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Brief report

Lack of association between the LPR and VNTR polymorphisms of the serotonin transporter gene and cocaine dependence in a Spanish sample



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ABSTRACT

We genotyped the LPR and VNTR polymorphisms of the serotonin transporter gene in 504 cocaine-dependent patients and 508 controls. No association was detected with either polymorphism or with any haplotype combination. This study provided no evidence that these polymorphisms confer susceptibility to cocaine dependence in our sample.

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1. Introduction

Cocaine dependence is a complex psychiatric disorder with both genetic and environmental factors involved. Different lines of investigation support a role for the serotonergic system in drug dependence. Cocaine blocks the serotonin reuptake from the synaptic cleft through its binding to the serotonin transporter 5HTT, which increases the level of this neurotransmitter at the neuronal synapses (Filip et al., 2005). Interestingly, cocaine rewarding effects were only abolished in mice lacking the dopamine transporter *DAT* and at least one copy of *5HTT*, and chronic cocaine self-administration is associated with increased cortical

5HTT availability (Filip et al., 2005; Gould et al., 2011). Since altered 5-HT transmission is thought to increase susceptibility to dependence, polymorphisms in the *5HTT* gene may contribute to the individual's risk for addiction, disease evolution and treatment response. The most studied functional polymorphisms of the *5HTT* gene are the 5HTT-LPR (serotonin-transporter-linked polymorphic region) at the promoter region, which contains 14 (short, S) or 16 (long, L) copies of a 22–23 bp repeat element, and the 5HTT-VNTR (Variable Number of Tandem Repeats) in intron 2, with four variants containing 9–12 repeats of a 16–17 bp unit (9R–12R). Several studies have focused on these variants and reported no association with cocaine dependence, response to treatment or 5HTT levels in platelets (Patkar et al., 2001, 2002, 2004; Mannelli et al., 2005). Although clinical implications of *5HTT* polymorphisms in cocaine dependence are still unknown, postmortem brain studies showed that the serotonin transporter binding was lower in the dorsal raphe of cocaine users and reported an effect of the LPR genotype on both serotonin transporter binding and mRNA

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levels (Little et al., 1998). Several studies also showed that the LPR polymorphism is consistently associated with alcohol dependence, effect that increased in individuals with psychiatric comorbidities and early onset (reviewed by Herman and Balogh, 2012). However, studies performed in nicotine and opioid dependence have displayed conflicting results (Herman and Balogh, 2012).

Interestingly, Vasiliou et al. (2012) have recently demonstrated *in vitro* that 5HTT-LPR and 5HTT-VNTR modulate the 5HTT transcription in response to cocaine by altering the binding of different transcription factors and inducing chromatin modifications. Gene reporter experiments showed that the LPR-VNTR haplotypes (allele combination in the same chromosome) S-12R and L-10R increased by two or six fold, respectively, the basal transcription levels in the presence of cocaine *in vitro*.

Under the hypothesis that haplotype combinations of these two 5HTT polymorphisms, not investigated to date, may alter the expression of the serotonin transporter after cocaine exposure and, therefore, contribute to the genetic susceptibility to cocaine dependence, we performed a case-control association study in an ethnically homogeneous sample of 504 Spanish Caucasian cocaine-dependent patients and 508 sex-matched unrelated controls. In line with previous studies, we also assessed the presence/absence of psychotic symptoms as well as the time between initial consumption and dependence onset, with the background idea of reducing heterogeneity of the sample (Cubells et al., 2000; Fernandez-Castillo et al., 2012)

