Association study between dopamine D3 receptor gene polymorphism and cocaine dependents

GP Messas (1,2); IV Meira-Lima (1); D Jardim (1);E Ikenaga (1);MD Turchi (3);
OF Franco (3); A Castelo (3); RR Laranjeira (2); H Vallada (1)

(1) Neuroscience Laboratory (LIM-27), Department of Psychiatry, School of Medicine, University of São Paulo, Brazil;
(2) UNIAD (Unity of Drug and Alcohol Research), Paulista School of Medicine, Federal University of São Paulo, Brazil;
(3) Department of Infection Diseases, Paulista School of Medicine, Federal University of Sao Paulo, Brazil.

Abstract

Previous investigations have shown that dopamine receptor sub-type 3 (DRD3) may be involved in the vulnerability to cocaine dependence. The present study investigates the Gly-9-Ser polymorphism of the DRD3 gene in 718 cocaine dependents and 782 healthy controls. No allelic or genotypic differences were found between the groups, when analyzing the whole sample. However, when subdividing the sample according to a maximum risk group of cocaine dependents (n=414), i.e. history of early onset of cocaine use and imprisonment records, an association with homozygosis was found ($p<0.001$). We concluded that the homozygosity of Gly-9-Ser polymorphism in the DRD3 gene may increase the vulnerability to develop cocaine dependence.

Keywords: cocaine dependence; dopamine receptor sub-type 3 gene (DRD3); association study; homozygosity

Cocaine affects particularly the dopaminergic system in the brain. The dopamine receptor sub-type 3 (DRD3), which is preferentially expressed in limbic areas, is associated with rewarding behaviour and could therefore be a plausible candidate gene for the vulnerability to develop cocaine dependence (1). Evidence from animal models has confirmed the role of this receptor in cocaine abuse. Nader & Mach (2) have shown that the self-administration by rhesus monkeys of dopamine D3 agonist 7-OH DPAT changes if animals are priorly exposed to cocaine. Xu (3) and Garner & Baker (4) have observed that DRD3 modulates animal responses to psychostimulants, including cocaine, and Pilla et al. (5) 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 (6;7), indicating that neuroadaptations in DRD3 receptor could be involved in the vulnerability to cocaine dependence (8).

A substitution of adenine for guanine in the first nucleotide of codon 9 of the gene encoding DRD3, determines a substitution of serine by glycine (Ser-9-Gly) (9). This site variation can be cleaved by the endonucleoase Ban I when the guanine is present. Although functional differences linked to this polymorphism have not been shown in human populations, an investigation in Chinese hamster ovary cells line found that the glycine allele has significantly higher affinity for dopamine than the serine allele (10). Studies about the role of this polymorphism in SNC function remain controversial. However, two results from schizophrenia studies could be informative about differential roles for Ser-9-Gly DRD3 polymorphism. A meta-analysis conducted by Dubertret et al. (11) showed a significant association between homozygosity and schizophrenics in Caucasians; Asherson et al. (12) found a positive association between homozygosity and schizophrenia only in males. These

results indicate a possible sex and ethnic effect for this polymorphism, at least in schizophrenia.

The investigations of Ser-9-Gly DRD3 polymorphism in drug dependence is still confined to a few studies. Parsian et al. (13) found no association between this polymorphism and alcohol dependence; Freimer et al. (14) reported a non-significant trend for an increase in homozygosity frequency in 124 cocaine dependents compared to 90 controls; Duaux et al. (15) could not find differences in frequencies of Ser-9-Gly DRD3 polymorphism and a population of opiate dependents. However, the authors found an excess of homozygosity in a sub-sample of the group, containing patients with a sensation-seeking score (from the Zückerman scale) above 24, indicating that the genetic vulnerability could be transmitted through this temperamental trait. Krebs et al. (16) also describe an association between homozygosity and a population of 36 schizophrenics with concomitant substance abuse. Comings et al. (17) studied 47 cocaine dependents and 305 controls and observed a reduction in heterozygosis frequency in dependents (29.8%) when compared to controls (46.9%) ($p<0.028$). This reduction is still more pronounced if analysis considers sub-samples using cocaine for more than 10 (25%) or 15 years (21.5%). Finally, Thome et al. (18) found an excess of Ser9 allele (allele 1) in a sample of alcohol dependents.

