ABCS HEALTH SCIENCES

ORIGINAL ARTICLE

OPEN ACCESS

No association between dopamine D2 receptor (*DRD2*) alleles and crack/cocaine dependence in Brazilians

Milena Binhame Albini Martini[1], Indiara Welter Henn[1], Ingra Taís Malacarne[1], Cleber Machado de Souza[2], Paula Cristina Trevilatto[1], Alexandre Rezende Vieira[3], Luciana Reis Azevedo-Alanis[1]

[1]Programa de Pós-Graduação em Odontologia, Escola de Ciências da Vida, Pontifícia Universidade Católica do Paraná (PUCPR) – Curitiba (PR), Brazil
[2]Escola de Medicina, Pontifícia Universidade Católica do Paraná (PUCPR) – Curitiba (PR), Brazil
[3]Departments of Oral Biology and Pediatric Dentistry, School of Dental Medicine, University of Pittsburgh, Pittsburgh, PA, USA

ABSTRACT

Introduction: The causal mechanisms behind crack/cocaine use are still unknown, but genetic influences are suggested. **Objective:** To investigate the relationship between the genetic polymorphism *TaqI* (rs1800497) in the dopamine D2 receptor (*DRD2*) gene and susceptibility to crack/cocaine dependence in a group of addicts to crack/cocaine and a non-addicted group. **Methods:** The case group (n=515) was composed of crack/cocaine-dependent men and the control group (n=106) comprised men who were considered not dependent on crack/cocaine. The oral hygiene habits, decayed, missing, and filled teeth index, gingival index, and plaque index were evaluated. The reference single nucleotide polymorphism (rs1800497 C/T) of the *DRD2* gene was genotyped by a real-time polymerase chain reaction technique. Student's t-tests for independent samples or the non-parametric Mann-Whitney test were used to compare groups regarding quantitative variables. **Results:** The case group showed a mean time of 9.91±7.03 years of crack use, and 61.06±92.96 stones/week. The socio-demographic profile of the sample was White, single men, with basic education, blue-collar worker, smoker, and reporting alcohol use. There was a high frequency of gingival inflammation, plaque accumulation, and caries experience. For all genetic models tested, there was no significant difference in the genotypic frequency in rs1800497 of the *DRD2* gene, between case and control groups (p>0.05). **Conclusion:** The genetic variant in the *DRD2* did not increase the vulnerability to develop crack/cocaine dependence. The complex genetic nature of crack/cocaine dependence and a large variation of *DRD2* allele frequencies, depending on the population group sampled, could be one explanation for the no association.

Keywords: Crack Cocaine; Biomarkers; Polymorphism; Genetic.

INTRODUCTION

The World Health Organization (WHO) has pointed to Brazil as one of the nations where the consumption of stimulants, such as intranasal (powder) or smoked coca (crack, merla, or oxy), is increasing, while in other countries it is decreasing[1]. Some reasons for the high consumption of crack/cocaine in Brazil are as follows: (i) the geographic position, neighboring the world's largest cocaine producers, Peru, Colombia, and Bolivia, (ii) the young population (about 35% of Brazilians is 15 to 34 years of age)[2], (iii) the socio-economic raise in the last decade in Brazil, which reflects in higher purchasing power, and (iv) the low cost of crack/cocaine in the country[1,3].

How to cite this article: Martini et al. No association between dopamine D2 receptor (DRD2) alleles and crack/cocaine dependence in Brazilians. ABCS Health Sci. 2022;47:e022219 https://doi.org/10.7322/abcshs.2020207.1652

Received: Dec 18, 2020
Revised: Apr 20, 2021
Approved: Apr 21, 2021

Corresponding author: Luciana Reis Azevedo-Alanis - Programa de Pós-Graduação em Odontologia, Escola de Ciências da Vida - Pontifícia Universidade Católica do Paraná - Rua Imaculada Conceição 1155 – Prado Velho – CEP: 80215-182 Curitiba (PR), Brazil - Email: l.azevedo@pucpr.br

Declaration of interests: nothing to declare
Funding: CAPES

The causal mechanisms behind substance use are still largely unknown, but genetic influences are suggested[4]. Some epidemiological studies have shown that about half of the individual risk for addiction to cocaine or other drugs is due to genetics[5,6].

