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

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ABSTRACT

Drug addiction is a complex neuropsychiatric disorder in which environmental and genetic factors are involved. 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 have genotyped the Int8 and the 3'UTR VNTRs of the dopamine transporter gene (*DAT1/SLC6A3*), the *Taq*IA (rs1800497) and *Taq*IB (rs1079597) SNP polymorphisms within the dopamine receptor D2 gene (*DRD2*) 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 VNTR of the *DAT1* gene in the group of cocaine abusers (P=0.016). However, no significant association was 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.

Keywords: cocaine dependence; case-control association study*; DAT1; SLC6A3*; dopamine transporter; DBH; DRD2

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; Volkow *et al.*, 2002). In addition, according to the "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 the dopamine transporter (DAT1) (Ballon *et al.*, 2007; Gelernter *et al.*, 1994; Guindalini *et al.*, 2006; Persico *et al.*, 1993), the dopamine receptors DRD2 (Ballon *et al.*, 2007; Gelernter *et al.*, 1999; Messas *et al.*, 2005; Noble *et al.*, 1993; Persico *et al.*, 1996), DRD3 (Ballon *et al.*, 2007; Block *et al.*, 2009; Comings *et al.*, 1999; Freimer *et al.*, 1996; Messas *et al.*, 2005) and DRD4 (Ballon *et al.*, 2007) and dopamine beta hydroxylase (DBH) (Cubells *et al.*, 2000; Guindalini *et al.*, 2008; Kalayasiri *et al.*, 2007), 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* (*Taq*IA and *Taq*IB 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.

MATERIAL 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 DSM-IV TR criteria (Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition Text Revision). 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 donnors that had never injected drugs intravenously. Both patients and controls were Spanish and Caucasian. Genomic DNA was extracted from peripheral blood lymphocites using the *salting-out* method (Miller *et al.*, 1988).

Fourty and 30-bp VNTRs in the 3'UTR and in intron 8 of the *DAT1* gene were genotyped as previously described (Qian *et al.*, 2004; Brookes *et al.*, 2006). Genotyping of the *Taq*IA (rs1800497) and *Taq*IB (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 was assessed for all genotypes using the HWE software (www2.biology.ualberta.ca/jbrzusto/hwenj.html). Genetic Power Calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/) was used to estimate genetic power of the sample. Genotype and allele frequencies were compared between cases and controls using the Fisher exact test. Genotypes and alleles of VNTRs with a frequency under 0.05 were grouped in a single class. Odds ratios (OR) and confidence intervals (CI) were estimated using SPSS v14.0. Logistic regression was used to adjust by age. Haplotypes were estimated using the UNPHASED software. The significance threshold was set at $2P<0.0038$ after the multiple comparison correction of Bonferroni

considering genotype and allele comparisons for 6 different polymorphisms and recessive model for those showing a trend.

RESULTS

All the studied polymorphisms were in Hardy-Weinberg equilibrium in both cases and controls. Genetic power calculations ranged from 36 to 48%. When we compared genotype and allele distributions of the *DAT1*, *DRD2* and *DBH* polymorphic variants between cocaine dependence patients and control subjects no significant differences were detected for the *DRD2* and *DBH* genes (Table 1). Instead, an overrepresentation of the 5R/5R genotype of the Int8 VNTR in *DAT1* was noticed in cocaine addicts (P = 0.016, OR = 4.02 (95% CI = 1.3-12.4)), and was also significant when adjusted by age (P = 0.015, OR = 1.13 (95% CI = 1.02 - 1.26)). However these differences were not statistically significant after 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 (data not shown). 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 previously been found associated with cocaine dependence (Cubells *et al.*, 2000), but no significant association was detected in our sample (data not shown).

DISCUSSION

In the present 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 work also demonstrated 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 appropriated cell lines, the 6R allele 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 subjects may exhibit a differential response via 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 overepresented in patients.

No significant association was found between cocaine dependence and the 3'UTR VNTR of *DAT1*, the *Taq*IA and *Taq*IB of *DRD2* and the 19-bp insertion/deletion and c.444G>A of *DBH*. A complete coverage of those genes,

however, is required to discard their involvement in this psychiatric disorder. As reviewed previously (Ballon *et al.*, 2007), no association has been reported between cocaine addiction and the 3'UTR VNTR polymorphism in the *DAT1* gene (Ballon *et al.*, 2007; Gelernter *et al.*, 1994; Guindalini *et al.*, 2006; Persico *et al.*, 1993), 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 Taq*1A and *Taq*1B 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 Taq*1A 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 Bal*I polymorphism (Ballon *et al.*, 2007; Block *et al.*, 2009; Comings *et al.*, 1999; Freimer *et al.*, 1996; Messas *et al.*, 2005) highlight the need for more extensive association studies in terms of sample size and genetic coverage.