Some other studies, not directly investigating drug dependence, also reached similar associations between homozygosity and Gilles de la Tourette (19) and gambling (20). As impulsive conditions seem to be related to vulnerability to cocaine dependence (21), these results could reinforce the hypothesis of a role for this polymorphism.

In the light of these findings, the Gly-9-Ser polymorphism in the DRD3 gene was investigated for cocaine dependence in a case-control study design, with especial focus on its homozygosity.

Seven hundred and eighteen cocaine dependents and 782 healthy controls were included, in a total of 1500 subjects. The frequencies of the alleles or the genotypes of Ser-9-Gly DRD3 polymorphism in both groups does not differ significantly, as shown in t**able 1**. However, there is some statistical significance between cocaine dependent and control groups in terms of age ($p<0.001$), ethnicity ($p<0.001$) and sex ($p<0.001$). A logistic regression analysis involving these parameters and the genotypes or the alleles of Gly-9-Ser DRD3 polymorphism was also negative.

A further statistical analysis sub-dividing the sample in the ones with a previous history of imprisonment and age at beginning of cocaine use under 18 (median age of beginning) was performed. This sub-group, named maximum risk group, may have a genetic predisposition for impulsive behaviour. The statistical analysis of the maximum risk group (n = 414 subjects) presented a significant association between cocaine dependence and homozygosity (OR=1.912; CI=1.452-2.518; $p<0.001$; r2=0.025). No association between the alleles and cocaine dependence was found. Finally, a logistic regression for this analysis was performed, where the association between cocaine dependence and homozygosity was maintained (OR=1.796; CI=1.302-2.477; $p<0.001$).

We investigated the role of Gly-9-Ser polymorphism in DRD3 gene in cocaine dependence. The results of the analysis involving the whole sample do not support it as a susceptibility locus to develop this disorder. However, if the drug availability within a population is facilitated, it may increases the number of drug abuser or dependents, and therefore including the ones with a less pronounced genetic vulnerability, diluting possible genetic effects. This population heterogeneity could even be responsible for the lack of

consistency in association studies found in the literature (23). Determining more homogeneous sub-groups in terms of biological risk could be a strategy for tackling the problem. One of the conditions apparently linked to the vulnerability to cocaine dependence is behavioral desinhibition (21). Behavioral desinhibition has been shown to play an important role in the prediction of early drug abuse (24), indicating a possible vulnerability for this population. To test the hypothesis of an association between Ser-9Gly DRD3 polymorphism and behavioral desinhibition, we selected from the original cocaine dependence group those ones with a lifetime history of imprisonment and early beginning of cocaine use (under 18 years old – median age). Our results may support the hypothesis of the inheritability of desinhibition traits. We observed association between homozygosity and a sub-group of cocaine dependents (i.e. maximum risk group) at a level of significance of $p<0.001$, suggesting that the homozygosity of Ser-9-Gly polymorphism in cocaine dependence could be accounted for temperamental traits linked to disinihibition and not as a directly transmitted factor (genetic effect).

Other results in the literature reinforces this view. Comings et al (17) not only found an association between homozygosity and cocaine dependence but also reported a stronger association in a sub-sample with ten years of cocaine use, as well as the strongest association in a sub-group of fifteen years of use. Duaux et al. (15) found association with homozyosity in a sub-group of opiate dependents with a high score of sensation seeking. Comings et al (19; 20) reported associations between homozygosity and impulsive behaviors, like pathological gambling or Tourette syndrome. Although none of these conditions could be taken exclusively as behavioral desinhibition, all of them reveal a background of behavioral extroversion. The description of a behavioral facilitatory system, modulator of externalizing behaviors (aggressive and sexual), integrated in mesolimbic dopaminergic system (25) strengthens the value of investigating the hypothesis of a relationship between this

polymorphism and behavioral extraversion. Funcional differences in a dopaminergic-linked behavioral facilitatory system, due to homozygosity of Bal I polymorphism, could elevate the risk of developing several psychiatric conditions, including cocaine dependence. Environmental and developmental factors, in this hypothesis, would lead genetic load to one or another phenotypic expression. Our study did not investigate directly this hypothesis. However, our results, when taken together with the others shown above, may give an important hint for understanding genetic vulnerability to cocaine dependence as well as the search for relevant vulnerability intermediate phenotypes.