One of the major neurobiological systems involved in cocaine addiction is the brain reward pathway. Cocaine also has effects on other neurotransmitter systems, such as norepinephrine and serotonin, but dopamine is the most important neurotransmitter[7]. Cocaine increases dopamine neurotransmission at the expense of dopamine reserves, which might be viewed as a positive reinforcer, creating a vicious cycle in which the pleasure neurotransmitter is emptied. Furthermore, cocaine produces rebound and uncontrollable effects[8]. The continued use of the drug leads to the depletion of postsynaptic dopamine receptors, requiring greater synaptic levels of dopamine to maintain the synaptic boost that provides the equivalent effect[9]. Each dopamine receptor, dopamine transporter, and enzymes involved in the synthesis and metabolism of dopamine are proteins. Thus, the genes encoding these proteins are interesting targets for the study of susceptibility to cocaine dependence.

Genetic polymorphisms of the dopaminergic system include single nucleotide polymorphisms (SNPs) and length polymorphisms of the receptors, transporters, and metabolizing enzymes. The dopamine D2 receptor gene (*DRD2*) is localized in the q22-q23 region on chromosome 11. One SNP in *DRD2* is rs1800497, which presents a C-to-T transition, and produces an amino-acid change (Glu for Lys) in position 713. This exchange between those bases may significantly reduce the specificity of receptor binding[10].

The dopamine D2 receptor was the first to be studied in 1990; there was an increased frequency of the A1 allele (thymine) of the *Taq*I polymorphism in alcoholics[11]. Among the genetic variants associated with smoking, the *DRD2 Taq*I variant is one of the most widely studied[12]. The first study evaluating cocaine dependence investigated the TaqI variant of *DRD2* and found a higher frequency of the A1 allele (thymine)[13] in cocaine users compared to non-users. Three subsequent studies also evaluated this association but they did not confirm that finding[14-16].

Scientists currently view cocaine addiction as a brain disease[17], a notion that modifies the original idea that addicted individuals merely have character deficiencies or a lack of willpower[8]. Even now, very little is known about the distribution of genetic variants that modulate hypothalamic-pituitary-adrenal axis deregulation in users who are resistant to crack/cocaine dependence compared to those who have become dependent. The definition of a genomic profile that brings a predictable response to crack/cocaine exposure could reveal new information regarding dependence. The *Taq*I polymorphism has been associated with addictive disorders such as alcoholism and polysubstance abuse[18,19] and here we proposed to analyze a sample of individuals dependent on crack/cocaine.

Therefore, the objective of this study was to investigate the relationship between the genetic polymorphism *Taq*I (rs1800497) in the dopamine D2 receptor (*DRD2*) gene and the susceptibility to crack/cocaine dependence in a group of addicts to crack/cocaine and a non-addicted control group with the previous contact with crack/cocaine.

METHODS

Design

This study included a cohort of male individuals who have used crack/cocaine and compared crack/cocaine dependents based on ASSIST[20] with individuals who have used crack/cocaine but were not dependent.

Participants

This study was approved by the local Committee of Ethics in Research (number 908.511). Using a statistic proportion sample calculation for a confidence interval of 95% and a maximum error of 6%, assuming a p=(1-p)=0.5, the sample size was based on the incidence of hospitalization of crack/cocaine users in clinics of chemical addiction rehabilitation located in the metropolitan region of Curitiba, south of Brazil Hospital San Julian, *Instituto the Pesquisa and Tratamento of Alcoolismo* (IPTA), and *Centro the Atenção Psicossocial Álcool and Drogas* (CAPS-AD) of approximately 1,680 male patients, resulting in 443 patients. All the subjects signed a free consent agreement before participating in the study.

The inclusion criteria for the case group were individuals that were admitted in the rehabilitation clinics, with a score of 16 or over for crack/cocaine on the screening test of smoking, alcohol, and other drug use[20] and with a maximal admission time of 15 days.

The sample was recruited as follows: Case group: 515 crack/cocaine dependents were recruited from Hospital San Julian, IPTA, and CAPS-AD; Control group: 106 not crack/cocaine dependents, but with the previous contact with crack/cocaine, were recruited from IPTA and CAPS-AD.

Measures

The subjects answered an interview with their personal, medical, and dental history, describing age, ethnicity, marital status, education level, job description, consumption, time, and quantity of alcohol, tobacco, and crack/cocaine use. For the job description, we used the Brazilian classification, and to evaluate the monthly income we used the demographic screening used by the *Instituto Brasileiro de Geografia e Estatística* (IBGE)[2]. Smoking and alcohol use were classified as present or absent and rated by the time of use (years), frequency, and quantity. The smoking habit was

classified as mild (1 to 9 cigarettes/day), moderate (10 to 19 cigarettes/day), and severe (20 or more cigarettes/day). The alcohol use was classified as mild (1 to 100 mL/week), moderate (101 to 300 mL/week), and severe (≥301 mL/week). The use of crack/cocaine was assessed by time of use (years), frequency (week), and quantity (1 stone=0.25g).

