Polymorphism of 5HT\textsubscript{2A} Serotonin Receptor Gene Is Implicated in Smoking Addiction


1Geriatrics and Gerontology Institute, PUCRS, Av Ipiranga 6690, Porto Alegre, Brazil
2Biochemistry Department, UFRGS, Rua Ramiro Barcelos 2600, Porto Alegre, Brazil
3Behavioral Neurobiology Research Group, Biomedical Research Institute, PUCRS, Av Ipiranga 6690, Porto Alegre, Brazil
4School of Medicine, PUCRS, Av Ipiranga 6690, Porto Alegre, Brazil
5Institute of Biosciences, PUCRS, Av Ipiranga 6690, Porto Alegre, Brazil

Smoking behavior is influenced by genetic factors. Polymorphisms affecting the dopaminergic system have been linked to smoking habits. The aim of this study was to investigate if the T102C polymorphism of the 5-HT\textsubscript{2A} receptor gene is related to tobacco use, since this receptor modulates the mesolimbic dopamine system and the C allele is associated with reduced receptor gene expression. A sample of 625 subjects were genotyped and classified according to their smoking behavior (never, former, or current smokers). We found differences in the distribution of the genotypes when the current smokers were compared with the never + former smokers, suggesting that T102C polymorphism is associated with maintenance, but not with initiation of the smoking habit. The CC genotype was more frequent in the current smokers than in the never + former smokers (\(\chi^2 = 6.825, P = 0.03\)). The odds ratio of being a current smoker with a CC genotype was 1.63, 95% CI 1.06–2.51.

KEY WORDS: smoking; addiction; dependence; serotonin; 5HT\textsubscript{2A} receptors; polymorphisms; T102C polymorphism

INTRODUCTION

Smoking behavior is influenced by genetic factors and several reports have shown the importance of them in smoking initiation as well as in nicotine dependence. A population-based investigation of 1,898 female twins [Kendler et al., 1999] demonstrated that genetic factors are more important than environmental ones in smoking initiation (78 vs. 22%) and in the development of nicotine dependence (72 vs. 28%). Another study with 3,356 Vietnam-era veteran male twins [True et al., 1999] showed that inheritability for nicotine dependence was 60.3%. Polymorphisms affecting the dopaminergic system (D\textsubscript{2} and D\textsubscript{3} dopamine receptor genes and dopamine transporter gene) have been linked to smoking-related behavior [Arinami et al., 2000]. Serotonin (5-HT), a neurotransmitter that regulates many cerebral functions, may also be involved. Serotonergic system polymorphisms, such as the tryptophan hydroxylase gene [Lerman et al., 2001; Sullivan et al., 2001] and 5-HT transporter gene [Ishikawa et al., 1999], have been linked to smoking behavior. However, studies verifying the relationship between serotonergic receptors and tobacco addition are not performed. Currently, serotonergic receptors are divided into seven groups (5-HT\textsubscript{1–7}). The type 2 receptors are categorized into three sub-types (A, B, and C). The 5-HT\textsubscript{2A} receptor gene is located at chromosome 13. A T102C polymorphism has been described in this receptor, where the mutation involves the replacement of a cytosine by a thymine [Peroutka, 1998]. This substitution does not determine a change of amino acid in the receptor molecule, making it a silent polymorphism. Nevertheless the C allele determines a differential gene expression [Polesskaya and Sokolov, 2002], whereby a diminished synthesis of 5-HT\textsubscript{2A} receptors results.

Many studies have shown that 5-HT\textsubscript{2A} receptors play a role in schizophrenia and alcohol dependence, two disorders that are strongly associated with smoking behavior. Therefore, we investigated whether T102C gene polymorphism of the 5-HT\textsubscript{2A} receptor could be associated with the development and maintenance of the smoking habit.

