

Journal of Psychopharmacology

<http://jop.sagepub.com/>

Dopamine DRD2/ANKK1 Taq1A and DAT1 VNTR polymorphisms are associated with a cognitive flexibility profile in pathological gamblers

Ana B Fagundo, Fernando Fernández-Aranda, Rafael de la Torre, Antonio Verdejo-García, Roser Granero, Eva Penelo, Manel Gené, Carme Barrot, Cristina Sánchez, Eva Alvarez-Moya, Cristian Ochoa, Maria Neus Aymamí, Mónica Gómez-Peña, Jose M Menchón and Susana Jiménez-Murcia

J Psychopharmacol published online 18 September 2014

DOI: 10.1177/0269881114551079

The online version of this article can be found at:

<http://jop.sagepub.com/content/early/2014/09/12/0269881114551079>

Published by:



<http://www.sagepublications.com>

On behalf of:



British Association for Psychopharmacology

Additional services and information for *Journal of Psychopharmacology* can be found at:

Email Alerts: <http://jop.sagepub.com/cgi/alerts>

Subscriptions: <http://jop.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

>> OnlineFirst Version of Record - Sep 18, 2014

What is This?



Dopamine DRD2/ANKK1 Taq1A and DAT1 VNTR polymorphisms are associated with a cognitive flexibility profile in pathological gamblers

Ana B Fagundo^{1,2}, Fernando Fernández-Aranda^{1,2,3}, Rafael de la Torre^{2,4}, Antonio Verdejo-García^{5,6,7}, Roser Granero^{2,8}, Eva Penelo⁸, Manel Gené³, Carme Barrot³, Cristina Sánchez³, Eva Alvarez-Moya¹, Cristian Ochoa¹, Maria Neus Aymamí¹, Mónica Gómez-Peña¹, Jose M Menchón^{1,2,9} and Susana Jiménez-Murcia^{1,2,3}

Journal of Psychopharmacology
1–8

© The Author(s) 2014
Reprints and permissions:
sagepub.co.uk/journalsPermissions.nav
DOI: 10.1177/0269881114551079
jop.sagepub.com



Abstract

Like drug addiction, pathological gambling (PG) has been associated with impairments in executive functions and alterations in dopaminergic functioning; however, the role of dopamine (DA) in the executive profile of PG remains unclear. The aim of this study was to identify whether the DRD2/ANKK1 Taq1A-rs1800497 and the DAT1-40 bp VNTR polymorphisms are associated with cognitive flexibility (measured by Wisconsin Card Sorting Test (WCST) and Trail Making Test (TMT)) and inhibition response (measured by Stroop Color and Word Test (SCWT)), in a clinical sample of 69 PG patients. Our results showed an association between DA functioning and cognitive flexibility performance. The Taq1A A1+ (A1A2/A1A1) genotype was associated with poorer TMT performance ($p < 0.05$), while DAT1 9-repeat homozygotes displayed better WCST performance ($p < 0.05$) than either 10-repeat homozygotes or heterozygotes. We did not find any association between the DRD2 or DAT1 polymorphisms and the inhibition response. These results suggested that pathological gamblers with genetic predispositions toward lower availability of DA and D2 receptor density are at a higher risk of cognitive flexibility difficulties. Future studies should aim to shed more light on the genetic mechanisms underlying the executive profile in PG.

Keywords

Addiction, cognition, cognitive flexibility, dopamine, dopamine receptor, dopamine transport, executive functions, gambling, genetics, inhibition response, pathological gambling, polymorphism

Introduction

Pathological gambling (PG) is the diagnostic term used to describe excessive and interfering patterns of gambling (American Psychological Association, 2000). PG is considered a behavioral or non-substance-related addiction, since pathological gamblers share certain common features with drug users, including tolerance, withdrawal and repeated unsuccessful attempts to restrain or stop their habit (Prakash et al., 2012). Like drug addiction, PG is also associated with executive function impairments. Executive functions (EF) are higher-order, cognitive capacities that allow persons to orient toward the future, display self-control

and effectively have goal-oriented behavior (Stuss and Alexander, 2000). In this regard, pathological gamblers show a dysfunctional executive profile characterized by deficits in cognitive flexibility, inhibition response, planning and decision-making (Goudriaan et al., 2006; Lawrence et al., 2009).

Of all the signals involved in EF, dopamine (DA) has been the most thoroughly investigated (Savitz et al., 2006). The anatomical distribution of DA projections, which originate in the ventral tegmental area of the midbrain and project to the prefrontal cortex (PFC), anterior cingulate cortex and basal forebrain (Bannon and

¹Department of Psychiatry, University Hospital of Bellvitge (IDIBELL), Barcelona, Spain

²CIBER Fisiopatología Obesidad y Nutrición (CIBEROBn), Instituto de Salud Carlos III [Carlos III Health Institute], Barcelona, Spain

³School of Medicine, University of Barcelona, Barcelona, Spain

⁴Neuroscience Research Program, 'Del Mar' Hospital Medical Research Institute (IMIM), Barcelona, Spain

⁵School of Psychology and Psychiatry, Monash University, Melbourne, VIC, Australia

⁶Institute of Neuroscience and Department of Clinical Psychology, University of Granada, Granada, Spain

⁷Red de Trastornos Adictivos [Network for Addictive Diseases], Instituto de Salud Carlos III, Madrid, Spain

⁸Department of Psychobiology and Methodology, Autonomous University of Barcelona, Barcelona, Spain

⁹CIBER Salud Mental (CIBERSam), Instituto Salud Carlos III, Barcelona, Spain

Corresponding author:

Susana Jiménez-Murcia, Departamento de Psiquiatría y CIBEROBn, Hospital Universitario de Bellvitge, c/ Feixa Llarga s/n, 08907 Barcelona, Spain.