The clinical standards evaluated were oral hygiene habits (do you brush your teeth? do you floss? have you been to the dentist?), decayed, missing, and filled teeth (DMFT) index, gingival index (GI), plaque index (PI). For the data analyses, the higher frequency of GI and PI for each tooth was considered. GI and PI were classified as absent (degree 0) or present (degree 1, 2, and 3)[21].

Genetic Analysis

DNA from the participants of the study was obtained through a mouthwash with 3% glucose solution and scrapings of the buccal mucosa for 60 seconds. The DNA was purified with 10 M of ammonium acetate and 1 mM EDTA. Genotyping methods included restriction fragment length polymorphism, allele-specific amplification, and real-time polymerase chain reaction (PCR). The reference SNP (rs1800497 C/T) of the *DRD2* gene was genotyped by the real-time PCR technique (Applied Biosystems 7500 Real-Time PCR System), with the use of technology TaqMan™ (Applied Biosystems). This marker was chosen concerning the previous studies[12,16,22]. For genotyping procedures for the *DRD2 Taq*IA (rs1800497), we used the same method with specific primer pairs. TaqIA: forward: 59-CCG TCG ACG GCT GGC CAA GTT GTC TA-39, reverse: 59-CCG TCG ACC CTT CCT GAG TGT CAT CA-39. The rs1800497 was assessed for genotypic modes of transmission (additive, dominant and recessive models). The dominant model was assembled using the homozygous genotype for less frequent allele (T) with heterozygous compared to the homozygous genotypes.

Statistical Analyses

Statistical data were processed and analyzed using the SPSS, version 22 (IBM SPSS Statistics, Chicago, IL). Haploview 4.2 was used to estimate the Hardy-Weinberg equilibrium for the genetic marker.

Quantitative variables were described by mean and standard deviation, or by the median, minimum and maximum value. Qualitative variables were described by frequencies and percentages. Student's *t*-test for independent samples or the non-parametric Mann-Whitney test was used to compare the groups about quantitative variables. As a measure of association, odds ratios (ORs) with a 95% confidence interval were estimated. Values of $p<0.05$ indicated statistical significance.

For the multivariate analysis, firstly, the regression model, considering as a response variable the group of crack/cocaine dependents (case group), was adjusted to include explicative variables presenting *p*-values <0.20 in the univariate analysis (i.e. age, ethnicity, marital status, education levels, job description, number of cigarettes/day, alcohol use, alcohol quantity/week, use of dental floss, DMFT Index, and genetic variable). After this initial process, the final model of this logistic regression analysis was obtained by gathering the explanatory variables that remained significant.

A random-effects meta-analysis model was performed to estimate the odds ratios of the allele frequency for this study and the 4 previously published studies in White ethnicity (Brazilian and European American) and Black ethnicity (African American)[13-16]. The heterogeneity test was used as a measure for meta-analysis using Cochrane's Review Manager (RevMan 5, version 5.3, Copenhagen, Netherlands). We measured the heterogeneity for all 5 studies combined (Figure 1; I^2 =63%, p=0.15).

RESULTS

Socio-demographic profile

A total of 621 men participated in this study. The case group was composed of 515 crack/cocaine users, with a mean time of 9.91±7.03 years of crack use, 61.06±92.96 stones/week (1 stone: 0.25g), and 142.33±147.80 days of hospitalization. The socio-demographic profile of the sample is described in Table 1.

