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Study of polymorphic variants of the serotonin 2A receptor gene (*5-HT2A*) and its possible effects on smoking habits of a population from northeastern Brazil

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ABSTRACT. Previous studies have revealed a genetic component, including genetic polymorphisms in the serotonergic pathway, particularly in the serotonin receptor gene (*5-HT2A*). The aim of this study was to investigate associations of the T102C (rs6313) and A-1438G (rs6311) polymorphisms with tobacco use in a population from northeastern Brazil. We evaluated these polymorphisms in 135 nonsmokers and 135 smokers using polymerase chain reaction-restricted fragment length polymorphism. The distribution of allele and genotype frequencies and associations of polymorphisms with smoking were assessed with the chi-squared (χ^2) test, the Fisher exact test, and odds ratio (OR) with a 95% confidence interval (CI). There were no differences in the distribution of genotype and allele frequencies between nonsmokers and smokers for A-1438G (P = 0.80) and T102C (P = 0.35). However, these

polymorphisms were significantly associated with habit frequency (A/G: P = 0.02, OR = 6.87, 95%CI = 1.23-38.31, P = 0.04; A/G+G/G: P = 0.04, OR = 3.67, 95%CI = 1.06-12.75, P = 0.07), age of onset (C/C: P = 0.02, OR = 3.26, 95%CI = 1.17-9.07, P = 0.03, and nicotine dependence level (A/G: P = 0.02, OR = 3.28, 95%CI = 1.17-9.18, P = 0.04; A/G+G/G: P = 0.04, OR = 2.81, 95%CI = 1.13-6.99, P = 0.04; T/C: P = 0.03, OR = 3.12, 95%CI = 1.13-8.57, P = 0.04; T/C+C/C: P = 0.02, OR = 3.06, 95%CI = 1.22-7.70, P = 0.02). Therefore, these polymorphisms may not contribute significantly to smoking initiation, they do appear to be associated with habit maintenance.

Key words: Tobacco smoking; Polymorphisms; Serotonin

INTRODUCTION

Tobacco smoking continues to pose a serious public health problem, since the adverse effects of nicotine on different organ systems result in many diseases that are associated with high morbidity and mortality including strokes, hypertension, myocardial infarction, and chronic obstructive pulmonary disease. It is estimated that there are 1.3 billion smokers worldwide, with male prevalence. In Brazil, approximately 16.7 million and 11.2 million smokers are males and females, respectively (Menezes, 2004; Torres and Godoy, 2004; Santos, 2007).

Contributions of both genetic and environmental factors are relevant to the initiation and maintenance of the smoking habit. However, genetic aspects are considered to be relatively more important to smoking initiation and nicotine dependence (do Prado-Lima et al., 2004; Chatkin, 2006). Genetic polymorphisms of the serotonergic pathway, such as the serotonin receptor-type 2A gene (*5-HT2A*) have been associated with smoking behavior (Arinami et al., 2000).

Nicotine increases the activity of neurons in the ventral tegumental area (VTA), which is related to rewards effects, and supports the release of dopamine in this system. This effect results from the ratio of nicotine to nicotinic cholinergic receptors (nAChR) present in cell bodies and VTA dopaminergic neuronal endings. It is also known that the serotonin receptor type 2A modulates dopaminergic activity (Benowitz, 1996; Di Chiara, 2000; do Prado-Lima et al., 2004; White et al., 2011).

Serotoninergic receptors are classified into seven groups (5-HT 1-7). The type 2 receptors are classified into three subtypes: A, B, and C (do Prado-Lima et al., 2004). The 5-HT2A receptor gene is located on chromosome 13 (13q14-q21) and consists of three exons and two introns, extending over more than 63 kb (Sorlí et al., 2008; Choi et al., 2010). This gene has two known functional single nucleotide polymorphisms (SNPs), T102C (rs6313) and A-1438G (rs6311), which were related to smoking habit (do Prado-Lima et al., 2004, Polina et al., 2009). The -1438A-G polymorphism is located in the promoter region and is in moderate linkage disequilibrium (LD) with the T102C polymorphism in the first exon (Saiz et al., 2009; Choi et al., 2010; Hranilovic et al., 2010). Previous studies have shown that the -1438G allele results in a decrease in promoter activity, whereas the 102C allele also determines gene expression changes resulting in decreased synthesis of 5-HT2A receptors (do Prado-Lima et al., 2004; Kim et al., 2008; Hranilovic et al., 2010).