Email: sjimenez@bellvitgehospital.cat

Roth, 1983), offers a reasonable basis for suggesting a role for DA in EF (Floresco and Magyar, 2006). In addition, psychopharmacological studies show that administration of dopaminergic drugs might result in opposite effects on cognitive performance (Cools and D'Esposito, 2011), mainly on executive tasks (Mattay et al., 2003; Mehta et al., 2004). In fact, administration of DA drugs is associated with a positive or negative effect on executive functions, depending on if the baseline executive efficiency level is low or high. These findings led to a proposed model in which the association between cognition and DA follows an 'inverted-U-shaped' function, defining an optimal DA level depending on the cognitive task (Cools and D'Esposito, 2011). In humans, evidence supporting this theory comes from studies allowing for genetic differences between individuals (Frank and Fossella, 2011).

Genes regulating DA transmission are of obvious interest in pathological gambling, due to the compelling evidence that the mesocorticolimbic DA system is a core component of the natural reward system, and is directly or indirectly activated by all abused drugs and behavioral addictions (Hyman et al., 2006; Zack and Poulos, 2009). Specifically, genetic differences in DA are associated with drug addiction, including amphetamine (Mattay et al., 2003) or alcohol use (Noble, 2000). As for PG, alterations in DA functioning have been proposed as underlying the reward capacity of gambling, by activating the release of DA both in healthy controls and in pathological gamblers (Zack and Poulos, 2009). There is also evidence of an association between the DA response and unpredictable reward in PG patients, whom display an appreciably higher heart rate response and increased plasma levels of DA while gambling (Meyer et al., 2004; Joutsa et al., 2012).

Typically, the executive-related effects of DA are attributed to modulation of the prefrontal cortex (PFC); however, recent studies highlight a complementary role for DA in the striatum, in executive functioning (Leber et al., 2008). Dopaminergic functioning in the striatum depends on both the level of DA available (which in turn depends on DA reuptake via the dopamine transporter [DAT]), and on DA receptor binding and activity, mainly D2 receptors (Cromptley et al., 2006). The DA receptor D2 (DRD2) is a G protein-coupled receptor located on postsynaptic dopaminergic neurons and is associated with a protective effect on cognition (Kempainen et al., 2003). The *DRD2* gene encoding this receptor is located on chromosome 11q23, and most studies have focused on the Taq1A (rs1800497) polymorphism (Neville et al., 2004; Noble, 2003), which was recently shown to map in the neighboring ankyrin repeat and 'kinase domain containing 1' (*ANKK1*) gene (Neville et al., 2004). The prevalence of the mutated A1 allele (amino acid: K, nucleotide base: T) is 28%, and A1 carriers show a 30–40% reduction in D2 receptor density (Ritchie and Noble, 2003). The A1 allele has been associated with EF, with A1 carriers showing both higher and lower performance in impulsivity (Eisenberg et al., 2007; White et al., 2008), inhibition response (Markett et al., 2011; Reuter et al., 2005) and cognitive flexibility (Markett et al., 2011; Stelzel et al., 2010).

The DAT is a protein that plays a decisive role in DA functioning, while removing DA from the extracellular space (Bannon et al., 2001). A variable number tandem repeat (VNTR) polymorphism of the *DAT1* gene (SLC6A3), resulting in variants that range from 3–13 repeats (with the 9- and 10-repeats occurring most commonly (Bannon et al., 2001). It was studied in depth and its length related to DAT expression and DA availability (Mill et al., 2002). The 9-repeat allele is associated with lower

DAT expression and, as a result, with higher levels of DA in the synaptic cleft (Mill et al., 2002). It has been demonstrated that 9-repeat carriers have better EF performance, including working memory, attention processes and impulsivity (Loo et al., 2003; Brehmer et al., 2009; Simon et al., 2011). Neuroimaging studies also suggest that 10-repeat allele homozygotes show increased activation in brain areas underlying executive tasks, indicating that executive functioning requires greater effort in this group (Braet et al., 2011; Gordon et al., 2013).

Despite evidence of the involvement of the dopaminergic system in EF, there appear to be no genetic studies linking EF performance with DRD2 and DAT functioning in pathological gamblers. We aimed to investigate the association between cognitive flexibility (measured by Wisconsin Card Sorting Test (WCST) and Trail Making Test (TMT)) and inhibition response (measured by Stroop Color and Word Test (SCWT)), and polymorphisms in genes encoding DRD2/ANKK1 (Taq1A-rs1800497) and DAT (DAT1 VNTR) in a PG sample.

