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Title: The role of COMT functional and promoter polymorphisms in cocaine addiction: analysis of a large Brazilian case control sample

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Corresponding Author: Dr Homero Vallada, MD, PhD

Corresponding Author's Institution: University of Sao Paulo Medical School (ProGene - LIM23)

First Author: Nadia S Cunha, Pharmacist

Order of Authors: Nadia S Cunha, Pharmacist; Camila S Guindalini, PhD; Quirino Cordeiro, MD; Guilherme Messas, MD, PhD; Ronaldo Laranjeira, MD, PhD; David Collier, PhD; Gerome Breen, PhD; Homero Vallada, MD, PhD

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the sample. This study suggests that genetic variation in COMT might play a role in development of cocaine addiction in the Brazilian population.

The role of *COMT* functional and promoter polymorphisms in cocaine addiction: analysis of a large Brazilian case control sample

Nádia Sousa Cunha¹, Camila Guindalini^{1,2}, Quirino Cordeiro¹, Guilherme Messas³,
Ronaldo Laranjeira³, David Collier², Jerome Breen², Homero Vallada^{1,2}

¹ Department of Psychiatry, University of Sao Paulo Medical School (ProGene, LIM23),
Brazil;

² Section of Molecular Genetics, Social Genetic and Developmental Psychiatry Centre
and Division of Psychological Medicine, Institute of Psychiatry, London, UK;

³ UNIAD (Unit of Drug and Alcohol Research, Department of Psychiatry, Federal
University of São Paulo, Brazil);

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Correspondence should be addressed to:

Homero Vallada

Instituto de Psiquiatria do Hospital das Clínicas

Faculdade de Medicina da Universidade de São Paulo

CEP: 05403-010

Sao Paulo-SP, Brasil

Phone/Fax: ++ 55 11 3069-7129

E-mail: hvallada@usp.br

Abstract

Genetic factors play a significant role in the development of cocaine addiction. Catechol-O-methyltransferase (COMT) is a major enzyme for the metabolism of dopamine, the neurotransmitter most directly related to the reinforcing properties of cocaine. In the present study, we genotyped three COMT SNPs [Val 108/158 Met (rs4680), rs737865, and rs165599] in a Brazilian sample comprised of 711 cocaine dependents and 862 healthy controls. Significant case-control differences were not observed for any individual SNP. Haplotype analyses showed that the haplotype comprised of alleles G-A-A of markers rs737865, rs4680 and rs165599, respectively, was over-represented in the cases (4%) compared to the controls (2%) ($z=2.99$; $p=0.02$). Moreover, when the markers rs737865/rs4680 were analyzed separately, the haplotype containing only alleles G-A was also significantly associated with cocaine abuse ($z=3.46$; $p=0.006$). 71 markers for genetic admixture analyses were also performed in the whole sample, and no difference was observed in case and controls, indicating that there is no significant population stratification in the sample. This study suggests that genetic variation in COMT might play a role in development of cocaine addiction in the Brazilian population.

Keywords

COMT, cocaine addiction, SNPs, polymorphism

Introduction

Cocaine addiction is a complex behaviour which probably arising from the interaction of genetic and environmental risk factors. Twins studies indicate that the heritability of cocaine addiction is around 80% and fits a complex polygenic model (Kendler & Prescott 1998; Kendler et al 2000). Cocaine potently binds the dopamine transporter and this blockade of dopamine reuptake is perhaps the key factor leading to cocaine addiction (Horowitz et al, 2000). Outside reuptake, the most important mechanism for the termination of dopamine is its metabolic degradation (Tao et al 2004), which is performed by Catechol-O-Methyltransferase (COMT) and the monoamine oxidases.

COMT catalyses the O-methylation of catecholamine neurotransmitters and catechol hormones including dopamine, thereby inactivating them (Saito et al 2001). The COMT gene (*COMT*) is located chromosome 22q11, and encodes two COMT isoforms, one soluble (S-COMT) and the other membrane-bound (MB-COMT) which has 50 additional C-terminal amino acids (Mannisto et al 1999). Lotta et al (1995) identified a G->A polymorphism in exon 4 (rs4680), which causes a Val->Met change at aa position 108 in S-COMT or 158 in MB-COMT. Met homozygotes have low enzyme activity and thermolability versus Val/Val carriers, and heterozygotes display intermediate levels (Saito et al 2001). In addition, Saito et al (2001) described 33 single nucleotide polymorphisms (SNPs) in the COMT gene, which were distributed in the 5' flanking region, introns, exons and in the 3' flanking region. SNPs in intron 1 (rs737865) and in the 3' flanking region (rs165599) have been reported to contribute to

allelic expression differences and to be associated with other psychiatric disorders (Chen et al 2004).

Several studies have examined the Val/Met polymorphism and psychiatric phenotypes, such as alcoholism, bipolar disorder, obsessive-compulsive disorder and schizophrenia (Craddock et al 2006). Although COMT plays an important role in dopaminergic pathway involved in drug reward, no genetic studies examining the influence of Val/Met polymorphism or any other variant in this gene in cocaine addiction has been conducted to date. To evaluate the role of *COMT* in cocaine addiction, we conducted an association study using three *COMT* SNPs [Val 108/158 Met (rs4680), rs737865 and rs165599] in ~700 cocaine abusers and ~850 controls subject from São Paulo in south east Brazil.