MATERIALS AND METHODS

Subjects

The Ethics Committee of the Pontifical Catholic University of Rio Grande do Sul approved the study protocol. Informed consent was obtained from all individuals whose information was collected prospectively. The study was structured considering the checklist for reporting and appraising of genetic diseases associations proposed by Little et al. [2002]. The study population included in the analyses was comprised of 625 unrelated, free-living individuals, recruited from three different sources:

i. 167 subjects from an epidemiological study of aging and non-transmissible disease developed in the city of Gravataí, Porto Alegre metropolitan area (prospective data).
ii. 376 subjects from a health spa facility near the metropolitan area of Porto Alegre (retrospective data from clinical records).
iii. 82 subjects from the smoking treatment program of the Säo Lucas Hospital, Porto Alegre (prospective data).

We excluded first or second-degree relatives of subjects previously included to avoid genetic frequency bias. Alves-Silva...
et al. [2000] and Parra et al. [2003] studying the ancestry of the Southern Brazilian region population emphasized that the massive inter-ethnic crosses occurred in 500 years of Brazilian history and underline that the large urban areas, such as Porto Alegre Metropolitan area (3.5 millions in habitants) do not present significant isolated ethnic groups. For this reason, we consider the sample source as a unique population, and no stratification is presented here. To test possible genetic or genotypic frequency differences from the two first samples, we used a chi-square test. We did not find any statistical differences and we assumed that these populations had the same genetic contributions.

The timing recruitment period, sample collection, and analysis occurred between 2001 March until 2002 July. Clinical and laboratory staff was blind to genotype and smoking condition respectively, during all experimental procedures. All subjects completed a self-report questionnaires including demographic characteristics (age, gender) and smoking history (smoker only). Smoking history variables included age at smoking initiation, current smoking rate, and nicotine dependence. Nicotine dependence was measured using the Fagerstrom Test of Nicotine Dependence (FTND) [Heatherton et al., 1991].

Smoking Status

Subjects were classified into three categories regarding their smoking habits currently. Current smokers were individuals smoking 10 cigarettes or more per day for at least 3 months. Former smokers were individuals who had stopped smoking for at least 2 years. Non smokers were individuals who had never smoked. Subjects who did not fit into these categories were excluded. Nicotine dependence was measured using the FTDN.

Molecular Analysis

The 5-HT_{2A} receptor polymorphism was determined from DNA isolated from lymphocytes using an extraction kit. Genotyping of the T102C polymorphism was done according to the polymerase chain reaction (PCR) method by Warren et al. [1993] with minor modifications as described by Tsai et al. [1999]. Briefly, standard PCR was carried out in a 25-µl volume containing 100 ng genomic DNA, 200 µM of each dNTP, 1.5 mM MgCl₂, 250 nM of the sense (5'-TGTGCTACAAGTTCTGGCTT-3'), and anti-sense (5'-GTGCCAGTTTTTCTCTAGGG-3') primers, and 0.6 U DNA Taq polymerase. The PCR products were digested with HpaII (B). The digestion products were separated by electrophoresis and visualized under UV light. The 102T allele PCR products remained uncut, with a single DNA band of 342 bp, whereas the 102C allele showed two bands of 342 bp and 186 bp.

Statistical Analysis

The allele and genotype frequencies were tested to equilibrium by the Hardy–Weinberg law. The significance of allele frequency or genotype distribution among volunteers with different smoking habits was examined by non-parametric chi-square test or Fischer’s exact test (two-tailed). Multivariate analyses, including sex and age effects, were conducted with multiple logistic regression methods and estimates of conditional relative risk and 95% confidence interval (CI). Statistical analyses were performed by means of the SPSS/PC Statistical Package Version 9.0 (SPSS, Inc., IL). All P-values were two-tailed. A value of \( P < 0.05 \) was considered statistically significant. To test interventional factors we performed a multivariate analysis using the Forward Wald logistic regression.

RESULTS

Table I shows the demographic data for all individuals, along with the genotype and allele distribution of the T102C polymorphism for the 5-HT_{2A} receptor gene and the frequency of smoking status.