Methods and materials

Study sample

Our initial sample comprised 69 consecutive Caucasian patients seeking treatment at the PG Unit within the Department of Psychiatry, University Hospital of Bellvitge in Barcelona, Spain. Results for the DAT1 phenotype were not obtained for three of the participants, because of analytical reasons (total study sample $n = 66$). PG was diagnosed according to the *Diagnostic and Statistical Manual of Mental Disorders, Version 4 (DSM-IV-TR)* criteria (American Psychiatric Association, 2000); we also assessed patients with the South Oaks Gambling Screen (SOGS) (Lesieur and Blume, 1987). All participants were males aged between 18–65 years, whom spoke Spanish as their first language. We enrolled participants between November 2005 and September 2007.

Exclusion criteria were:

- History of chronic medical illness or neurological condition that might affect cognitive function;
- Head trauma, learning disability or mental retardation;
- Lifetime history of an Axis I mental disorder, according to the *DSM-IV-TR* (American Psychiatric Association, 2000);
- History of substance abuse in the previous 3 months;
- Age under 18 or over 65 (to preclude neuropsychological deficits associated with age).

Our research procedures were explained in full to the participants, and all subjects gave written informed consent, prior to enrollment. The procedures were approved by the Ethical Committee of the University Hospital of Bellvitge.

Neuropsychological assessment

As described in a previous study (Alvarez-Moya et al., 2011), we determined verbal intelligent quotient (IQ) using the vocabulary subtest of the Wechsler Adult Intelligence Scale-III (WAIS-III) (Wechsler, 1997). For the purpose of this study, we included the

following tests (for further information on these tests, see the Supplementary material):

1. Stroop Color and Word Test, or SCWT (Golden, 1978): It measures interference control, flexibility and attention. The main outcome variable is the 'interference score'. Higher scores on this variable indicate better capacity for response inhibition.
2. Wisconsin Card Sorting Test or WCST (Heaton 1981): It measures planning capacity, cognitive flexibility and capacity of shifting among stimuli. The main outcome is the number of categories completed: Higher scores indicate better cognitive flexibility and conceptualization. We also considered the number of errors and the number of cards used until the first category was successfully completed (initial conceptualization), as both these variables are considered predictors of WCST results and mental set flexibility (Gligorovic and Buha, 2013).
3. The Trail-Making Test or TMT (Reitan, 1958): It measures motor speed, attention and cognitive flexibility. The test consists of two parts ((a) and (b)). Higher scores on Part (a) suggest deficits in motor speed and attention; while higher scores on Part (b) suggest set-shifting difficulties. In order to control for individual differences in speed of processing and attention, we generated a score based on the subtraction of time to complete Part (a) from the time to complete Part (b):
TMT (a)–(b)
Higher scores suggested difficulties with cognitive flexibility.

Genotyping and analysis methods

DNA was extracted from the blood sample and used as a template for the polymerase chain reaction (PCR). We performed genotyping of the Taq1A (rs1800497) DRD2 single nucleotide polymorphism (SNP) by real time PCR ($n = 69$), as follows: We used primers 5'-CCG TCG ACC CTT CCT GAG TGT CAT CA-3' and 5'-CCG TCG ACG GCT GGC CAA GTT GTC TA-3' to amplify a 310 base pair (bp) polymorphic fragment site of the *ANKK1* gene. PCR product was digested with five units of TaqI for 22 h at 65°C, to reveal three genotypes:

- Predominant homozygote (CC), indicated by two fragments (130 and 180 bp);
- Heterozygote (CT), indicated by three fragments (130, 180 and 310 bp); and
- Rare homozygote (TT), indicated by the uncleaved (310 bp) fragment.

The VNTR region of the *DAT1* gene was amplified from the genomic DNA using PCR. Results were not obtained for three of the participants ($n = 66$). In research on genetic polymorphisms, a non-amplified locus is not something exceptional and could be due to different causes; however, according to our rigorous quality control in the application of the methods, the most likely hypothesis is that the PCR product was not amplified by the presence of mutations in the region of the primers' hybridization. To demonstrate this, it would be necessary to sequence the region, which goes

further than the objectives of the present study. Primers were used as follows: forward, 5-TGTGGT GTA GGG AAC GGC CTG AG-3 and reverse, 5-CTT CCT GGA GGT CAC GGC TCA AGG-3. The PCR amplifications (25 l) were performed in a top-heated thermal cycler (Model GeneAmp System 9700, Applied Biosystems, Foster City, CA) for 35 cycles, and contained 1.5 μ L $MgCl_2$, 200 microM each of dNTP, 1.5 μ L each of the primer, and 0.25 units of AmpliTaq DNA polymerase. PCR cycles were: 95°C for 30 sec, 65°C for 30 sec and 72 °C for 30 sec. There was a 15-min pre-incubation at 95°C before starting the cycles, and 5 min at 72°C after the completion of the cycles. The PCR products were separated on a 2.5% agarose gel and stained with ethidium bromide.

Statistical analysis

Analyses were carried out with SPSS20 for Windows. We explored the association between genetic indicators and cognitive outcomes with analysis of variance (ANOVA) procedures, adjusted for patient age and academic level. When significant effects emerged in the ANOVA, pair-wise comparisons of DRD2/ANKK1 Taq1A and DAT1 VNTR polymorphism means were estimated through post-hoc comparisons, including Bonferroni's correction for multiple tests (with the level of significance fixed at .05), and Cohen's-d coefficient were used to measure the effect size of mean differences (moderate effect size was considered for $|d| > 0.5$ and good effect size for $|d| > 0.8$). We used radar charts to represent group performance across the main cognitive domains (cognitive measures were plotted through z-scores).

