

Association study between the *DAT1*, *DBH* and *DRD2* genes and cocaine dependence in a Spanish sample

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Drug addiction is a complex neuropsychiatric disorder involving the environmental and genetic factors. Genetic and physiological evidences suggest that the dopaminergic system may play an important role in cocaine abuse and dependence. Several association studies have focused on dopaminergic genes. We genotyped the Int8 and 3'UTR variable number of tandem repeats of the dopamine transporter gene (*DAT1/SLC6A3*), the *TaqIA* (rs1800497) and *TaqIB* (rs1079597) SNP polymorphisms within the dopamine receptor D2 gene and the 19-bp insertion/deletion and c.444G>A (rs1108580) polymorphisms of the dopamine β -hydroxylase gene (*DBH*) in a Spanish sample of 169 patients with cocaine addiction and 169 sex-matched controls. The case-control study showed a nominal overrepresentation of the 5R/5R genotype of the Int8 variable number of tandem repeats within *DAT1* in cocaine abusers ($P=0.016$). However, no significant associations were detected when *DAT1* haplotype frequencies or polymorphisms within the other

dopaminergic genes were considered. Sample size is limited and further studies should be performed in a larger cohort. *Psychiatr Genet* 20:317–320 © 2010 Wolters Kluwer Health | Lippincott Williams & Wilkins.

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Introduction

Cocaine addiction is a complex psychiatric disorder that results from the interaction of different genetic and environmental factors. The dopaminergic system plays an important role in drug addiction. Cocaine blocks the dopamine transporter (*DAT1*) (Volkow *et al.*, 1996) and this binding causes an increase of dopamine in the synapse that results in stimulation of the reward system and reinforcement (Volkow *et al.*, 1999, 2002). In addition, according to 'the reward deficiency syndrome' hypothesis (Comings and Blum, 2000), high dopamine reuptake or high levels of dopamine degradation, as well as low density of dopamine receptors, could predispose to cocaine addiction.

In this regard, several association studies in cocaine dependence have focused on dopaminergic genes, such as genes encoding *DAT1* (Persico *et al.*, 1993; Gelernter *et al.*, 1994; Guindalini *et al.*, 2006; Ballon *et al.*, 2007), the dopamine receptor D2 (*DRD2*) (Noble *et al.*, 1993; Persico *et al.*, 1996; Gelernter *et al.*, 1999; Messas *et al.*, 2005; Ballon *et al.*, 2007), *DRD3* (Freimer *et al.*, 1996; Comings *et al.*, 1999; Messas *et al.*, 2005; Ballon *et al.*, 2007; Bloch *et al.*, 2009) and *DRD4* (Ballon *et al.*, 2007) and dopamine β hydroxylase (*DBH*) (Cubells *et al.*, 2000; Kalayasiri *et al.*, 2007; Guindalini *et al.*, 2008), and displayed conflicting results.

We aimed to study several polymorphisms in *DAT1* [two variable number of tandem repeats (VNTRs) in the 3' untranslated region (3'UTR) and in intron 8], *DRD2* [*TaqIA* and *TaqIB* single nucleotide polymorphisms (SNPs) in 3'UTR and in intron 1, respectively] and *DBH* (19-bp insertion/deletion in 5'UTR and c.444G>A in exon 2) in an ethnically homogeneous sample of 169 Spanish Caucasian cocaine-dependent patients and 169 sex-matched unrelated healthy controls.

Materials and methods

The patient sample consisted of 169 cocaine-dependent patients [mean age 37 ± 7 years and 84% males ($n = 142$)] recruited and evaluated at the Drugs Unit of the Hospital Universitari Vall d'Hebron (Barcelona, Spain) according to *Diagnostic and Statistical Manual of Mental Disorders*, Fourth Edition Text Revision criteria. One hundred and sixty-nine sex-matched unrelated controls (mean age 39 ± 9 years) were obtained at the Blood and Tissues Bank of the Hospital Universitari Vall d'Hebron. All of them were non-smoker blood donors that had never injected drugs intravenously. Both patients and controls were Spanish and Caucasian, with the last names of the parents being of Spanish origin. Genomic DNA was extracted from peripheral blood lymphocytes using the *salting-out* method (Miller *et al.*, 1988).

Forty and 30-bp VNTRs in the 3'UTR and in intron 8 of the *DAT1* gene were genotyped as described earlier (Qian *et al.*, 2004; Brookes *et al.*, 2006). Genotyping of the *TaqIA* (rs1800497) and *TaqIB* (rs1079597) SNPs within the *DRD2* gene as well as the 19-bp insertion/deletion and the c.444G > A (rs1108580) SNP of the *DBH* gene has also been described earlier (Tan *et al.*, 2003; Yamamoto *et al.*, 2003; respectively).