Materials and Methods

Clinical

Patients

Subjects were recruited from inpatient and outpatient units for the treatment of drug dependence in São Paulo, Brazil. All patients above 17 years old attending these units from August 1997 to October 1998 were selected, excluding those with a history of psychotic symptoms at the moment of the interview. The diagnosis was given based on data collected using a questionnaire designed for the Brazilian population (Dunn & Laranjeira 2000). Seven hundred and eleven (n=711) cocaine dependents were included

in this study, aged between 17-56 years (mean age 26.7 years, SD \pm 7.2), of which 95.8% were male (Guindalini et al 2006).

Controls

Controls were recruited from the Blood Unit of Hospital das Clinicas (School of Medicine – University of São Paulo). Exclusions were a past history of drug abuse or recent use of abusive substance, a history of psychiatric inpatient treatment or a current psychiatric condition. Eight hundred and thirty nine (n=862) subjects, aged between 18-60 (mean age 31.6, SD \pm 7.2), 68.3% male, were included as healthy controls. All participants provided written informed consent and were subject to a brief screening interview. Ethical approval for the study was obtained from the Ethics Committee at the Hospital das Clinicas, University of São Paulo Medical School (CAPPesq).

Genotyping

COMT polymorphisms

Genomic DNA was extracted through standard methods from blood samples collected in tubes containing EDTA. We selected the SNPs Val 108/158 Met (rs4680), rs737865 and rs165599 in the *COMT* for this investigation. Genotyping of these SNPs was performed blind to clinical status using an amplifluor assay and under contract by K-Biosciences (Cambridge, UK; <http://www.kbioscience.co.uk/>).

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Markers for Population Stratification Analysis

In order to detect the presence of genetic stratification in our sample, we selected a total of 71 (64 SNPs and 7 microsatellites) ancestry informative markers, e.g. markers that exhibit large allele frequency differences among the three main Brazilian ancestral populations (Europeans, Africans and Native American). The genotyping of SNPs selected for this study was performed by Kbiosciences as before (14 SNPs) or blind to status using allele-specific PCR with molecular beacons (Myakishev et al 2001) and under contract by Prevention Genetics (Marshfield, USA; <http://www.preventiongenetics.com>) (50 SNPs). Names, primers and conditions for the microsatellites used can be obtained on request. Briefly, they were genotyped using fluorescently labelled primers and an ABI3100 capillary electrophoresis system (Guindalini 2006).

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Statistical Analysis

We analyzed Hardy-Weinberg equilibrium using the HWE program (Ott 1988). The SPSS (v 13.0) software was used to calculate χ^2 test to analyze the SNPs distributions between the samples of cases and controls. Logistic regression analysis was used to derive odds ratio. In addition, pair-wise LD was calculated using LD pairs program from GC utilities and GENECOUNTING software v2.0 was used to estimate haplotype frequencies (Zhao et al 2002). The effect of population stratification was tested using the ADMIXMAP program (McKeigue et al 2000, McKeigue, 2005).

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Results

All polymorphisms were in Hardy-Weinberg equilibrium in cases and controls. No significant case-control differences were found with respect to genotypes or allelic distribution for any SNP (Table 1).

Pairwise linkage disequilibrium analyses demonstrated significant linkage disequilibrium between markers rs737865 and rs4680 ($D'=0.56$; $p<0.0001$) and markers rs4680 and rs165599 ($D'=0.71$; $p<0.0001$) but not between markers rs737865 and rs165599 ($D'=0.05$; $p<0.05$). Haplotype analyses for the three markers, and for each pairwise combination were also conducted. Overall, no significant association was observed between cocaine addiction and the three marker haplotype ($\chi^2=11.8$; $df=7$; $p>0.05$) (Table2) and the rs4680/rs165599 haplotype ($\chi^2=4.7$; $df=3$; $p>0.05$). However, the overall test for the haplotypes containing the markers rs737865/rs4680 demonstrated marginal evidence for association ($\chi^2=7.56$; $df=3$; $p<0.05$). Individual haplotype analyses showed that the haplotype comprised of alleles G-A-A of markers rs737865, rs4680 and rs165599, respectively, was over-represented in the cases (4%) compared to the controls (2%) ($z=2.99$; $p=0.02$) (Table2). Moreover, when the markers rs737865/rs4680 were analyzed separately, the haplotype containing only alleles G-A was also significantly associated with cocaine abuse ($z=3.46$; $p=0.006$).

The program ADMIXMAP was used to correct the association tests for the presence of population stratification in the sample. The score test calculated by ADMIXMAP verifies the association of the outcome variable with the alleles at each locus, adjusting for age, sex and admixture; by averaging over the posterior distribution

of individual admixture proportions as estimated using the observed ancestry informative marker genotypes. The score tests did not provide evidence for association between cocaine addiction and any individual SNP markers, after correction for admixture (data not shown).