The aim of this study was to investigate the association of these polymorphisms with some relevant aspects related to smoking habits in a population of smokers in Parnaíba city, State of Piauí, Brazil.

MATERIAL AND METHODS

Study population

The patient sample consisted of 270 individuals, 135 nonsmokers (never smoked) and 135 smokers, which were being preferably treated in state and municipal institutions in the region of Parnaíba, Piauí, Brazil. The control group had a mean age (± SD) of 60 (± 20) years, with a prevalence of approximately 1.49 men for every woman. In the smoking group, the average age was 51 (± 21), with a prevalence of approximately 1.75 men for every woman. All participants completed a questionnaire to assess sociodemographic data and detailed smoking history (frequency of habit, age of onset of regular habit, number of cigarettes smoked per day, attempts to quit, and abstinence time upon abandonment). The subjects were classified with respect to the level of nicotine dependence as low, medium, and high dependency, according the Fagerström test (Kirchenchtejn and Chatkin, 2004).

Smoking status was determined by adapting the classification system used by Sieminska et al. (2009). Current smokers were defined as individuals who, at the time of survey, smoked frequently (daily and at least three times per week) or occasionally (less than three times per week). A nonsmoker was defined as someone who never smoked. The study protocol was approved by the Ethics Committee of the Federal University of Piauí, Brazil. All participants signed an informed consent form.

DNA extraction and genotyping

DNA was isolated from peripheral blood using the Wizard® Genomic DNA Purification kit (Promega, Inc., USA) according to manufacturer instructions. Genotyping was performed using polymerase chain reaction followed by enzymatic digestion with restriction endonucleases (PCR-RFLP).

The cycling conditions for the polymorphism A-1438G were: 4 min at 94°C; 35 cycles of 30 s at 94°C, 30 s at 60°C, and 45 s at 72°C; and a final extension at 72°C for 4 min. For the T102C polymorphism, cycling conditions were: 5 min at 95°C; 35 cycles of 1 min at 95°C, 2 min at 56°C and 2 min at 72°C; and a final extension at 72°C for 4 min.

For the A-1438G polymorphism, PCR was performed using the following primers: 5'-AACCAACTTATTTCCTACCAC-3' (forward) and 5'-AAGCTGCAAGGTAGCAACAGC-3' (reverse), generating a 469-bp product. For the T102C polymorphism, PCR was performed using the following primers: 5'-CAAGGTGAATGGTGAGCAGA-3' (forward) and 5'-GGCATTCTGCAGCTTTTTCT-3' (reverse), forming a 399-bp PCR product.

Both PCR products were digested with 2 U *Hpa*II enzyme (NEB, USA) at 37°C for 16 h, revealing the standard band of 469 bp (allele A) and two bands of 244 and 225 bp (allele G), for the A-1438G polymorphism, and 399 bp (allele T) and two bands of 223 and 176 bp (allele C) for the T102C polymorphism.

The SNP fragments were separated on 2.5% agarose gel and stained with Red Gel®.

Statistical analysis

The allele and genotype frequencies were determined by simple counting. The Hardy-Weinberg equilibrium analysis and comparisons between gene frequencies and population characteristics obtained through the questionnaire were performed using the chi-squared (χ^2) test, the Fisher exact test, and odds ratio (OR). The smoker group was stratified according to genotypes in relation to some characteristics associated with smoking behaviors, such as frequency of habit, number of cigarettes smoked daily, age of onset, attempts to quit, abstinence time following abandonment and dependence level. We adopted a significance threshold level of $P < 0.05$. Data analysis was conducted using BioStat 5.3 (Mamirauá Institute, Brazil). The statistical analyses for LD were performed using the Haploview 4.2 program (Barrett et al., 2005).