Polymorphisms in the *DBH* gene coding for dopamine-beta hydroxylase, 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 previously associated with cocaine-induced paranoia and low DBH activity in plama (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 previous 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, gender, 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 in order 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, 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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REFERENCES

Ballon N, Leroy S, Roy C, Bourdel MC, Olie JP, Charles-Nicolas A, *et al.* (2007). Polymorphisms TaqI A of the DRD2, BalI of the DRD3, exon III repeat of the DRD4, and 3' UTR VNTR of the DAT: association with childhood ADHD in male African-Caribbean cocaine dependents? *Am J Med Genet B Neuropsychiatr Genet* **144B**:1034-1041.

Bloch PJ, Nall AH, Weller AE, Ferraro TN, Berrettini WH, Kampman KM, et al. (2009). Association analysis between polymorphisms in the dopamine D3 receptor (DRD3) gene and cocaine dependence. *Psychiatr Genet* [Epub ahead of print]

Brookes KJ, Mill J, Guindalini C, Curran S, Xu X, Knight J, *et al.* (2006). A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. *Arch Gen Psychiatry* **63**:74-81.

Comings DE, Blum K. (2000). Reward deficiency syndrome: genetic aspects of behavioral disorders. *Prog Brain Res* **126**:325-341.

Comings DE, Gonzalez N, Wu S, Saucier G, Johnson P, Verde R, *et al.* (1999). Homozygosity at the dopamine DRD3 receptor gene in cocaine dependence. *Mol Psychiatry* **4**:484-487.

Cubells JF, Kranzler HR, McCance-Katz E, Anderson GM, Malison RT, Price LH, *et al.* (2000). A haplotype at the DBH locus, associated with low plasma dopamine beta-hydroxylase activity, also associates with cocaine-induced paranoia. *Mol Psychiatry* **5**:56-63.

Freimer M, Kranzler H, Satel S, Lacobelle J, Skipsey K, Charney D, *et al.* (1996). No association between D3 dopamine receptor (DRD3) alleles and cocaine dependence. *Addict Biol* **1**: 281-287.

Gelernter J, Kranzler H, Satel SL. (1999). No association between D2 dopamine receptor (DRD2) alleles or haplotypes and cocaine dependence or severity of cocaine dependence in European- and African-Americans. *Biol Psychiatry* **45**:340-345.

Gelernter J, Kranzler HR, Satel SL, Rao PA. (1994). Genetic association between dopamine transporter protein alleles and cocaine-induced paranoia. *Neuropsychopharmacology* **11**:195-200.

Guindalini C, Howard M, Haddley K, Laranjeira R, Collier D, Ammar N, *et al.* (2006). A dopamine transporter gene functional variant associated with cocaine abuse in a Brazilian sample. *Proc Natl Acad Sci U S A* **103**:4552-4557.

Guindalini C, Laranjeira R, Collier D, Messas G, Vallada H, Breen G. (2008). Dopamine-beta hydroxylase polymorphism and cocaine addiction. *Behav Brain Funct* **4**:1.

Kalayasiri R, Sughondhabirom A, Gueorguieva R, Coric V, Lynch WJ, Lappalainen J, *et al.* (2007). Dopamine beta-hydroxylase gene (DbetaH) -1021C-->T influences self-reported paranoia during cocaine self-administration. *Biol Psychiatry* **61**:1310-1313.

Messas G, Meira-Lima I, Turchi M, Franco O, Guindalini C, Castelo A, *et al.* (2005). Association study of dopamine D2 and D3 receptor gene polymorphisms with cocaine dependence. *Psychiatr Genet* **15**:171-174.

Miller SA, Dykes DD, Polesky HF. (1988). A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* **16**:1215.

Noble EP, Blum K, Khalsa ME, Ritchie T, Montgomery A, Wood RC, *et al.* (1993). Allelic association of the D2 dopamine receptor gene with cocaine dependence. *Drug Alcohol Depend* **33**:271-285.

Persico AM, Bird G, Gabbay FH, Uhl GR. (1996). D2 dopamine receptor gene TaqI A1 and B1 restriction fragment length polymorphisms: enhanced frequencies in psychostimulant-preferring polysubstance abusers. *Biol Psychiatry* **40**:776-784.