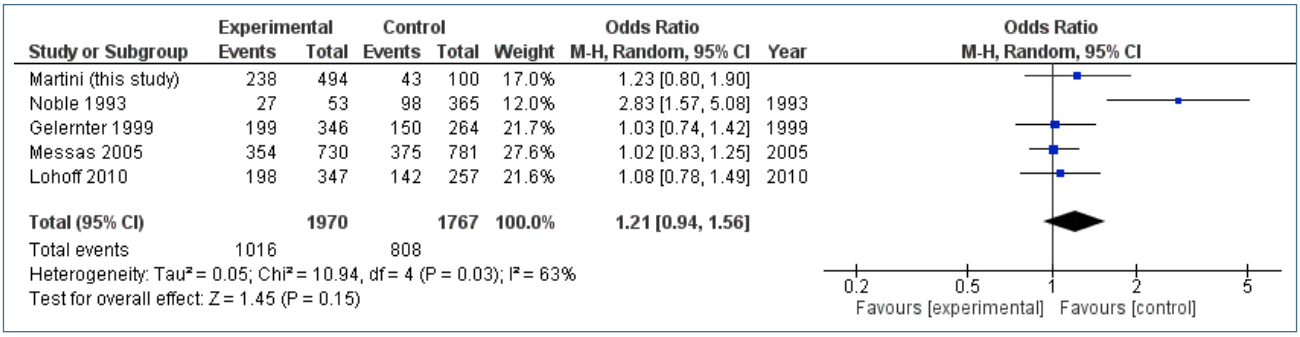

Figure 1: Meta-analysis of the genetic polymorphism *Taq*I (re1800497 – 11q22-q23) in the *DRD2* gene in individuals susceptible to crack/cocaine dependence.

Table 1: Epidemiological data of the sample.

Variable		Case Group n=515	Control Group n=106	P-Value	OR (CI 95%)
Age (years)	Mean±SD	33.83±8.765	35.28±9.611	0.083	
Ethnicity	n (%)			0.001*	
White		326 (63.5)	82 (77.4)		
Black		157 (30.5)	14 (13.2)		
Others		32 (6.0)	10 (9.4)		
Marital status	n (%)			0.035*	
Single		352 (68.3)	64 (60.4)		
Married		71 (13.8)	12 (11.3)		
Divorced		41 (7.9)	19 (17.9)		
Cohabitation		51 (10.0)	11 (10.4)		
Education levels	n (%)			0.014*	
Basic Education		274 (53.1)	56 (52.9)		
Secondary		192 (37.3)	29 (27.4)		
Higher		49 (9.6)	21 (19.7)		
Job description	n (%)			0.123	
Armed Forces and Police		6 (1.2)	5 (3.8)		
Members of public authority		14 (2.8)	7 (5.8)		
Science Professionals		10 (2.0)	3 (2.0)		
Middle-level technicians		35 (6.8)	9 (7.7)		
Administrative services		18 (3.6)	9 (7.7)		
Sellers of commerce		102 (20.1)	22 (19.2)		
Agriculture and forestry		21 (4.2)	5 (3.8)		
Industrial services		107 (20.9)	13 (12.5)		
Maintenance and Repair		195 (38.4)	40 (37.5)		
Smoker	n (%)			0.907	1.042 (0.524–2.072)
Yes		463 (89.9)	95 (89.5)		
No		52 (10.1)	11 (10.5)		
Tobacco use (years)	Mean±SD	15.94±9.123	16.66±10.538	0.753	
Number of cigarettes/day	n (%)			0.126	
Mild – (1 to 9)		92 (19.9)	23 (24.2)		
Moderate – (10 to 19)		112 (24.2)	27 (28.4)		
Severe – (≥20)		259 (55.9)	45 (47.4)		
Use of alcohol	n (%)			0.457	1.238 (0.704–2.177)
Yes		442 (85.8)	88 (83.0)		
No		73 (14.2)	18 (17.0)		
Alcohol use (years)	Mean±SD	16.14±9.012	18.51±9.518	0.034*	
Alcohol quantity/week (mL)	n (%)			0.042*	
Mild – (1 to 100)		35 (7.9)	4 (4.6)		
Moderate – (101 to 300)		57 (12.9)	6 (6.8)		
Severe (≥301)		350 (79.2)	78 (88.6)		

*p<0.05

Clinical Parameters

All the results of the clinical parameters evaluated are shown in Table 2.

Genetic Analysis

The polymorphisms evaluated were in Hardy-Weinberg equilibrium (Table 3). There was no statistically significant difference in the genotypic frequency in rs1800497 of the *DRD2* gene, for all genetics models, between case and control groups. The full data is described in Table 3.

Multivariate analysis

The regression was significantly associated to crack/cocaine dependence, after adjustment for licit drugs (number of cigarettes/day and alcohol use/years).