RESULTS

The genotypic and allelic frequencies were in Hardy-Weinberg equilibrium for both SNPs.

The allele and genotype frequencies for the SNP A-1438G did not differ significantly between smokers and controls. The -1438A allele frequency for the control group was 16.30%, whereas it was 18.52% in the smoking group (P = 1.00). The A/A, A/G, and G/G genotype frequencies in the control group were 16.30, 45.93, and 37.77%, respectively, whereas these frequencies were 18.52, 42.22, and 39.26%, respectively, in the smoking group (P = 0.80). Using the AA genotype as reference, the OR analysis showed that the genotypes A/G, G/G, and A /G+G/G were not associated with an increased risk of smoking in the population studied (A/G: OR = 1.23, 95%CI = 0.6-2.43, P = 0.65; G/G: OR = 1.09, 95%CI = 0.54-2.18, P = 0.93; A/G+G/G: OR = 1.16, 95%CI = 0.6-2.19, P = 0.74) (Table 1).

Table 1. Genetic characteristics of smoker and control groups.

Variables	Smokers	Controls	P*	OR (95%CI)	P
A-1438G	N = 135 (%)	N = 135 (%)			
Genotype					
AA	25 (18.52)	22 (16.30)			
AG	57 (42.22)	62 (45.93)		1.23 (0.62-2.43)	0.65
GG	53 (39.26)	51 (37.77)	0.80	1.09 (0.54-2.18)	0.93
AG+GG				1.16 (0.62-2.19)	0.74
Allele					
A	107 (39.63)	106 (39.26)			
G	163 (60.37)	164 (60.74)	0.93	0.98 (0.69-1.39)	1.00
T1O2C	N = 135 (%)	N = 135 (%)			
Genotype					
TT	24 (17.78)	27 (20.0)			
TC	61 (44.18)	62 (45.93)	0.35	0.90 (0.46-1.73)	0.89
CC	50 (37.4)	46 (34.07)		0.81 (0.41-1.61)	0.68
TC+CC				0.86 (0.46-1.59)	0.75
Allele					
T	109 (40.37)	116 (42.96)			
C	161 (59.63)	154 (57.04)	0.93	1.11 (0.79-1.56)	0.60

*P value from the chi-square or Fisher exact tests. OR = odds ratio; CI = confidence interval.

The allele and genotype frequencies for the SNP T102C did not differ significantly between the smoking and control groups. The 102T allele frequency in the control group was

42.96%, whereas it was 40.37% in the smoking group (P = 0.93). The T/T, T/C, and C/C genotype frequencies in the control group were 20, 45.93, and 34.07%, respectively, whereas in the smoking group they were 17.78, 45.18, and 37.04%, respectively (P = 0.35). Using the T/T genotype as reference, OR analysis showed that the genotypes T/C, C/T, and C/C+T/T were not associated with an increased risk of smoking in the population studied (T/C: OR = 0.90, 95%CI = 0.46-1.73, P = 0.89; C/C: OR = 0.81, 95%CI = 0.41-1.61, P = 0.68; T/C+C/C: OR = 0.86, 95%CI = 0.46-1.59, P = 0.75) (Table 1).

The genotypes were also investigated for associations with some particular features of smoking behavior, including the frequency of the habit, age of onset, number of cigarettes consumed daily, attempts to quit, abstinence time, and the level of nicotine dependence.