2. Methods

A total of 504 cocaine dependent patients (35.2 ± 7.9 years; 80% males ($n=403$)) were recruited and evaluated according to the *Diagnostic and Statistical Manual of Mental Disorders* (DSM-IV TR) criteria for cocaine dependence at the Drug Unit of the Psychiatry Department of the Hospital Universitari Vall d'Hebron (Barcelona, Spain) and the Mental Health division of the Fundació Althaia de Manresa (Barcelona, Spain). Other drug dependences were assessed in 460 patients (91.3%): alcohol dependence was present in 27.4% of the patients, cannabis dependence in 27.8%, opiate dependence in 18.9%, benzodiazepine dependence in 7.2%, amphetamine or methamphetamine dependence in 1.7% and comorbid dependence was absent in 47.4%. Eighty-nine percent of the patients were evaluated for the presence ($n=263$) or absence ($n=187$) of psychotic symptoms, and 87% ($n=442$) reported age at initial consumption and age at dependence onset. The control sample consisted of 508 unrelated blood donors (48.9 ± 17.4 years;

79.9% males ($n=406$)) recruited at the Blood and Tissues Bank of the Hospital Universitari Vall d'Hebron (Barcelona, Spain). They were negative for intravenous drug use, but data on cocaine use was not available. Patients and controls were Spanish, Caucasian and sex-matched. Population stratification was previously discarded (Fernandez-Castillo et al., 2013).

Genomic DNA was extracted from peripheral blood lymphocytes using the salting-out method (Miller et al., 1988). The 5HTT-LPR polymorphism was amplified by PCR (primers: 5'-GGCGTTGCCGCTCTGAATGC-3' and 5'-GAGGGACTGAGCTGGA-CAACCAC-3'), and fragment sizes were determined by agarose gel electrophoresis. The 5HTT-VNTR polymorphism was amplified by PCR (primers: 5'-[6FAM]-GTCAG-TATCACAGGCTGGCAG-3' and 5'-TGTTCCTAGTCTTACGCCAGT-3'), and the different alleles were called with the ABI PRISM 310 Genetic Analyzer and the Peak Scanner Software Version 1.0 (Applied Biosystems, California, USA).

Hardy-Weinberg equilibrium (HWE) was assessed using the HWE software (<http://www.biology.ualberta.ca/jbrzusto/hwenj.html>). Genotype, allele and haplotype frequencies were compared between cases and controls using the Fisher's exact test with the SPSS v15.0 software (SPSS Inc., Chicago, Illinois, USA). Genotypes and alleles with frequencies < 0.05 were collapsed in a single class. We also assessed the association between the two variations and comorbid alcohol, opiate or cannabis dependence, the presence of psychotic symptoms as well as the time between initial consumption and dependence onset. *P*-values were adjusted by age using logistic regression models. Haplotypes were assigned to individuals with the PHASE 2.0 software (Stephens et al., 2001).

3. Results

The two 5HTT sequence variations were in HWE in both cases and controls (Table 1a). The comparison of genotype and allele distributions for the two sequence variations did not show significant differences between cocaine-dependent patients and controls ($p > 0.05$; Table 1a), not even when the clinical sample was subdivided according to the presence or absence of psychotic symptoms, comorbid alcohol, opiate or cannabis dependence or when considering the time between initial consumption and dependence onset (data not shown).

Since a functional effect in cultured cells in the presence of cocaine was previously described for the S-12R and L-10R allelic combinations (Vasiliou et al., 2012), we further evaluated association between these specific haplotypes and cocaine dependence. However, no evidence for an overrepresentation of any of these allelic combinations was detected in our sample (Table 1b). No differences were observed neither in the presence or absence of psychotic symptoms, comorbid dependence to other drugs or

Table 1
(a) Genotypic and allelic distributions of the 5HTT-LPR and 5HTT-VNTR polymorphisms of the 5HTT gene in 504 cocaine-dependent patients and 508 controls and (b) distribution of the 5HTT-LPR-VNTR haplotypes.