Discussion

Cocaine addiction is likely to be a result of complex interplay between genetic and environmental factors (Uhl et al 1995). COMT is important in dopamine metabolism in the brain it has been show that COMT may be differentially expressed in cocaine dependents. We examined the association of three genetic polymorphisms encoding COMT and cocaine addiction. This is the first association study between the val/met SNP, rs165599, or rs737865 in a cocaine addiction cases and controls.

Significant evidence for an association between the individual markers and cocaine addiction was not detected. These results differ from other published studies which have examined the functional polymorphism Val 108/158 Met (rs4680) association with other drugs. Li et al (2004), Horowitz et al (2000), Vandenberg et al (1997) all reported a higher frequency of the high activity Val allele, in populations of methamphetamine, heroin and polysubstance abusers, respectively. The low activity Met allele has also been associated with alcoholism in a family based approach (Wang et al 2001) and in a type 1 alcoholic population from Finland (Tiihonen et al 1999).

On the other hand, our haplotype analyses showed an overall association between the haplotypes of SNPs rs737865/rs4680 and cocaine addiction and an over-representation of the G-A-A (rs737865-rs4680-rs165599) and G-A (rs737865-rs4680)

haplotypes in the case group. This represents a different haplotype than that reported by Shifman et al (2002) as being associated with schizophrenia risk, the GGG haplotype.

Population stratification is a major concern and one of the most cited reasons for conflicting results in genetic association studies, in particular when an admixed population is under study (Spielman & Ewens 1996; McKeigue et al 2000). The presence of population stratification may lead to increase rates of false-positive and/or false-negative results. However, the occurrence of population stratification was evaluated in our sample and correction for its effect did not affect the association test results, suggesting that a false negative or positive result due to this is unlikely.

In conclusion, we found some evidence for associations between the G-A-A haplotype of SNPs rs737865-rs4680-rs165599 and between the G-A haplotype (rs737865-rs4680) and cocaine addiction, suggesting that genetic variation in the COMT gene may play a role in development of cocaine addiction in the Brazilian population, although these were associations with small effect sizes and will require replication. In addition, we did not find any evidence suggesting that the aal08/aal58 VAL/MET functional polymorphism (rs4680), on its own, can influence risk for cocaine addiction and dependence.

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	Genotype-wise								Allele-wise				
		AA		AG		GG			A		G		
rs737865	Controls	493	59%	290	35%	53	6%	p=0.11	1276	76%	396	24%	p=0.0001
	Cases	385	55%	278	40%	37	5%		1048	75%	352	25%	
rs4680		GG		GA		AA			G		A		
	Controls	296	35%	417	50%	124	15%	p=0.55	1009	60%	248	40%	p=0.0001
	Cases	257	37%	330	47%	113	16%		844	60%	226	40%	
rs165599		AA		AG		GG			A		G		
	Controls	257	31%	399	48%	173	21%	p=0.77	913	55%	346	45%	p=0.0001
	Cases	210	31%	324	47%	154	22%		744	54%	308	46%	

Table 1: Genotypic and allelic distribution in cases and controls

ID	Haplotype			Cases	Controls	Emp p
1	A	G	A	10.4%	11.1%	0.602
2	A	G	G	29.1%	27.7%	0.403
3	A	A	A	30.4%	30.9%	0.769
4	A	A	G	5.0%	6.6%	0.128
5	G	G	A	9.0%	10.8%	0.158
6	G	G	G	11.8%	10.7%	0.400
7	G	A	A	4.0%	2.2%	0.025
8	G	A	G	0.3%	0.0%	0.117

Table 2: Estimated commonest haplotype frequencies among cases and unrelated controls. Id : haplotype identifier; Cases: haplotype frequency estimates from cases; Controls: haplotype frequency estimates from controls; Emp p: empirical p value

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All author names (in order of appearance on the manuscript title page): Nádia Sousa Cunha, Camile Guindalini, Quirino Cordeiro, Guilherme Messas, Ronaldo Laranjeira, David Collier, ~~Marcelo~~ Jerome Braun, Homero Vallada

Corresponding author name: Homero Vallada

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Author Name (Printed)	Author Signature	Date
1. <u>Nádia Sousa Cunha</u>	<u>[Signature]</u>	<u>27th October 2006</u>
2. <u>Camile Guindalini</u>	<u>[Signature]</u>	<u>27th October 2006</u>
3. <u>Quirino Cordeiro</u>	<u>[Signature]</u>	<u>27 Oct</u>
4. <u>Guilherme Messas</u>	<u>[Signature]</u>	<u>27 Oct</u>
5. <u>Ronaldo Laranjeira</u>	<u>[Signature]</u>	<u>27 Oct</u>
6. <u>David Collier</u>	<u>[Signature]</u>	<u>27 Oct</u>
7. <u>Jerome Braun</u>	<u>[Signature]</u>	<u>27 Oct</u>
8. <u>Homero Vallada</u>	<u>[Signature]</u>	<u>26th October 2006</u>
9. _____	_____	_____
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