For the A-1438G SNP, the AA genotype was used as reference for comparisons. The A/G and A/G+G/G genotypes were associated with frequency of the habit, and the AG genotype showed a high risk of frequent use, whereas the A/G+G/G genotypes showed marginal risk. On the other hand, there was no significant association between frequency of habit and GG genotype (A/G: P = 0.02, OR = 6.87, 95%CI = 1.23-38.31, P = 0.04; A/G+G/G: P = 0.04, OR = 3.67, 95%CI = 1.06-12.75, P = 0.07; G/G: P = 0.27, OR = 4.00, 95%CI = 0.87-18.35, P = 0.13). Regarding the age of onset, the A/G, G/G, and A/G+G/G genotypes showed no association or high risk for early habit formation (before 17 years old) (A/G: P = 0.60, OR = 0.69, 95%CI = 0.25-1.88, P = 0.64; G/G: P = 0.09, OR = 2.31, 95%CI = 0.86-6.18, P = 0.14; A/G+G/G: P = 0.65, OR = 1.27, 95%CI = 0.51-3.14, P = 0.75). The genotypes A/G, G/G, and A/G+G/G also indicated no association for the following comparisons: daily consumption of up to 20 cigarettes per day (A/G: P = 0.72, OR = 1.36, 95%CI = 0.34-5.14, P = 0.91; G/G: P = 1.00, OR = 0.93, 95%CI = 0.25-3.37, P = 0.82; A/G+G/G: P = 0.99, OR = 1.11, 95%CI = 0.33-3.69, P = 0.89), quitting attempts (A/G: P = 0.46, OR = 0.64, 95%CI = 0.24-1.74, P = 0.53; G/G: P = 0.79, OR = 1.19, 95%CI = 0.42-3.34, P = 0.94; A/G+G/G: P = 0.81, OR = 0.85, 95%CI = 0.33-2.16, P = 0.92), and abstinence period (A/G: P = 0.29, OR = 2.45, 95%CI = 0.64-9.29, P = 0.32; G/G: P = 1.00, OR = 1.04, 95%CI = 0.31-3.48, P = 0.81; A/G+G/G: P = 0.55, OR = 1.49, 95%CI = 0.48-4.59, P = 0.68). Regarding nicotine dependence level, the A/G and A/G+G/G genotypes showed a significant association with low dependence, but the G/G genotype did not (A/G: P = 0.02, OR = 3.28, 95%CI = 1.17-9.18, P = 0.04; G/G: P = 0.11, OR = 2.41, 95%CI = 0.88-6.62, P = 0.13; A/G+G/G: P = 0.04, OR = 2.81, 95%CI = 1.1-6.99, P = 0.04) (Table 2).

For the T102C SNP, the TT genotype was used as reference for comparisons. There was no significant association between the C/C and T/C+C/C genotypes and frequency of habit. On the other hand, the G/G genotype showed a marginal association (T/C: P = 0.05; OR = 5.90, 95%CI = 1.00-34.69, P = 0.08; C/C: P = 0,71; OR= 1,46, 95%CI = 0.37-5,77, P = 0.85; T/C+C/C: P = 0.22; OR = 2.57, 95%CI = 0.70-9.37, P = 0.27).

Regarding the age of onset, the C/C genotype showed a significant association for early onset of habit (C/C: P = 0.02, OR = 3.26, 95%CI = 1.17-9.07, P = 0.03) unlike the T/C and T/C+C/C genotypes (T/C: P = 0.59, OR = 0.71, 95%CI = 0.25-1.97, P = 0.69; T/C+C/C: P = 0.49, OR = 1.46, 95%CI = 0.58-3.71, P = 0.55). There was no association between T/C, C/C, and T/C+C/C with: daily consumption of up to 20 cigarettes per day (T/C: P = 0.27, OR = 2.41, 95%CI = 0.65-8.81, P = 0.31; C/C: P = 0.99, OR = 1.19, 95%CI = 0.35-4.06, P = 0.97; T/C+C/C: P = 0.52, OR = 1.68, 95%CI = 0.54-5.19, P = 0.54), quitting attempts (T/C: P = 0.80, OR = 0.82, 95%CI = 0.30-2.23, P = 0.89; C/C: P = 1.00, OR = 1.06, 95%CI = 0.37-2.99,

