism in a population of opiate dependents. However, the authors found an excess of homozygosity in a subsample of the group containing patients with a higher sensation-seeking score (Zückerman scale), indicating that the genetic vulnerability could be associated with this subgroup of dependents. Krebs *et al.* (1998) described an association between homozygosity of the same polymorphism and a population of schizophrenics with substance abuse. Finally, Comings *et al.* (1999) observed a reduction in heterozygosity frequency in cocaine dependents when compared with controls ($P < 0.028$). This reduction is still more pronounced if the analysis considers subsamples of those using cocaine for more than 10 or 15 years.

In the light of these findings, the DRD2 *Taq*I A and the DRD3 *Bal*I (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

Study participants

Participants in this study came from seven distinct units dedicated to the treatment of drug dependence in São Paulo, Brazil. Six of them are inpatient units and only one of them is exclusively an outpatient unit; however, only the severe cases from this outpatient unit were included in the study. All patients aged over 17 years 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 and Laranjeira, 1999). A diagnostic of cocaine dependence was based on previous clinical records and on the data from the questionnaire. A total of 730 cocaine dependents were included in this study, aged between 17 and 46 years (mean age: 26.7 years, SD ± 7.2), of whom 95.6% were male. The whole group had the following ethnic makeup: 65.3% Caucasians, 21% black, 12.7% mulatto and 1% Asians.

Controls

Controls were recruited from the Blood Unit of Hospital das Clinicas, Faculty of Medicine, University of São Paulo. Each blood donor completed a questionnaire investigating contagious diseases and the use of any kind of drug. Participants showing a past history of drug abuse or having a recent use of a substance of potential abuse were excluded. During blood donation, a short interview was conducted, excluding participants with a history of a lifetime psychiatric inward treatment or those suffering from a psychiatric condition at the moment of the interview. All these participants agreed to take part in the study, and signed an informed consent. A total of 781 participants, aged between 18 and 60 (mean age: 31.7 years, SD ± 10), 69.2% male, were included as controls. The whole group had the following ethnic makeup: 50.3% Caucasian, 39.9% mulatto, 8.8% black and 1% Asians.

Genotyping

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

Dopamine D2 receptor gene TaqI A polymorphism

Genotyping was carried out using polymerase chain reaction (PCR)-based restriction analysis described by Grandy *et al.* (1993) with primers 5′-CCGTCGACGGC TGGCCAAGTTGCTCTA and 5′-CCGTCGACCCTT CCTGAGTGTCATCA. PCR products (310 bp) were digested using *Taq*I A with the A1 allele uncut and the A2 allele cut into 130-bp and 180-bp fragments.

Dopamine D3 receptor gene BalI polymorphism (Ser9Gly)

PCR was performed as described (Lannfelt *et al.*, 1992). PCR primer sequences used were 5′-GCTCTATCTC-CAACTCTCACA and 5′-AAGTCTACTCACCCTCCAG GTTA. The 362-bp products were digested with *Msc*I, an isoschizomer of *Bal*I, resulting in products of either 304 bp (allele 1 or Ser9) or 206 and 98 bp (allele 2 or Gly9). Constant bands of 47 and 11 bp were also produced.

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

Statistics

Allelic and genotypic distribution of the variants studied among patients and control groups were analysed with

Pearson's χ^2 test and a multivariate logistic regression implemented in the statistical package SPSS for Windows, version 10.0 (SPSS Inc., Chicago, Illinois, USA). A test for deviations from the Hardy–Weinberg equilibrium (HWE) was performed using the HWE program (Ott, 1999). For all statistic tests, the level of significance adopted was $\alpha < 0.5$ or 5%.

Results

Genotypic distribution of the DRD2 *Taq*I A and the DRD3 *Bal*I polymorphisms were consistent with a Hardy–Weinberg equilibrium. The allelic frequencies and genotypic distribution of DRD2 and DRD3 gene polymorphisms in Cocaine dependents and controls are shown in Table 1.

No significant difference was observed in the frequency of allele and genotype distribution between patients and control groups for DRD2 gene (genotype: $\chi^2 = 0.08$, df = 2, $P = 0.9$; allele: $\chi^2 = 0.01$, df = 1, $P = 0.9$) and for DRD3 gene (genotype: $\chi^2 = 0.4$, df = 2, $P = 0.8$; allele: $\chi^2 = 0.0$, df = 1, $P = 0.9$). We also analysed a subgroup comprising only male Caucasian patients ($n = 438$) compared with a similarly stratified subgroup of controls ($n = 252$) and the association was also negative (DRD2 genotype: $\chi^2 = 2$, df = 2, $P = 0.35$; DRD2 allele: $\chi^2 = 2$, df = 1, $P = 0.15$, and DRD3 genotype: $\chi^2 = 0.72$, df = 2, $P = 0.69$; DRD3 allele: $\chi^2 = 0.05$, df = 1, $P = 0.82$).

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 and controls. All these models were controlled for age, sex and ethnicity. However, no association was found between cocaine dependents and the studied gene polymorphisms.

Table 1 Genotypic and allelic frequencies for the dopamine D2 receptor gene (DRD2) *Taq*I A and dopamine D3 receptor gene (DRD3) *Bal*I polymorphisms in cocaine dependents and controls

Variable	Cocaine dependents $n=730$	Controls $n=781$
DRD2 TaqI alleles[a]		
A1	414 (0.28)	441 (0.28)
A2	1046 (0.72)	1121 (0.72)
Genotype[b]		
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[c]		
1	774 (0.53)	828 (0.53)
2	686 (0.47)	734 (0.47)
Genotypes[d]		
1–1	212 (0.29)	233 (0.30)
1–2	350 (0.48)	362 (0.46)
2–2	168 (0.23)	186 (0.24)

[a]$\chi^2=0.01$, df=1, P=0.9; [b]$\chi^2=0.08$, df=2, $P=0.9$; [c]$\chi^2=0.01$, df=1, $P=0.9$; [d]$\chi^2=0.4$, df=2, $P=0.8$.

Discussion

Dopamine is one of the most important neurotransmitters involved in the rewards pathways in the brain, and is thought to be crucial for reinforcing properties of cocaine and other substances of abuse (Comings and 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 *Taq*I A or DRD3 *Bal*I alleles and cocaine dependence in a Brazilian sample. Cocaine increases synaptic availability of dopamine by blocking the dopamine transporter. Therefore, the gene coding for dopamine transporter protein (DAT1) is also a good candidate gene to include in our further genetic investigations.

However, if the drug availability within a population is facilitated, it may increase the number of drug abusers or dependents, and therefore include the ones with a less pronounced genetic vulnerability, diluting any 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 are consistent with previous studies, which have shown no association of DRD2 and DRD3 alleles with the phenotype of cocaine dependence (O'Hara *et al.*, 1993; Berrettini and Persico, 1996; Freimer *et al.*, 1998, Gelernter *et al.*, 1999).

Population stratification could also be a potential explanation for the present negative findings; indeed, a large variation of DRD2 allele frequencies is found, 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, sex and ethnicity in our logistic regression analysis. In addition, we have also analysed a subgroup of our sample consisting of only male and Caucasian participants, and we similarly failed to demonstrate an association. It is important to stress that considering a frequency of 25% for the allele A1 of DRD2 gene polymorphism in the population, this subgroup of male Caucasians had more than 95% power to detect an association that confers an odds ratio of 2.0 to develop cocaine dependence.

We concluded that the studied genetic variants in the D2 and D3 receptors do not increase the vulnerability to develop cocaine dependence in our sample; however, it is still possible that a subgroup of 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.

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