DISCUSSION

Drug addiction is a multifactorial condition affected by psychological, physiological, pharmacological, genetic, and environmental variables[23]. Dopamine is one of the most important

Table 2: Clinical Parameters of participants

Variable		Case Group n=515	Control Group n=106	P-value	OR (CI 95%)
Do you brush your teeth?	n (%)			0.947	0.970 (0.393–2.392)
Yes		485 (94.2)	100 (94.3)		
No		30 (5.8)	6 (5.7)		
Do you floss?	n (%)			0.005*	0.526 (0.335–0.825)
Yes		113 (22.0)	37 (34.9)		
No		402 (78.0)	69 (65.1)		
Have you been to the dentist?	n (%)			0.456	0.774 (0.394–1.521)
Yes		448 (87.0)	95 (89.6)		
No		67 (13.0)	11 (10.4)		
Gingival Inflammation	n (%)			0.760	0.819 (0.227–2.954)
Presence		503 (97.7)	103 (97.2)		
Absence		12 (2.3)	3 (2.8)		
Bacterial Plaque	n (%)			0.221	0.644 (0.316–1.310)
Presence		479 (93.0)	94 (89.5)		
Absence		36 (7.0)	11 (10.5)		
DMFT	Mean±SD	12.95 (6.69)	12.33 (7.43)	0.170	
DMFT Index	n (%)			0.093	0.696 (0.455–1.064)
Less than 12		260 (50.5)	63 (59.4)		
12 or more		255 (49.5)	43 (40.6)		
Teeth Decayed	Mean±SD	4.43 (3.48)	3.06 (3.45)	<0.001*	
Teeth Missing	Mean±SD	4.72 (6.27)	5.20 (7.02)	0.009*	
Teeth Filled	Mean±SD	3.81 (3.59)	3.98 (3.79)	0.632	

*p<0.05

Table 3: Results of the univariate analysis for the *DRD2* gene tag SNPs (rs1800497) between the case group (n=494) and the control group (n=100).

		Group	Classification			P-Value	OR (CI95%)	HW
			TT	TC	CC			
rs1800497	Additive	Case	39 (7.9)	199 (40.3)	256 (51.8)			1.000
		Control	7 (7.0)	36 (36.0)	57 (57.0)	0.639	-	0.7889
			CC	TT/TC				
rs1800497	Dom T	Case	256 (51.8)	238 (48.2)	-			
		Control	57 (57.0)	43 (43.0)	-	0.344	0.811 (0.526–1.252)	
			TT	TC/CC				
rs1800497	Rec T	Case	39 (7.9)	455 (92.1)	-			
		Control	7 (7.0)	93 (93.0)	-	0.760	1.139 (0.494–2.624)	

Dom: dominant model; Rec: recessive model; OR: odds ratio; CI: confidence interval; HW: Hardy-Weinberg equilibrium.

neurotransmitters acting in the reward pathways in the brain[15,24,25]. Genes encoding dopaminergic receptors are interesting targets for the study of susceptibility to crack/cocaine dependence. Therefore, in the present study, the role of *DRD2* polymorphism (rs1800497) was investigated in crack/cocaine dependence.

Our data do not support an allelic association between *DRD2* and crack/cocaine dependence. This result is consistent with the studies that followed the first one, which has shown no association of *DRD2* alleles with the phenotype of cocaine dependence[14-16]. This finding can be explained either by a weak effect of some still unmapped *DRD2* polymorphism on the phenotype or by no effect of *DRD2* on the phenotype[14]. Our data suggest that *DRD2* is not involved in crack/cocaine dependence; however, additional markers are needed for complete gene coverage to comprehensively rule out the role of the gene in crack/cocaine dependence[16]. Our findings show the same effect previously reported[26] that showed that the first genetic association study reports an effect much stronger than subsequent studies. Both bias and genuine undetected population substructure might explain why early association studies tend to overestimate the disease protection or predisposition conferred by a polymorphism. In the particular case of the *DRD2 Taq*I variant, when the first report of an association with alcoholism was published[27], a critique[28] regarding the comparison group used for the association studies (unscreened versus screened controls for alcoholism) was suggested. This was one of the reasons to carefully select a comparison group for our study that has been exposed to crack/cocaine.

The first study evaluating cocaine dependence investigated the *DRD2* gene and showed a strong association of the alleles Al and Bl (T and C) with cocaine dependence. The prevalence of the T allele in White cocaine-dependent subjects was significantly higher than in non-substance abusing controls[13]. Persico et al.[18] also confirmed this association in White polysubstance users, including cocaine. The positive findings may have been due to some set of phenomena not necessarily including genetic variation in the *DRD2* locus causing change to get in responsibility for dependence on other drugs. A subsequent study also evaluated this association in European American and African American subjects but did not confirm such association[14]. A study with a sample of 730 Brazilian participants investigated polymorphisms of *DRD2* and *DRD3* and found no association with cocaine dependence[15], as well as the study developed with 347 cocaine addicts and 257 non-dependent afro-descendants[16].