P = 0.88; T/C+C/C: P = 1.00, OR = 0.92, 95%CI = 0.36-2.34, P = 0.94), and abstinence period (T/C: P = 0.06, OR = 3.96, 95%CI = 0.99-15.76, P = 0.09; C/C: P = 1.00, OR = 0.85, 95%CI = 0.25-2.90, P = 0.95; T/C+C/C: P = 0.54, OR = 1.67, 95%CI = 0.53-5.23, P = 0.55). Regarding the degree of nicotine dependence, the T/C and T/C+C/C genotypes showed significant association and high risk for low-nicotine dependence, whereas the association with the C/C showed a tendency but did not reach statistical significance (T/C: P = 0.03, OR = 3.12, 95%CI = 1.13-8.57, P = 0.04; C/C: P = 0.05, OR = 3.00, 95%CI = 1.05-8.53, P = 0.06; T/C+C/C: P = 0.02, OR = 3.06, 95%CI = 1.22-7.70, P = 0.02) (Table 3).

Table 2. Analysis related to habit according to polymorphism A-1438G.

Genotype	Frequency of habit [N (%)]		P value*	OR (95%CI)	P
	Frequent[a]	Occasional[b]			
A/A	20 (14.81)	5 (3.70)			
A/G	55 (40.74)	2 (1.50)	0.02*	6.87 (1.23-38.31)	0.04*
G/G	48 (35.55)	5 (3.70)	0.27	4.00 (0.87-18.35)	0.13
A/G+G/G			0.04*	3.67 (1.06-12.75)	0.07
	Age of onset (years old) [N (%)]				
	<17	≥17			
A/A	9 (6.67)	16 (11.85)			
A/G	16 (11.85)	41 (30.37)	0.60	0.69 (0.25-1.88)	0.64
G/G	30 (22.22)	23 (17.04)	0.09	2.31 (0.86-6.18)	0.14
A/G+G/G			0.65	1.27 (0.51-3.14)	0.75
	Cigarette consumption/day [N (%)]				
	≤20	>20			
A/A	21 (15.56)	4 (2.96)			
A/G	50 (37.04)	7 (5.18)	0.72	1.36 (0.34-5.14)	0.91
G/G	44 (32.59)	9 (6.67)	1.00	0.93 (0.25-3.37)	0.82
A/G+G/G			0.99	1.11 (0.33-3.69)	0.89
	Quitting attempts [N (%)]				
	Some attempt	Never			
A/A	17 (12.59)	8 (5.93)			
A/G	33 (24.44)	24 (17.78)	0.46	0.64 (0.24-1.74)	0.53
G/G	38 (28.15)	15 (11.11)	0.79	1.19 (0.42-3.34)	0.94
A/G+G/G			0.81	0.85 (0.33-2.16)	0.92
	Abstinence period [N (%)]				
	≤6 months	>6 months			
A/A	11 (12.50)	6 (6.82)			
A/G	27 (30.68)	6 (6.82)	0.29	0.64 (0.24-1.74)	0.53
G/G	25 (28.41)	13 (14.77)	1.00	1.19 (0.42-3.34)	0.94
A/G+G/G			0.55	0.85 (0.33-2.16)	0.92
	Nicotine-dependence level [N (%)]				
	Low	Medium-High			
A/A	14 (10.37)	11 (8.15)			
A/G	46 (34.07)	11 (8.15)	0.02*	3.28 (1.17-9.18)	0.04*
G/G	40 (29.63)	13 (9.63)	0.11	2.41 (0.88-6.62)	0.13
A/G+G/G			0.04*	2.81 (1.13-6.99)	0.04*

*P value from the Fisher exact test. [a]Daily and at least 3 times per week. [b]Less than 3 times per week. OR = odds ratio; CI = confidence interval.

The LD analysis showed that the alleles for A-1438G and T102C SNPs were moderately associated ($D' = 0.79$, $r^2 = 0.57$). Four haplotypes were constructed from these variations and only one showed a significant difference between the control group and the smoker group

(haplotype GT, P = 0.007). For example, the frequency of the GC haplotype, which carries the two alleles with the highest frequencies that are considered to be at risk, was 50.8% in the smoking group and 56.3% in the control group (P = 0.59; 10,000 permutations) (Table 4).