Polymorphism	HWE		Genotypes				Alleles							
	Cases <i>P</i>	Controls <i>P</i>	Cases <i>N</i> (%)			Controls <i>N</i> (%)			<i>P</i>	<i>P</i> -adj				
(a)														
5HTT-LPR	0.13	0.37	LL 137 (27.2)	LS 235 (46.6)	SS 132 (26.2)	LL 147 (28.9)	LS 243 (47.8)	SS 118 (23.2)	0.54	0.48	0.31	0.23		
5HTT-VNTR	0.58	0.21	10R/10R 58 (11.5)	10R/12R 206 (40.9)	12R/12R 224 (44.4)	Others ^a 16 (3.2)	10R/10R 64 (12.6)	10R/12R 200 (39.4)	12R/12R 230 (45.3)	Others ^a 14 (2.8)	0.91	0.55	0.93	0.41
			Cases <i>N</i> (%)				Controls <i>N</i> (%)				<i>P</i>	<i>P</i> -adj		
(b) 5HTT-LPR-VNTR Haplotype														
L-10R			L-10R carriers 228 (46.7)		Non carriers 260 (53.3)		L-10R carriers 272 (44.9)		Non carriers 272 (55.1)		0.61	0.98		
S-12R			S-12R carriers 334 (68.4)		Non carriers 154 (31.6)		S-12R carriers 330 (66.8)		Non carriers 164 (33.2)		0.59	0.35		
L-10R+S12R			L-10R or S-12R carriers 449 (92.0)		Non carriers 39 (8.0)		L-10R or S-12R carriers 442 (89.5)		Non carriers 52 (10.5)		0.19	0.16		

HWE: Hardy-Weinberg Equilibrium; LPR: Linked Polymorphic Region; VNTR: Variable Number of Tandem Repeats. *P*-adj: *p*-value adjusted by age.

^a Genotypes and alleles with frequencies under 0.05 were grouped in a single class.

when we considered the time between initial consumption and dependence onset (data not shown).

4. Discussion

It is well established that the serotonergic system, and more specifically the serotonin transporter (5HTT), plays an important role in cocaine dependence (Filip et al., 2005). Studies performed in knockout mice showed that 5HTT modulates cocaine rewarding and adverse effects (Filip et al., 2005).

In addition, a recent *in vitro* study demonstrated that cocaine treatment results in differential binding of transcription factors and epigenetic variations that correlate with specific genotypes of the 5HTT-LPR and 5HTT-VNTR polymorphisms at the serotonin transporter gene (5HTT). This work also showed that certain combinations of these two polymorphisms seem to increase the expression of the gene after cocaine exposure (Vasiliou et al., 2012). Based on this study, we hypothesized that haplotype combinations of these two polymorphisms, not investigated yet, could contribute to the susceptibility to cocaine dependence by altering the expression of the serotonin transporter after cocaine exposure. Thus, we performed a case-control association study in a Caucasian sample of cocaine-dependent patients and healthy controls. This study provides evidence for the lack of association between 5HTT polymorphisms and cocaine dependence or related phenotypes, such as cocaine-induced psychotic symptoms, comorbid dependence to other drugs or the time between initial consumption and dependence onset. To our knowledge, this is the first association study in cocaine dependence that considers the combination of the 5HTT-LPR and 5HTT-VNTR polymorphisms and is in agreement with previous studies that evaluated these variations separately in small cohorts (Patkar et al., 2001, 2002). Other studies also obtained negative results when testing the involvement of these two polymorphisms in the response to pharmacological treatment in cocaine-dependent individuals, or in the 5HTT levels in platelets (Patkar et al., 2004; Mannelli et al., 2005). Thus, in line with previous reports, the present study does not support the contribution of these two sequence variants to cocaine dependence and, thus, their clinical relevance. However, additional polymorphisms within the 5HTT gene may warrant further investigation. In this regard, Enoch et al. (2010) assessed a SNP within the 5HTTLPR-L variant (L_A and L_C) and identified a significant association between cocaine and/or heroin dependence and the low 5HTT-LPR activity genotype group (SS, SL_C , $L_C L_C$) (Enoch et al., 2010). Since we did not consider this SNP in our analysis, we cannot rule out that this sequence variation may account for the absence of association reported in the present study. Further studies considering this polymorphism are required to discard the involvement of 5HTT-LPR, alone or in combination with the 5HTT-VNTR variation, in cocaine dependence.

In conclusion, our results do not support association between the 5HTT-LPR and 5HTT-VNTR polymorphisms of the 5HTT gene, encoding the serotonin transporter, and cocaine dependence.

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