Results

Our study patients' sociodemographic characteristics and gambling-related variables are presented in Table 1. No statistical differences for the DRD2/ANKK1 Taq1A and DAT1 VNTR polymorphisms were obtained, except for level of education ($p = 0.021$ for the DRD2/ANKK1) and age ($p = 0.001$ for DAT1 VNTR). These two variables were included as covariates in the ANOVA comparisons. The outcomes of this study did not achieve significant associations with the gambling severity (measured by the SOGS-total score): correlation coefficients with $|r|$ lower than 0.14, ranging between $r = 0.03$ for WCST-total errors to $r = 0.13$ for TMT-B.

Role of DRD2/ANKK1 Taq1A (rs1800497)

The genotype frequency for the *DRD2/ANKK1* Taq1A polymorphism was as follows: A1/A1, $n = 7$; A1/A2, $n = 25$ and A2/A2, $n = 37$. Homozygotes for the minor allele were grouped together with heterozygotes, i.e. the *DRD2/ANKK1* Taq1A A1+ group consisted of A1/A1 (TT) and A1/A2 (CT) genotypes. This analysis is consistent with the literature and a dominant model of inheritance (Noble, 2003). The genotype data were in Hardy-Weinberg equilibrium ($\chi^2 = 0.78$; $p = 0.38$).

DRD2/ANKK1 Taq1A (rs1800497) polymorphism was associated with verbal IQ and cognitive flexibility performance (Table 2). Evaluation of mean scores indicated that the A1+ genotype was associated with higher vocabulary scores ($p < 0.05$), considered a measure of IQ, but also with higher scores on the TMT (Part (b); $p < 0.05$), suggesting difficulties with cognitive flexibility. No signifi-

Table 1. Sociodemographics and clinical variables.

	Total		DAT1 VNTR				p	
	(n = 69)	<i>TaqIA</i> (rs1800497)	A1+ (n = 32)	A1- (n = 37)	p	9R-9R (n = 7)		9R-10R (n = 27)
Age (yrs); mean (SD)	35.84 (10.69)	37.63 (11.99)	34.30 (9.32)	41.43 (13.43)	.199	40.33 (10.50)	31.34 (8.46)	.001
Duration gambling disease (yrs); mean (SD)	5.18 (6.01)	6.21 (7.71)	4.32 (4.05)	13.67 (13.66)	.212	4.92 (4.38)	3.88 (3.65)	.120
Education (yrs); mean (SD)	10.78 (3.15)	10.47 (3.08)	11.05 (3.22)	10.00 (2.38)	.445	10.70 (3.21)	11.00 (3.35)	.799
Education level (%)	72.1%	87.10%	59.46%	100%	.026	74.07%	67.74%	.515
Primary								
Secondary	22.1%	12.90%	29.73%	0%		18.52%	25.81%	
University	5.9%	0%	10.81%	0%		7.41%	6.45%	
Socioeconomic level (%)	3.2%	0%	5.71%	0%	.089	3.85%	3.57%	.596
High								
Mean-high	4.8%	0%	8.57%	0%		7.69%	3.57%	
Mean	11.1%	14.29%	8.57%	0%		7.69%	17.86%	
Mean-low	60.3%	53.57%	65.71%	66.67%		50.00%	64.29%	
Low	20.6%	32.14%	11.43%	33.33%		30.77%	10.71%	
Civil status (%)	38.2%	25.81%	48.65%	14.29%	.127	25.93%	54.84%	.097
Single								
Married/couple	52.9%	61.29%	45.95%	71.43%		66.67%	35.48%	
Divorced/separated	8.8%	12.90%	5.41%	14.29%		7.41%	9.68%	
Employment status	86.8%	87.10%	86.49%	85.71%	.941	92.59%	80.65%	.421
(% employed)								
Total number of problematic games	1.58 (1.12)	1.47 (1.14)	1.68 (1.11)	2.29 (1.98)	.447	1.26 (0.81)	1.75 (1.08)	.117
Smoker (% yes)	76.2%	72.41%	79.41%	71.43%	.516	60.87%	87.10%	.084
Gambling variables								
Maximum bets (Euros); mean (SD)	1.36 (0.97)	690.0 (1107)	891.8 (1672)	901.4 (1218)	.577	1082.5 (2086)	574.3 (734)	.785
Mean bets (Euros); mean (SD)	797.2 (1428)	168.8 (287)	247.9 (564)	80.0 (70)	.510	301.6 (683)	165.6 (201)	.403
Cumulative debt (Euros); mean (SD)	209.0 (448)	14,546.0 (24,789)	8534.8 (12,723)	10,741.7 (18,825)	.302	19,640.0 (26,759)	7380.8 (14,835)	0.69
SOGS total score; mean (SD)	10.94 (2.64)	11.29 (2.69)	10.65 (2.60)	10.00 (3.92)	.321	11.19 (2.17)	10.97 (2.73)	.795

SD: standard deviation; yrs: years.

Table 2. Association between the *DRD2/ANKK1 Taq1A (rs1800497)* and *DAT1 VNTR* polymorphisms and cognitive variables, after ANOVA adjusted by age, years of education and substance use.