Table 3. Analysis related to habit according to polymorphism T102C.

Genotypes	Frequency of habit [N (%)]		P value*	OR (95%CI)	P
	Frequent[a]	Ocassional[b]			
T/T	20 (14.81)	4 (2.96)			
T/C	59 (43.70)	2 (1.50)	0.05	5.90 (1.00-34.69)	0.08
C/C	44 (32.59)	6 (4.44)	0.71	1.46 (0.37-5.77)	0.85
T/C+C/C			0.22	2.57 (0.70-9.37)	0.27
	Age of onset (years old) [N (%)]				
	<17 anos	≥17 anos			
T/T	8 (5.93)	16 (11.85)			
T/C	16 (11.85)	45 (33.34)	0.59	0.71 (0.25-1.97)	0.69
C/C	31 (22.96)	19 (14.07)	0.02*	3.26 (1.17-9.07)	0.03*
T/C+C/C			0.49	1.46 (0.58-3.71)	0.55
	Cigarette consumption/day [N (%)]				
	≤20	>20			
T/T	19 (14.07)	5 (3.70)			
T/C	55 (40.74)	6 (4.45)	0.27	2.41 (0.65-8.81)	0.31
C/C	41 (30.37)	9 (6.67)	0.99	1.19 (0.35-4.06)	0.97
T/C+C/C			0.52	1.68 (0.54-5.19)	0.54
	Quitting attempts [N (%)]				
	Some attemp	Never			
T/T	16 (11.85)	8 (5.93)			
T/C	38 (28.15)	23 (17.04)	0.80	0.82 (0.30-2.23)	0.89
C/C	34 (25.18)	16 (11.85)	1.00	1.06 (0.37-2.99)	0.88
T/C+C/C			1.00	0.92 (0.36-2.34)	0.94
	Abstinence period [N (%)]				
	≤6 months	>6 months			
T/T	10 (11.36)	6 (6.82)			
T/C	33 (37.50)	5 (5.68)	0.06	3.96 (0.99-15.76)	0.09
C/C	20 (22.73)	14 (15.91)	1.00	0.85 (0.25-2.90)	0.95
T/C+C/C			0.54	1.67 (0.53-5.23)	0.55
	Nicotine-dependence level [N (%)]				
	Low	Medium-High			
T/T	13 (9.63)	11 (8.15)			
T/C	48 (35.55)	13 (9.63)	0.03*	3.12 (1.13-8.57)	0.04*
C/C	39 (28.89)	11 (8.15)	0.05	3.00 (1.05-8.53)	0.06
T/C+C/C			0.02*	3.06 (1.22-7.70)	0.02*

*P value from the Fisher exact test. [a]Daily and at least 3 times per week. [b]Less than 3 times per week. OR = odds ratio; CI = confidence interval.

Table 4. Haplotype frequency for the A-1438G and T102C SNPs in the smoker and control groups.

Haplotypes	Smoker (%)	Control (%)	P	P*
GC	56.3	50.8	0.19	0.59
AT	36.3	33.0	0.41	0.84
GT	4.0	10.0	0.007*	0.04*
AC	3.3	6.3	0.10	0.41

*Adjusted P values after 10,000 permutations.

DISCUSSION

Tobacco smoking is currently a worldwide public health problem given the possible complications related to the habit. Thus, studies related to this behavior are of great importance for establishing more effective therapies and better preventive measures.

The importance of the genetic component in the etiology of smoking behavior has been well established in twin studies (Kendler et al., 1999; True et al., 1999). Previous studies have reported associations of genetic polymorphisms in the serotonin pathway with addictive behaviors, including smoking behavior (Ishikawa et al., 1999; Lerman et al., 2001; do Prado-Lima et al., 2004).