To investigate more precisely the relationship between the genetic polymorphism *Taq*I (rs1800497) in the *DRD2* gene and the susceptibility to crack/cocaine dependence, we decided to use a meta-analytical approach. This approach reinforced that the genetic polymorphism *Taq*I (rs1800497) in the *DRD2* gene is probably not a relevant risk factor in the susceptibility to crack/cocaine dependence.

In the present study, the number of cigarettes/day and alcohol use (years) was significantly associated to crack/cocaine dependence. We can observe the presence of heavy alcoholic subjects and smokers in this study. Crack/cocaine users are usually multiple drug users or have a history of consuming other substances. The history of crack/cocaine users demonstrates that most of them started with the consumption of licit drugs (tobacco and alcohol) at an early age and with heavy use[29,30].

Several studies report that the majority of crack/cocaine users are young, single, men, with low educational levels and monthly income[30,31], and, on average, they had smoked crack/cocaine for 10 years[21,31]. This is by the profile of the present studied sample. Young subjects are the majority among crack/cocaine users, probably due to the vulnerability to death by external causes, mainly violent deaths[30].

The quality of life and lifestyle adopted by the users seem to define their oral condition because the abusive consumption of drugs is considered a risk factor for oral diseases in the worldwide population[32]. In crack/cocaine dependents of the present study, it was observed a decreased frequency in flossing and a higher decayed teeth index when compared to the control group, showing a positive association with crack/cocaine addiction. Furthermore, missing teeth showed a negative association with crack/cocaine dependence, to Cury et al.[33], which reflects the typical lack of self-care in drug users. This could be a consequence of higher access to dental treatment by the control group compared to the case group. These findings are by Albini et al.[21], who described that poor oral hygiene was predominant in crack/cocaine users, with high DMFT index, decayed teeth, missing teeth, presence of gingival inflammation, and dental biofilm. Besides that, the lack of dental care may have contributed to the results[33,34].

It is necessary to emphasize some limitations of this study. First, the group of addicts studied did not represent all drug addicts, as it only included subjects that had sought help and were hospitalized. Second, recognizing that sex is an important factor in substance abuse studies, for logistical reasons (the setting of our study did not treat females, who are treated elsewhere), only males were included in the present study, which excludes the possibility of finding sex-specific associations. Finally, we must recognize that the status of controls was established by self-report and therefore some participants in the control group could have hidden or underestimated exposure to substances of abuse.

Conclusion

Based on our original data, we conclude that the studied genetic variant in the *DRD2* (rs1800497) does not increase the chance of developing cocaine dependence. The complex genetic nature of crack/cocaine dependence and population stratification could be a potential explanation for the no association of the T alleles

with cocaine dependence in the present findings; indeed, a large variation of *DRD2* allele frequencies is found, depending on the population group sampled[35,36]. Studies using additional cohorts of ethnicity and carefully defined addiction phenotypes are needed for the confirmation and identification of new targets for the prevention of crack/cocaine dependence[17].

ACKNOWLEDGMENTS

The authors would like to thank the Hospital San Julian, Instituto de Pesquisa e Tratamento do Alcoolismo (IPTA) and Centro de Atenção Psicossocial Álcool e Drogas (CAPS-AD), for the opportunity of conducting this work in its facility, and Patrícia Tolentino da Rosa de Souza, for revising the statistical analysis.

REFERENCES

1. United Nations Office on Drugs and Crime (UNODC). World drug report: 2014. Vienna: United Nations, 2014.

2. Instituto Brasileiro de Geografia e Estatística (IBGE). Sidra. Available from: https://sidra.ibge.gov.br/tabela/3705

3. Abdalla RR, Madruga CS, Ribeiro MIP, Caetano R, Laranjeira R. Prevalence of cocaine use in Brazil: data from the II Brazilian National Alcohol and Drugs Survey (BNADS). Addict Behav. 2014;39(1):297-301. http://doi.org/10.1016/j.addbeh.2013.10.019