	<i>Taq1A (rs1800497)</i>					<i>DAT1 VNTR</i>					<i>p</i> ^a	F (df = 2; 61)	Post-hoc analysis
	A1+ (n = 32)	A1- (n = 37)	F (df = 1; 65)	<i>p</i> ^a	Cohen d	9R-9R (n = 7)	9R-10R (n = 27)	10R-10R (n = 32)	F (df = 2; 61)	<i>p</i> ^a			
Vocabulary	37.03 (7.08)	34.54 (7.90)	4.27	.043	0.54^b	32.86 (7.78)	34.96 (7.87)	36.66 (7.44)	0.95	.392			
SCWT													
Interference	-1.20 (10.05)	2.08 (9.94)	1.29	.261	0.29	-1.79 (6.73)	0.51 (10.28)	0.51 (10.63)	0.27	.762			
WCST													
Total correct	71.42 (13.30)	68.84 (10.74)	0.77	.383	0.23	82.00 (10.32)	67.48 (12.36)	68.71 (10.55)	4.42	.017	9-9 > 9-10; φ = 14.28; <i>p</i> = 0.024 9-9 > 10-10; φ = 14.14; <i>p</i> = .022		
Total errors	42.15 (24.02)	39.38 (24.39)	0.01	.934	0.02	27.83 (15.09)	51.38 (22.52)	37.21 (24.85)	2.90	.065			
Pers errors	22.55 (15.82)	21.36 (14.44)	0.23	.637	0.11	12.67 (8.12)	31.67 (20.15)	19.50 (13.78)	3.79	.030	9-9 < 9-10; φ = 18.72; <i>p</i> = .029		
Non-pers errors	13.09 (7.96)	19.94 (14.10)	0.84	.362	0.24	15.17 (8.61)	19.71 (11.92)	17.04 (14.49)	0.47	.624			
TMT													
TMT-A	31.35 (13.30)	24.86 (7.18)	2.70	.106	0.40	25.97 (11.92)	30.82 (9.99)	26.56 (11.27)	0.64	.532			
TMT-B	104.2 (76.75)	66.09 (24.97)	4.50	.038	0.54^b	74.09 (29.46)	94.08 (58.92)	72.63 (51.80)	0.38	.685			

^aSignificant contrast (.05 level).^bModerate to high effect size (|d| > 0.50).

ANOVA: Analysis of variance; pers: perseverative; SCWT: Stroop Color and Word Test; TMT: Trail-making Test; WCST: Wisconsin Card Sorting Test.

cant association was found between inhibition response performance (SCWT) and the *DRD2/ANKK1* polymorphism (Table 2).

Role of *DAT1 VNTR*

The genotype frequency for the *DAT1 VNTR* 40 bp was as follows: 9/9, $n = 7$; 9/10, $n = 27$; 10/10, $n = 32$. The genotype data were in Hardy-Weinberg equilibrium ($\chi^2 = 0.13$; $p = 0.71$).

The *DAT1* polymorphism was also associated with cognitive flexibility performance. Nine-repeat homozygotes displayed more WCST total correct responses ($p = 0.02$), and made fewer perseverative errors ($p = 0.03$) than heterozygotes and subjects with two copies of the 10-repeat allele, suggesting better cognitive flexibility performance. We found no significant association between inhibition response performance (SCWT) and the *DAT1* polymorphism (Table 2).

Discussion

This study set out to examine the association between DA polymorphisms and executive functions in PG patients. A significant association was observed between DA-related genes (i.e. *DRD2/ANKK1* and *DAT1*) and cognitive flexibility. Our results showed that greater availability of DA content and D2 receptor density were related to higher cognitive flexibility in PG patients. Interestingly, we did not observe genetic effects on response inhibition. Although DA modulation was previously related to cognitive performance in humans (Markett et al., 2011; Stelzel et al., 2010), this is, to the best of our knowledge, the first time that the dopaminergic system has been associated with executive functioning in a PG sample.

Our results suggest that *DAT* and *DRD2* have similar effects on cognitive flexibility in PG patients. Our finding of individual differences in DA associated with cognitive flexibility corroborates those of both animal (Floresco et al., 2006) and human studies (Jocham et al., 2009; Klein et al., 2007), suggesting that A1 carriers are less able to adjust their behavior, based on feedback obtained from the preceding trials. These data also support the pharmacological evidence that a *DRD2* agonist decreases task-switching performance and impairs cognitive flexibility (Mehta et al., 2004).

Interestingly, although both WCST and TMT are classical neuropsychological tests for measuring cognitive flexibility, a different association with the *DRD2* and *DAT* polymorphisms was observed. These differences might be partially explained by dissimilarities in task complexity and the cognitive functions required for their performance: Whereas optimal performance on the TMT is based on preservation of the capacity of set shifting, attention processing and processing speed; the WCST also reflects strategic planning, organized searching, the ability to use environmental feedback to adjust cognitive sets and goal-oriented behavior (Nyhus and Barceló, 2009).