Herein, we analyzed the association of *5HT2A* A-1438G and T102C SNPs with tobacco smoking in a population of northeastern Brazil. To our knowledge, this is the first study to assess polymorphisms in this population. The results showed that genotypic and allelic frequencies for both polymorphisms were similar in smoker and control groups ($P > 0.05$). Furthermore, the allele frequencies of these polymorphic variants were not associated with a significant risk of smoking (A-1438G: OR = 0.98, 95%CI = 0.69-1.39, P = 1.00; T102C: OR = 1.11, 95%CI = 0.79-1.56, P = 0.60). These data suggest that these SNPs do not contribute significantly to smoking initiation in this population. However, after stratifying the smoker group according to characteristics related to the habit, associations were found between these polymorphic variants with the frequency of the habit and the level of nicotine dependence, thus suggesting a contribution to smoking behavior maintenance.

Association studies between these polymorphisms and smoking behavior have only emerged recently. Terayama et al. (2004) and Lee et al. (2005) ruled out a contribution of the A-1438G SNP in a Japanese and South Korean population, respectively. More recently, Polina et al. (2009) conducted a population study in southern Brazil, and demonstrated a contribution of the A allele to smoking status. Moreover, the number of cigarettes smoked per day was higher in individuals with co-occurrence of smoking and alcoholism than in non-alcoholic smokers. Our results differ from Polina et al. (2009) study, which suggested that the G allele could also be considered a risk allele.

Regarding the T102C SNP, data are discordant among different studies with respect to the attributed risk for genotypes. Previous data have suggested a contribution of the C allele to smoking behavior in a population in southern Brazil (do Prado-Lima et al., 2004). According to this study, the C/C genotype was associated with current smoking status and conferred a high risk to the habit compared to the C/T+T/T genotype. Moreover, the T/T genotype was associated with ever-smokers (who smoked at any time at the life but stopped) and nonsmokers (never smokers) status. Furthermore, individuals with the C/C genotype showed fewer attempts to quit compared to the T/T and T/C genotypes (31.4, 6.7, and 17.3%, respectively). Recently, a study conducted by White et al. (2011) in an Australian population suggested that the T/T genotype conferred a high risk of developing smoking behavior when compared to the C/C genotype (OR = 7.53, 95%CI = 1.58-35.89, P = 0.011), whereas the T/C genotype showed a nearly significant tendency (OR = 2.53, 95%CI = 0.87-7.32, P = 0.08). Thus, it was suggested that the T allele has a linear effect on the risk of becoming a smoker. Although T/T homozygotes have shown a greater predisposition to be smokers, there was no association with the number of cigarettes smoked per day.

Our findings contrast with those of do Prado-Lima et al. (2004) and White et al. (2011), since no association was suggested for development of the smoking behavior with these SNPs.

In addition, other conditions, which were not analyzed by these authors, pointed to a contribution of these polymorphic variants on the maintenance of the smoking habit, since a significant association of genotypes T/C and T/C+G/G was observed with a low-nicotine dependence.

According to Parsons et al. (2004) and Myers et al. (2007), the G allele appears to decrease the promoter activity. Polesskaya and Sokolov (2002) demonstrated that the expression of the C allele was approximately 20% lower than that of the T allele.

In behavioral sensitization tests, nicotine was shown to act primarily within the mesolimbic dopaminergic system, which is modulated by the 5HT2A receptor (Berke and Hyman, 2000; Millan et al., 2000; do Prado-Lima et al., 2004; Egerton et al., 2008). In this case, the decrease in 5HT2A receptors is favored by the G allele at position A-1438G and the C allele at position 102, which, may explain the possible association with persistence in tobacco addiction in this population in northeastern Brazil.

Thus, the present study suggests a possible genetic contribution of the serotonin pathway to the maintenance of smoking behavior, since it encompasses aspects hitherto not found in the literature. These results could contribute to adoption of public policies and more effective individualized therapeutic strategies that favor a reduction of the adverse effects of tobacco addiction.

CONCLUSIONS

Polymorphic variants of the 5HT2A appear to influence some aspects of smoking behavior, including the frequency of the habit and low dependence on nicotine. We cannot dismiss the possibility that our results may reflect the small sample size. Despite this potential limitation, the significant associations should not be disregarded and merit further investigation for clarification of these results.

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