Hardy–Weinberg equilibrium (HWE) was assessed for all genotypes using the HWE software (www2.biology.ualberta.ca/jbrzustolhwenj.html). Genetic Power Calculator (pugu.mgh.harvard.edu/~purcell/gpc) was used to estimate the statistical power of the sample assuming an odds ratio (OR) of 1.5, a disease prevalence of 0.03, a calculated average Minor Allele Frequency of 0.27 and a significance level of 0.05. Genotype frequencies under a codominant model and allele frequencies were compared between cases and controls using the Fisher's exact test. Genotypes and alleles of VNTRs with a frequency under 0.05 were grouped in a single class. OR and confidence intervals (CI) were estimated using SPSS v14.0 (SPSS Inc., Chicago, Illinois, USA). Logistic regression was used to adjust by age. The significance threshold was set at 2 *P* value of less than 0.0042 after the multiple comparison correction of Bonferroni considering genotype and allele comparisons for six different polymorphisms. When a nominal association was found, genotypes under recessive and dominant models were also compared. Haplotypes were estimated using the UNPHASED software (homepages.lshrm.ac.uk/frankdudbridge/software/unphased/).

Results

All the studied polymorphisms were in HWE in both cases and controls (Table 1). The minimal statistical power for the χ^2 test in our sample was 48%. When we

compared genotype and allele distributions of the *DAT1*, *DRD2* and *DBH* polymorphic variants between cocaine dependence patients and controls, no significant differences were detected for the *DRD2* and *DBH* genes (Table 1). Instead, a nominal association was identified under a codominant model for the Int8 VNTR in *DAT1* ($P = 0.047$), with an overrepresentation of the 5R/5R genotype in cocaine addicts [$P = 0.016$, OR = 4.02 (95% CI = 1.3–12.4)] that was also nominally significant when adjusting by age [$P = 0.015$, OR = 1.13 (95% CI = 1.02–1.26)]. However, these differences were not statistically significant after applying the Bonferroni correction. We further estimated *DAT1* haplotype frequencies considering the Int8 and the 3'UTR VNTRs but their comparison between cases and controls showed no significant differences (Table 2). Although the *DBH* polymorphisms did not show association signals in the single-marker analysis, we also tested the 19-bp deletion-c.444G > A haplotype as it had earlier been found associated with cocaine dependence (Cubells *et al.*, 2000), but no significant association was detected in our sample (Table 2).

Discussion

In this study a nominal association between cocaine dependence and the 5R/5R genotype of the Int8 VNTR polymorphism in the *DAT1* gene has been detected in a Caucasian Spanish population. A previous study in a Brazilian sample also found association between cocaine dependence and this polymorphism (Guindalini *et al.*, 2006). However, in this cohort the 6R allele (named allele 3 by the authors) and the 6R/6R genotype were more frequent in cases. This study also showed an influence of the 5R and 6R alleles on *DAT1* expression, as both showed decreased transcription levels when inserted into intronic or into 5' sequences of a reporter gene and transfected into appropriate cell lines, the 6R allele

Table 1 Genotypic and allelic distributions of six polymorphisms within the *DAT1*, *DRD2* and *DBH* genes in 169 cocaine-dependent patients and 169 controls from Spain

Gene	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>
<i>DAT1</i>	3'UTR VNTR	0.31	0.54	9R/9R	9R/10R	10R/10R	Others ^a	9R/9R	9R/10R	10R/10R	Others ^a	0.74	0.49		
	19 (11.2)			69 (40.8)	77 (45.6)	4 (2.4)	18 (10.7)	68 (40.2)	75 (44.4)	8 (4.7)					
	Int8 VNTR	0.06	0.55	5R/5R	5R/6R	6R/6R	Others ^a	5R/5R	5R/6R	6R/6R	Others ^a	0.047 ^b	0.13		
				15 (8.9)	52 (30.8)	100 (59.2)	2 (1.2)	4 (2.4)	56 (33.1)	108 (63.9)	1 (0.6)				
<i>DRD2</i>	TaqIA	0.24	1.00	CC	TC	TT		CC	TC	TT		0.56	0.68		
				123 (72.8)	40 (23.7)	6 (3.6)	117 (69.2)	48 (28.4)	4 (2.4)						
	TaqIB	0.67	0.37	AA	AG	GG		AA	AG	GG		0.55	0.90		
				2 (1.2)	30 (17.8)	137 (81.1)	0 (0)	32 (18.9)	137 (81.1)						
<i>DBH</i>	5'UTR Ins/del	0.65	0.63	in/in	in/del	del/del		in/in	in/del	del/del		0.16	0.09		
				42 (24.9)	88 (52.1)	39 (23.1)	58 (34.3)	79 (46.7)	32 (18.9)						
	c.444G>A	1	1.00	GG	GA	AA		GG	GA	AA		0.79	0.54		
				43 (25.4)	85 (50.3)	41 (24.3)	48 (28.4)	84 (49.7)	37 (21.9)						

DAT1, dopamine transporter; *DBH*, dopamine β -hydroxylase; *DRD2*, dopamine receptor D2; HWE, Hardy–Weinberg Equilibrium; VNTR, variable number of tandem repeats.

^aGenotypes and alleles with frequency under 0.05 were grouped in a single class.

^bFor the Int8 VNTR in *DAT1*, the comparison of the 5R/5R genotype versus all the others displayed a *P* value of 0.016 (not shown in the Table), with an odds ratio (OR) of 4.02 (95% Confidence Interval = 1.3–12.4) that was not statistically significant after the Bonferroni correction for multiple testing.