We failed to find an association between DA genes and the inhibition response. A number of studies associate DA functioning with behavioral inhibition (Congdon and Canli, 2008; Enoch and Goldman 2001), although some studies suggest that DA does not reach the extent and effect size originally hypothesized (Hack et al., 2011). Studies using the Stroop test found contradictory results, suggesting there is a positive association between haplotypes of

COMT and *DRD2* (Reuter et al., 2005), while no role of *DRD2* and *DAT* polymorphisms in the inhibition performance (Wohl et al., 2008). Interestingly, positive associations have been found between dopaminergic polymorphisms (*DRD2*, *DAT* and *COMT*) and other inhibition-based tasks, such as prepulse inhibition (Montag et al., 2011) and the stop signal task (Enoch and Goldman, 2001). These discrepant results highlight the importance of conducting further studies evaluating both the DA-related catabolic enzyme activity and receptor density, and conducting a more comprehensive assessment of the inhibition response.

Finally, we found an association between DA receptor density and verbal IQ. Patients with lower D2 binding potential had higher general verbal abilities. These results are in agreement with those showing a significant association between the *Taq1A* polymorphism and general cognitive ability (Bolton et al., 2010); however, the results of studies investigating the role of DA in general IQ are inconsistent: *Taq1A* has not been associated with intelligence capacity in middle-aged adults (Moises et al., 2001), but it was associated with increased IQ in young women (Tsai et al., 2002). It should be borne in mind that we used only one subtest (vocabulary) as an estimating measure of verbal IQ, as the main focus of our study was executive functioning. Therefore, these findings should be confirmed by studies conducting thorough general cognitive assessments in PG.

Although we did not find an association between severity of gambling behavior (measured by means of SOGS) and the studied polymorphisms, this finding should be interpreted with caution. The lack of a positive result does not necessarily mean that perhaps, if taking other more accurate clinical/psychometric measures of gambling severity, different results might be reached. The implications of these negative findings warrant additional attention and need further research.

This study has several important strengths, including the specific characteristics of the selected sample and its genetic approach. We comprehensively assessed clinical and psychopathological profiles in a group of PG individuals attended consecutively at a specialized PG unit. Molecular genetics, as applied in the present study, is an additional level of analysis above neuropsychological assessment, and it provides a practicable tool for the study of executive processes. Additionally, our study was specifically designed to comprehensively test executive dysfunction in PG, by using three well-validated executive tests; however, certain limitations of the study should also be borne in mind. First, no control group was included; however, the associations observed between DA functioning and executive profile were the opposite of those found in healthy control studies, suggesting a specific pattern of associations in PG patients. Specifically, the availability of *DAT* has been negatively correlated with cognitive flexibility performance in healthy volunteers (Hsieh et al., 2010) and the group with the A1 genotype of the *DRD2/ANKK1* scores significantly higher in both executive and memory tasks (Bartres-Faz et al., 2002; Tsai et al., 2002). Second, our data do not in any way demonstrate causality: Studies with a longitudinal design are required, in order to confirm the cause-effect relationship between DA functioning and executive function in PG. Third, our study sample size was small; thus, further studies using a bigger sample are desirable. Finally, given that cognitive flexibility and the inhibition response are polygenic EFs, future studies including serotonin, acetylcholine and brain-derived neurotrophic factor-related genes should be conducted in

order to shed more light on the genetic mechanisms underlying the EF profile in pathological gamblers.

In summary, our results provide novel information regarding the influence of DA-related genes on EF in PG. Pathological gamblers with genetic predispositions associated with poorer DA efficacy are at a higher risk of presenting difficulties with cognitive flexibility. This study is particularly timely, given the important public health impact of PG and the potential significance of accurately-defined endophenotypes and genotypes associated with this disorder. Identifying gene-environment interactions is an essential element in the study of PG as an addiction, which, by definition, relies on the exposure to an addictive agent and is therefore powerfully modulated by genetics and environmental features. Thus, our understanding of PG will be improved by the detection of genes that have a role in altered gambling-specific vulnerabilities, such as personality traits, cognitive functioning and reward processing.

Conflict of interest

The authors declare there are no conflict of interest.

Funding

This work was supported by the Fondo de Investigación Sanitaria (FIS) [foundation for health investigation] (grant number PI11/210), Ministerio de Economía y Competitividad [the Spanish ministry of economy and competitiveness] (grant number PSI2011-28349) and Agència de Gestió d'Ajuts Universitaris i de Recerca (AGAUR) (grant numbers 2014SGR1672 and 2009SGR718); plus ABF was supported by the Ministerio de Ciencia e Innovación Subprograma Juan de la Cierva [ministry of science and innovation, Juan de la Cierva subprogram] (grant number JCI-2011-09248). The Centros de Investigación Biomédica en Red (CIBER) programs are supported by Instituto de Salud Carlos III (ISCIII).