4. Waaktaar T, Kan K, Torgersen S. The genetic and environmental architecture of substance use development from early adolescence into young adulthood: a longitudinal twin study of comorbidity of alcohol, tobacco, and illicit drug use. Addiction. 2018;113(4):740-8. http://doi.org/10.1111/add.14076

5. Nestler EJ, Malenka RC. The addicted brain. Sci Am. 2004;290(3):78-85. http://doi.org/10.1038/scientificamerican0304-78

6. Agrawal A, Lynskey MT. Are there genetic influences on addiction: evidence from family, adoption and twin studies? Addiction. 2008;103(7):1069-81. http://doi.org/10.1111/j.1360-0443.2008.02213

7. Koob GF, Le Moal M. Drug abuse: hedonic homeostatic dysregulation. Science. 1997;278(5335):52-8. http://doi.org/10.1126/science.278.5335.52

8. Dackis CA, O'Brien CP. Cocaine dependence: a disease of the brain's reward centers. J Subst Abuse Treat. 2001;21(3):111-17. http://doi.org/10.1016/s0740-5472(01)00192-1

9. Lambert G, Karila L, Lowenstein W. Neuroimaging and cocaine: mapping dependence?. Presse Med. 2008;37(4 Pt 2):679-88. http://doi.org/10.1016/j.lpm.2007.08.014

10. Vereczkei A, Demetrovics Z, Szekely A, Sarkozy P, Antal P, Szilagyi A, et al. Multivariate analysis of dopaminergic gene variants as risk factors of heroin dependence. PLoS One. 2013;8(6):66592. http://doi.org/10.1371/journal.pone.0066592

11. Ponce G, Pérez-González R, Aragüés M, Palomo T, Rodríguez-Jiménez R, Jiménez-Arriero MA, et al. The ANKK1 kinase gene and psychiatric disorders. Neurotox Res. 2009;16(1):50-9. http://doi.org/10.1007/s12640-009-9046-9

12. Huang Y, Ren HT, Zou Q, Wang YQ, Zhang JL, Yu XL. Computational identification and characterization of miRNAs and their target genes from five A1 Cyprinidae fishes. Saudi J Biol Sci. 2017;24(6):1126-35. http://doi.org/10.1016/j.sjbs.2015.05.007

13. Noble EP, Blum K, Khalsa ME, Ritchie T, Montgomery A, Wood RC, et al. Allelic association of the D2 dopamine receptor gene with cocaine dependence. Drug Alcohol Depend. 1993;33(3):271-85. http://doi.org/10.1016/0376-8716(93)90113-5

14. Gelernter J, Kranzler H, Satel SL. No association between D2 dopamine receptor (DRD2) alleles or haplotypes and cocaine dependence or severity of cocaine dependence in Europeans and African-Americans. Biol Psychiatry. 1999;45(3):340-5. http://doi.org/10.1016/s0006-3223(97)00537-4

15. Messas G, Meira-Lima I, Turchi M, Franco O, Guindalini C, Castelo A, et al. Association study of dopamine D2 and D3 receptor gene polymorphisms with cocaine dependence. Psychiatr Genet. 2005;15(3):171-4. http://doi.org/10.1097/00041444-200509000-00006

16. Lohoff FW, Bloch PJ, Hodge R, Nall AH, Ferraro TN, Kampman KM, et al. Association analysis between polymorphisms in the dopamine D2 receptor (DRD2) and dopamine transporter (DAT1) genes with cocaine dependence. Neurosci Lett. 2010;473(2):87-91. http://doi.org/10.1016/j.neulet.2010.02.021

17. Yuferov V, Levran O, Proudnikov D, Nielsen DA, Kreek MJ. Search for genetic markers and functional variants involved in the development of opiate and cocaine addiction and treatment. Ann N Y Acad Sci. 2010;1187:184-207. http://doi.org/10.1111/j.1749-6632.2009.05275

18. Persico AM, Bird G, Gabbay FH, Uhl GR. D2 dopamine receptor gene TaqI A1 and B1 restriction fragment length polymorphisms: enhanced frequencies in psychostimulant-preferring polysubstance abusers. Biol Psychiatry. 1996;40(8):776-84. http://doi.org/10.1016/0006-3223(95)00483-1

19. Smith L, Watson M, Gates S, Ball D, Foxcroft D. Meta-analysis of the association of the Taq1A polymorphism with the risk of alcohol dependency: a HuGE gene-disease association review. Am J Epidemiol. 2008;167(2):125-38. http://doi.org/10.1093/aje/kwm281