Table 2 Haplotype analysis of the *DBH* and *DAT1* genes

Haplotype	Cases n (%)	Controls n (%)
<i>DBH</i> : 5'UTR 19-bp insertion/deletion – c.444G>A ^a		
Del-A	114 (33.6)	93 (27.8)
Del-G	52 (15.5)	50 (15)
Ins-A	53 (15.8)	63 (18.9)
Ins-G	119 (35.1)	128 (38.3)
<i>DAT1</i> : Int8 VNTR – 3'UTR VNTR ^b		
5R-9R	64 (20.9)	55 (17.9)
6R-9R	40 (13.1)	44 (14.3)
6R-10R	202 (66.0)	209 (67.8)

DAT1, dopamine transporter; *DBH*, dopamine β-hydroxylase; VNTR, variable number of tandem repeats.

^aOverall association $\chi^2=3.027$; d.f.=3; $P=0.3875$.

^bOverall association $\chi^2=0.9846$; d.f.=2; $P=0.6112$.

reaching lower values than the 5R allele. In addition, the 6R allele showed a further decreased expression upon cocaine treatment (Guindalini *et al.*, 2006). These data prompted the authors to suggest that 6R/6R individuals may exhibit a differential response through altered *DAT1* gene expression when exposed to cocaine. However, the conflicting results observed in association studies, including ours, suggest that the Int8 VNTR polymorphism may not be the only functional variation in the gene that is related to the tested phenotype, or that other elements such as environmental risk factors or genetic background have a distorting effect on the analyses. Alternatively, we cannot discard a false positive result in our study, since the single-marker results were not statistically significant after the Bonferroni correction for multiple testing, and the multiple-marker analysis of the Int8 and the 3'UTR VNTRs in the *DAT1* gene did not show any haplotype overrepresented in patients.

No significant association was found between cocaine dependence and the 3'UTR VNTR of *DAT1*, the *TaqIA* and *TaqIB* of *DRD2* and the 19-bp insertion/deletion and c.444G > A of *DBH*, although a complete coverage of these genes is required to properly assess their involvement in this psychiatric disorder. The comparison of allele and genotype frequencies at all the studied polymorphisms between our control cohort and several European populations showed similar figure (Franke *et al.*, 2008; Togsverd *et al.*, 2008; Schosser *et al.*, 2010; www.hapmap.org). As reviewed earlier (Ballon *et al.*, 2007), no association has been reported between cocaine addiction and the 3'UTR VNTR polymorphism in the *DAT1* gene (Persico *et al.*, 1993; Gelernter *et al.*, 1994; Guindalini *et al.*, 2006; Ballon *et al.*, 2007), although a positive association was detected in a subgroup of Caucasian cocaine addicts which also presented cocaine-induced paranoia (Gelernter *et al.*, 1994). Two studies reported a positive association with the *DRD2 Taq1A* and *Taq1B* variations (Noble *et al.*, 1993; Persico *et al.*, 1996), but others did not replicate this association (Gelernter *et al.*, 1999; Messas *et al.*, 2005). A more recent study described an association between cocaine dependence

with comorbid childhood ADHD and the *DRD2 Taq1A* polymorphism as well as a repeat in exon 3 of the *DRD4* gene (Ballon *et al.*, 2007). These conflicting results, together with those described between cocaine addiction and the *DRD3 BaI* polymorphism (Freimer *et al.*, 1996; Comings *et al.*, 1999; Messas *et al.*, 2005; Ballon *et al.*, 2007; Bloch *et al.*, 2009) highlight the need for more extensive association studies in terms of sample size and genetic coverage.

Polymorphisms in the *DBH* gene coding for DBH that catalyzes the conversion of dopamine to norepinephrine, have also been studied. The 19-bp deletion-c.444A haplotype of the *DBH* gene had been earlier associated with cocaine-induced paranoia and low DBH activity in plasma (Cubells *et al.*, 2000). In our sample we did not detect significant differences between cases and controls, although a slight overrepresentation of this allelic combination was observed in cases (33.6% in cases vs. 27.8% in controls; Table 2). Other polymorphisms within the gene have also been considered in earlier studies. In this regard, the -1021T > C (rs1611115) SNP in the *DBH* 5'UTR was associated with an increased propensity to paranoia over time during cocaine self-administration (Kalayasiri *et al.*, 2007) but showed no association with cocaine addiction (Guindalini *et al.*, 2008).

Limited sample size and heterogeneity at different levels may explain the observed conflicting results. In this regard, ethnicity, sex, comorbidity with other psychiatric disorders, environmental risk factors as well as different endophenotypes, such as cocaine-induced paranoia, may bias association results and might be important issues to consider in future studies to disentangle the genetic background of cocaine dependence.

In conclusion, we found nominal association between cocaine dependence and the 5R/5R genotype of the Int8 VNTR within the *DAT1* gene. Nevertheless, although our Spanish sample is ethnically homogeneous and cases and controls were individually sex-matched, sample size is still limited and further studies should be performed in a larger cohort.

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