References

- Alvarez-Moya EM, Ochoa C, Jimenez-Murcia S, et al. (2011) Effect of executive functioning, decision-making and self-reported impulsivity on the treatment outcome of pathologic gambling. *J Psychiatry Neurosci* 36: 165–175.
- American Psychiatric Association (APA) (2000) *DSM-IV-TR: Diagnostic and Statistical Manual of Mental Disorders*. Washington, DC: APA.
- Bannon MJ and Roth RH (1983) Pharmacology of mesocortical dopamine neurons. *Pharmacol Rev* 35: 53–68.
- Bannon MJ, Michelhaugh SK, Wang J, et al. (2001) The human dopamine transporter gene: Gene organization, transcriptional regulation and potential involvement in neuropsychiatric disorders. *Eur Neuro-psychopharmacol* 11: 449–455.
- Bartrés-Faz D, Junqué C, Serra-Grabulosa JM, et al. (2002) Dopamine DRD2 Taq I polymorphism associates with caudate nucleus volume and cognitive performance in memory impaired subjects. *Neuroreport* 13: 1121–1125.
- Bolton JL, Marioni RE, Deary IJ, et al. (2010) Association between polymorphisms of the dopamine receptor D2 and catechol-o-methyltransferase genes and cognitive function. *Behav Genet* 40: 630–638.
- Braet W, Johnson KA, Tobin CT, et al. (2011) fMRI activation during response inhibition and error processing: The role of the DAT1 gene in typically developing adolescents and those diagnosed with ADHD. *Neuropsychologia* 49: 1641–1650.
- Brehmer Y, Westerberg H, Bellander M, et al. (2009) Working memory plasticity modulated by dopamine transporter genotype. *Neurosci Lett* 467: 117–120.
- Congdon E and Canli T (2008) A neurogenetic approach to impulsivity. *J Pers* 76: 1447–1484.
- Cools R and D'Esposito M (2011) Inverted-U-shaped dopamine actions on human working memory and cognitive control. *Biol Psychiatry* 69 (12): e113–125.
- Cropley VL, Fujita M, Innis RB, et al. (2006) Molecular imaging of the dopaminergic system and its association with human cognitive function. *Biol Psychiatry* 59: 898–907.
- Eisenberg DT, Campbell B, Mackillop J, et al. (2007) Season of birth and dopamine receptor gene associations with impulsivity, sensation seeking and reproductive behaviors. *PLoS One* 2: e1216–e1225.
- Enoch MA and Goldman D (2001) The genetics of alcoholism and alcohol abuse. *Curr Psychiatry Rep* 3: 144–151.
- Floresco SB and Magyar O (2006) Mesocortical dopamine modulation of executive functions: Beyond working memory. *Psychopharmacology* 188: 567–585.
- Floresco SB, Magyar O, Ghods-Sharifi S, et al. (2006) Multiple dopamine receptor subtypes in the medial prefrontal cortex of the rat regulate set-shifting. *Neuropsychopharmacology* 31: 297–309.
- Frank MJ and Fossella JA (2011) Neurogenetics and pharmacology of learning, motivation and cognition. *Neuropsychopharmacology* 36: 133–152.
- Gligorovic M and Buha N (2013) Conceptual abilities of children with mild intellectual disability: Analysis of Wisconsin Card Sorting Test performance. *J Intellect Dev Disabil* 38: 134–140.
- Golden CJ (1978) *Stroop Color and Word Test: Manual for Clinical and Experimental Uses*. Chicago: Stoelting.
- Gordon EM, Devaney JM, Bean S, et al. (2013) Resting-state striato-frontal functional connectivity is sensitive to DAT1 genotype and predicts executive function. *Cereb Cortex* Epub ahead of print 22 August. DOI: 10.1093/cercor/bht229.
- Goudriaan AE, Oosterlaan J, De Beurs E, et al. (2006) Neurocognitive functions in pathological gambling: A comparison with alcohol dependence, Tourette syndrome and normal controls. *Addiction* 101: 534–547.
- Hack LM, Kalsi G, Aliev F, et al. (2011) Limited associations of dopamine system genes with alcohol dependence and related traits in the Irish Affected Sib Pair Study of Alcohol Dependence (IASPSAD). *Alcohol Clin Exp Res* 35: 376–385.
- Heaton RK (1981) *Wisconsin Card Sorting Test manual*. Odessa, FL: Psychological Assessment Resources.
- Hollingshead AB (1975) *Four-factor Index of Social Status*. New Haven, CT: Unpublished manuscript, Yale University, Department of Sociology.
- Hsieh PC, Yeh TL, Lee IH, et al. (2010) Correlation between errors on the Wisconsin Card Sorting Test and the availability of striatal dopamine transporters in healthy volunteers. *J Psychiatry Neurosci* 35: 90–94.
- Hyman SE, Malenka RC and Nestler EJ (2006) Neural mechanisms of addiction: The role of reward-related learning and memory. *Annu Rev Neurosci* 29: 565–598.
- Jocham GT, Klein A, Neumann J, et al. (2009) Dopamine DRD2 polymorphism alters reversal learning and associated neural activity. *J Neurosci* 29: 3695–3704.
- Joutsa J, Johansson J, Niemelä S, et al. (2012) Mesolimbic dopamine release is linked to symptom severity in pathological gambling. *Neuroimage* 60: 1992–1999.
- Kempainen N, Laine M, Laakso MP, et al. (2003) Hippocampal dopamine D2 receptors correlate with memory functions in Alzheimer's disease. *Eur J Neurosci* 18: 149–154.
- Klein TA, Neumann J, Reuter M, et al. (2007) Genetically-determined differences in learning from errors. *Science* 318: 1642–1645.
- Krugel LK, Biele G, Mohr PN, et al. (2009) Genetic variation in dopaminergic neuromodulation influences the ability to rapidly and flexibly adapt decisions. *Proc Natl Acad Sci USA* 106: 17951–17956.
- Lawrence AJ, Luty J, Bogdan NA, et al. (2009) Problem gamblers share deficits in impulsive decision-making with alcohol-dependent individuals. *Addiction* 104: 1006–1015.