20. WHO ASSIST Working Group. The Alcohol, Smoking, and Substance Involvement Screening Test (ASSIST): development, reliability, and feasibility. Addiction. 2002;97(9):1183-94. http://doi.org/10.1046/j.1360-0443.2002.00185.x

21. Albini MB, Couto ACF, Invernici MM, Martins MC, Lima AAS, Gabardo MCL, et al. Socio-demographic profile and oral health status of drug users in two municipalities of the State of Paraná, Brazil. Rev Odontol UNESP. 2015; 44:244-9. https://doi.org/10.1590/1807-2577.1068

22. Fernàndez-Castillo N, Ribasés M, Roncero C, Casas M, Gonzalvo B, Cormand B. Association study between the DAT1, DBH and DRD2 genes and cocaine dependence in a Spanish sample. Psychiatr Genet. 2010;20(6):317-20. https://doi.org/10.1097/YPG.0b013e32833b6320

23. Isaza C, Henao J, Beltrán L, Porras L, Gonazalez M, Cruz R, et al. Genetic variants associated with addictive behavior in Colombian addicted and non-addicted to heroin or cocaine. Colomb Med (Cali). 2013;44(1):19-25.

24. Powledge TM. Addiction and the brain: The dopamine pathway is helping researchers find their way through the addiction maze. BioScience. 1999;49(7):513-19. https://doi.org/10.2307/1313471

25. Comings DE, Blum K. Reward deficiency syndrome: genetic aspects of behavioral disorders. Prog Brain Res. 2000;126:325-41. https://doi.org/10.1016/S0079-6123(00)26022-6

26. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG. Replication validity of genetic association studies. Nat Genet. 2001;29(3):306-9. https://doi.org/10.1038/ng749

27. Gelernter J, Goldman D, Risch N. The A1 allele at the D2 dopamine receptor gene and alcoholism: a reappraisal. J Am Med Assoc. 1993;269(13):1673-7.

28. Noble EP, Blum K. Alcoholism and the D2 dopamine receptor gene. JAMA. 1993;270(13):1547. https://doi.org/10.1001/jama.1993.03510130053027

29. Guindalini C, Vallada H, Breen B, Laranjeira R. Concurrent crack and powder cocaine users from Sao Paulo: do they represent a different group? BMC Public Health. 2006;6:10. https://doi.org/10.1186/1471-2458-6-10

30. Duailibi LB, Ribeiro M, Laranjeira R. Profile of cocaine and crack users in Brazil. Cad Saude Publica. 2008;24(Suppl 4):545-57. https://doi.org/10.1590/s0102-311x2008001600007

31. Miguel AQC, Madruga CS, Cogo-Moreira H, Yamauchi R, Simões V, Silva CJ, et al. Sociodemographic characteristics, patterns of crack use, concomitant substance use disorders, and psychiatric symptomatology in treatment-seeking crack-dependent individuals in Brazil. J Psychoactive Drugs. 2018;50(4):367-72. https://doi.org/10.1080/02791072.2018.1436729

32. D'Amore MM, Cheng DM, Kressin NR, Jones J, Samet JH, Winter M, et al. Oral health of substance-dependent individuals: impact of specific substances. J Subst Abuse Treat. 2011;41(2):179-85. https://doi.org/10.1016/j.jsat.2011.02.005

33. Cury PR, Oliveira MG, de Andrade KM, de Freitas MD, Dos Santos JN. Dental health status in crack/cocaine-addicted men: a cross-sectional study. Environ Sci Pollut Res Int. 2017;24(8):7585-90. https://doi.org/10.1007/s11356-017-8404-z

34. Sordi MB, Massochin RC, Camargo AR, Lemos T, Munhoz EA. Oral health assessment for users of marijuana and cocaine/crack substances. Braz Oral Res. 2017;31:102. https://doi.org/10.1590/1807-3107BOR-2017.vol31.0102

35. Goldman D, Brown GL, Albaugh B, Robin R, Goodson S, Trunzo M, et al. DRD2 dopamine receptor genotype, linkage disequilibrium, and alcoholism in American Indians and other populations. Alcohol Clin Exp Res. 1993;17(2):199-204. https://doi.org/10.1590/1807-3107BOR-2017.vol31.0102

36. Castiglione CM, Deinard AS, Speed WC, Sirugo G, Rosenbaum HC, Zhang Y, et al. Evolution of haplotypes at the DRD2 locus. Am J Hum Genet. 1995;57(6):1445-56.