The contribution of homozygosity to cocaine dependence-maximum risk group is significant but small. Only 2.5% of the variance was due to homozygosity, a value close to 1.65% found by Comings et al (17), indicating that, regardless of population heterogeneity, only small effects can be expected to be found for this polymorphism.

We concluded that homozygosity for Ser-9-Gly polymorphism may increase the vulnerability to develop cocaine dependence, at least in specific sub-groups. In addition, behavioral disinhibition seems to be the trait responsible for mediating the genetic inheritance. A better definition of phenotypic categories strictly linked to genetic functional variations is urgently needed for scientific development. Instead of using clinically based categories (which is generally a complex behavior, like cocaine dependence, involving a web of gene-environmental interactions) researchers should look for biologically rooted categories, more prone to reveal genetic susceptibilities. Behavioral desinhibition, as an expression of a dimension of extroversion, emerges as a phenotypic candidate for future designs in the search for vulnerabilities in cocaine dependence.

Methods

Subjects

Subjects in this study came from seven distinct units dedicated to 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, being excluded 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 to the Brazilian population (26), where information about age of beginning and intensity of use of cocaine was registered. History of lifetime imprisonment was also recorded. Diagnostic of cocaine dependence was based in previous clinical records and from the data from the questionnaire. Seven hundred and eighteen cocaine dependents, aged between 17-46 years (mean age 26.7 years, SD ± 7.2), 95.7% were male and 70.5% were Caucasians.

Controls

Controls were recruited in Blood Unit of Hospital das Clinicas, from 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 are not allowed concluding donation. During the act of donation a short interview (conducted by GPM and by DJ) was conducted, 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 of the study, and signed an informed consent. Seven hundred and eighty four healthy control subjects, aged between 18-60 (mean age 31.7, SD ± 10), 69.7% were male and 50.3% were Caucasians.

Genotyping

Genomic DNA was extracted through standard methods from blood samples collected in tubes containing EDTA and amplified by polymerase chain reaction (PCR). PCR was realized in a total volume of 25 µl, containing 40-100 ng of genomic DNA, 12.5 pM of each primer, buffer with 2mM of MgCl 2, 10 mM of Tris, 125 mM of each dNTP, 1.25 µl of DMSO 5% and 1U of Taq polymerase. Primer sequences were 5' GCTCTATCTCCAACTCTCACA 3' (sense) and 5' AAGTCTACTCACCTCCAGGTA 3'(antisense). Samples were pretreated at 95 C for 5 minutes, followed by 30 cycles of denaturation at 95 C for 30 seconds, annealing at 56 C for 40 minutes and extension at 72 C for 40 seconds and a final extension at 72 C for 10 minutes. The PCR products were digested overnight with Ban I restriction enzymes, following the instructions of the manufacturer. The digested products were separated by electrophoresis in a 2% agarose gel and identified by U.V. light using ethidium bromide.

Statistics

Analysis investigating associations between allelic and genotypic polymorphism and phenotypes were conducted in two steps. A Pearson's chi-square test followed by a univariate regression analysis, from which the P and r2 values were calculated. For controlling all variables simultaneously a logistic regression analysis was also performed. For all statistic tests the level of significance adopted was $\alpha<.5$, or 5%. In addition, the Hardy-Weinberg equilibrium for the distribution of genotypes was estimated using HWE program (Ott, 1970).

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Table 1: Allelic and genotypic distribution of DRD3-*Bal I* polymorphism in 718 cocaine dependents and 782 controls.

	Genotypes			P value
	Gly/Gly	Gly/Ser	Ser/Ser	
Cocaine dependence	207(28.8%)	345(48.1%)	166(23.1%)	
				0.8
Controls	233(29.8%)	362(46.3%)	187(23.9%)	

	Allele		P value
	Gly	Ser	
Cocaine dependents	759	677	
			0.8
Controls	828	736	