- Leber A, Turk-Browne N and Chun M (2008) Neural predictors of moment-to-moment fluctuations in cognitive flexibility. *Proc Natl Acad Sci USA* 105: 13592–13597.
- Lesieur HR and Blume SB (1987) The South Oaks Gambling Screen (SOGS): A new instrument for the identification of pathological gamblers. *Am J Psychiatry* 144: 1184–1148.
- Loo SK, Specter E, Smolen A, et al. (2003) Functional effects of the DAT1 polymorphism on EEG measures in ADHD. *J Am Acad Child Adolesc Psychiatry* 42: 986–993.
- Markett S, Montag C, Walter NT, et al. (2011) On the molecular genetics of flexibility: The case of task-switching, inhibitory control and genetic variants. *Cogn Affect Behav Neurosci* 11: 644–651.
- Mattay VS, Goldberg TE, Fera F, et al. (2003) Catechol-O-methyl-transferase val158-met genotype and individual variation in the brain response to amphetamine. *Proc Nat Acad Sci USA* 100: 6186–6191.
- Mehta MA, Manes FF, Magnolfi G, et al. (2004) Impaired set-shifting and dissociable effects on tests of spatial working memory following the dopamine D2 receptor antagonist sulpiride in human volunteers. *Psychopharmacology* 176: 331–342.
- Meyer G, Schwertfeger J, Exton MS, et al. (2004) Neuroendocrine response to casino gambling in problem gamblers. *Psychoneuroendocrinology* 29: 1272–1280.
- Mill J, Asherson P, Browes C, et al. (2002) Expression of the dopamine transporter gene is regulated by the 3' UTR VNTR: Evidence from brain and lymphocytes using quantitative RT-PCR. *Am J Med Genet* 114: 975–979.
- Moises HW, Frieboes RM, Spelzhaus P, et al. (2001) No association between dopamine D2 receptor gene (DRD2) and human intelligence. *J Neural Transm* 108: 115–121.
- Montag C, Hartmann P, Merz M, et al. (2008) D2 receptor density and prepulse inhibition in humans: Negative findings from a molecular genetic approach. *Behav Brain Res* 187: 428–432.
- Neville MJ, Johnstone EC and Walton RT (2004) Identification and characterization of ANKK1: A novel kinase gene closely linked to DRD2 on chromosome band 11q23.1. *Hum Mutat* 23: 540–545.
- Noble EP (2000) Addiction and its reward process through polymorphisms of the D2 dopamine receptor gene: A review. *Eur Psychiatry* 15: 79–89.
- Noble EP (2003) D2 dopamine receptor gene in psychiatric and neurologic disorders and its phenotypes. *Am J Med Genet B Neuropsychiatr Genet* 116B: 103–125.
- Nyhus E and Barceló F (2009) The Wisconsin Card Sorting Test and the cognitive assessment of prefrontal executive functions: A critical update. *Brain Cogn* 71: 437–451.
- Prakash O, Avasthi A and Benegal V (2012) Should pathological gambling be considered an addictive disorder? *Asian J Psychiatr* 5: 211–214.
- Reitan RM (1958) The validity of the Trail-Making Test as an indicator of organic brain damage. *Percept Motor Skills* 8: 271–276.
- Reuter M, Peters K, Schroeter K, et al. (2005) The influence of the dopaminergic system on cognitive functioning: A molecular genetic approach. *Behav Brain Res* 164: 93–99.
- Ritchie T and Noble EP (2003) Association of seven polymorphisms of the D2 dopamine receptor gene with brain receptor-binding characteristics. *Neurochem Res* 28: 73–82.
- Savitz J, Solms M and Ramesar R (2006) The molecular genetics of cognition: Dopamine, COMT and BDNF. *Genes Brain Behav* 5: 311–328.
- Simon JR, Stollstorff M, Westbay LC, et al. (2011) Dopamine transporter genotype predicts implicit sequence learning. *Behav Brain Res* 216: 452–457.
- Stelzel C, Basten U, Montag C, et al. (2010) Frontostriatal involvement in task switching depends on genetic differences in d2 receptor density. *J Neurosci* 30: 14205–14212.
- Stuss DT and Alexander MP (2000) Executive functions and the frontal lobes: A conceptual view. *Psychol Res* 63: 289–298.
- Tsai SJ, Yu YW, Lin CH, et al. (2002) Dopamine D2 receptor and N-methyl-D-aspartate receptor 2B subunit: genetic variants and intelligence. *Neuropsychobiology* 45: 128–130.
- Wechsler D (1997) Wechsler Adult Intelligence Scale: Third edition administration and scoring manual. San Antonio, TX; Psychological Corporation.
- White MJ, Morris CP, Lawford BR, et al. (2008) Behavioral phenotypes of impulsivity related to the ANKK1 gene are independent of an acute stressor. *Behav Brain Funct* 4: 54–62.
- Wohl M, Boni C, Asch M, et al. (2008) Lack of association of the dopamine transporter gene in a French ADHD sample. *Am J Med Genet B Neuropsychiatr Genet* 147: 1509–1510.
- Zack M and Poulos CX (2009) Parallel roles for dopamine in pathological gambling and psychostimulant addiction. *Curr Drug Abuse Rev* 2: 11–25.