Persico AM, Vandenbergh DJ, Smith SS, Uhl GR. (1993). Dopamine transporter gene polymorphisms are not associated with polysubstance abuse. *Biol Psychiatry* **34**:265-267.

Qian QJ, Wang YF, Zhou RL, Yang L, Li J. (2004). [Association studies of G352A polymorphism of dopamine transporter gene in Han Chinese attention deficit hyperactivity disorder patients.]. *Beijing Da Xue Xue Bao* **36**:626-629.

Tan EK, Tan Y, Chai A, Tan C, Shen H, Lum SY, *et al.* (2003). Dopamine D2 receptor TaqIA and TaqIB polymorphisms in Parkinson's disease. *Mov Disord* **18**:593-595.

Volkow ND, Fowler JS, Wang GJ. (1999). Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. *J Psychopharmacol* **13**:337-345.

Volkow ND, Fowler JS, Wang GJ, Goldstein RZ. (2002). Role of dopamine, the frontal cortex and memory circuits in drug addiction: insight from imaging studies. *Neurobiol Learn Mem* **78**:610-624.

Volkow ND, Wang GJ, Fowler JS, Gatley SJ, Ding YS, Logan J, *et al.* (1996). Relationship between psychostimulant-induced "high" and dopamine transporter occupancy. *Proc Natl Acad Sci U S A* **93**:10388-10392.

Yamamoto K, Cubells JF, Gelernter J, Benkelfat C, Lalonde P, Bloom D, *et al.* (2003). Dopamine beta-hydroxylase (DBH) gene and schizophrenia phenotypic variability: a genetic association study. *Am J Med Genet B Neuropsychiatr Genet* **117**:33-38.

TABLES

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

		HWE		Genotypes								Alleles
				Cases N (%)				Controls N (%)				
Gene	Polymorphism	Cases P	Controls P								P	P
DAT1	3'UTR VNTR	0.31	0.54	9R/9R 19 (11.2)	9R/10R 69 (40.8)	10R/10R 77 (45.6)	freq < 0,05 4 (2.4)	9R/9R 18 (10.7)	9R/10R 68 (40.2)	10R/10R 75 (44.4)	freq < 0,05 8 (4.7)	0.74	0.49
	Int8 VNTR	0.06	0.55	5R/5R 15 (8.9)	5R/6R 52 (30.8)	6R/6R 100 (59.2)	freq < 0,05 2 (1.2)	5R/5R 4 (2.4)	5R/6R 56 (33.1)	6R/6R 108 (63.9)	freq < 0,05 1 (0.6)	0.05 *	0.13
DRD2	*Taq*IA	0.24	1.00	CC 123 (72.8)	TC 40 (23.7)	TT 6 (3.6)		CC 117 (69.2)	TC 48 (28.4)	TT 4 (2.4)		0.56	0.68
	*Taq*IB	0.67	0.37	AA 2 (1.2)	AG 30 (17.8)	GG 137 (81.1)		AA 0 (0)	AG 32 (18.9)	GG 137 (81.1)		0.55	0.90
DBH	5'UTR Ins/del	0.65	0.63	in/in 42 (24.9)	in/del 88 (52.1)	del/del 39 (23.1)		in/in 58 (34.3)	in/del 79 (46.7)	del/del 32 (18.9)		0.16	0.09
	c.444G>A	1	1.00	GG 43 (25.4)	GA 85 (50.3)	AA 41 (24.3)		GG 48 (28.4)	GA 84 (49.7)	AA 37 (21.9)		0.79	0.54

Genotypes and alleles with frequencies under 0.05 were grouped in a single class.
* For the Int8 VNTR, the comparison of the 5R/5R genotype vs others displayed a p-value of 0.016, with an odds ratio (OR) of 4.02 (95% CI: 1.3-12.4) which was not statistically significant after Bonferroni correction

Table 2. Haplotype analyisis of the *DBH* and *DAT1* genes

Haplotype	**Cases** ***n*** **(%)**	**Controls** ***n*** **(%)**
DBH: 5'UTR 19-bp insertion/deletion – c.444G>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)
DAT1: Int8 VNTR – 3'UTR VNTR **		
5R-9R	64 (20.9)	55 (17.9)
6R-9R	40 (13.1)	44 (14.3)
6R-10R	202 (66.0)	209 (67.8)

* Overall association $\chi^2 = 3.027$; df = 3; $P = 0.3875$.
** Overall association $\chi^2 = 0.9846$; df = 2; $P = 0.6112$.