The multivariate regression model analyses showed no association between 5-HT_{2A} polymorphism and smoking status based on gender or age.

We did not find differences in genotype distribution or in dose effect analyses when the individuals were divided into the three smoking categories (never, former, or current smokers) or when the current + former smokers were grouped and compared with the never smokers.

However, when the never smokers and the former smokers were grouped and compared with the current smokers, statistical differences became apparent among genotypes frequencies. The current smoker group had a high frequency of the CC genotype and a lower frequency of the TT genotype than the never + former smoker group. We also observed a dose effect considering T102C polymorphism and smoking habits. The CC genotype was associated with current smoking behavior and TT genotype was related to the smoking behavior of the never + former smoker group (Table II). These results suggest that this polymorphism is involved in the maintenance of the smoking habit and not with initiation of smoking. Therefore, further research is necessary to verify whether this polymorphism is related to the severity of tobacco dependence or response to treatment.

The occurrence of CC genotype seems to increase the risk to be a current smoker and to be an ever smoker (current + former smoking behavior). CC individuals have a 63% greater risk to be current smokers than the group of TT + TC subjects. On the other hand, the TT genotype seems to decrease the risk to be a current smoker, but not an ever smoker. Furthermore, the T allele seems to decrease the risk to be a current smoker but not an ever smoker (Table III).

We found that CC 5-HT_{2A} genotype subjects try to stop smoking less than other ones. That is, 31.4% (n=13) CC subjects did not try to quit before, whereas just 6.7% (n=2) and 17.3% (n=21) TT and CT subjects, respectively, did not try to stop smoking before the interview. This association was independent of the gender and age.

DISCUSSION

Our findings suggest an association between 5-HT_{2A} gene receptor polymorphism and maintenance of smoking but not smoking initiation. However, as the statistical association was between 0.03 and 0.05 range could have resulted by chance where the P of >0.01 is a chance finding. This depends on a priori probability. We attempted to minimize this by replicating the findings in three different subject groups, and we did not find significant differences among them. Of course, we cannot discard chance association, however some aspects related with the possible role of serotonergic system in tobacco addiction could help us if the association could be consistent.

<table>
<thead>
<tr>
<th>TABLE I. Demographic Characterization of Subjects</th>
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<tbody>
<tr>
<td>Age Mean ± SD (years)</td>
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<tr>
<td>Smoking status</td>
</tr>
<tr>
<td>Never (%)</td>
</tr>
<tr>
<td>Former (%)</td>
</tr>
<tr>
<td>Current (%)</td>
</tr>
<tr>
<td>Genotype</td>
</tr>
<tr>
<td>CC (%)</td>
</tr>
<tr>
<td>TC (%)</td>
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<tr>
<td>Allelic frequency</td>
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Previous studies have reported serotonergic system genes polymorphism association with addition, including smoking behavior as the Ishikawa et al. [1999] study. The authors described the association between serotonin transporter gene polymorphism in the upstream regulatory region associated with decreased transcription efficiency of the 5-HTT-gene promoter and smoking among Japanese males. The results suggested the presence of the L allele as significantly increased in smokers (37%) compared with that in nonsmokers (24%).

Additional studies analyzing others gene polymorphism serotonergic system-related were reported. Lerman et al. [2001] investigated the A799C polymorphism of tryptophan hydroxylase (the rate-limiting enzyme in the synthesis of 5-HT) gene and found an association with age of smoking initiation. They argued that a relationship between impulsive behavior and serotonergic abnormality could explain the finding that 799A allele individuals begin to smoke earlier than those with 799C alleles. Sullivan et al. [2001] studied the same polymorphism and found a similar association with smoking initiation and not with progression to nicotine dependence. They also detected the same association with another tryptophan hydroxylase gene polymorphism, the C218A.

As can be seen in the literature, until few years ago, the gene polymorphism association with tobacco addition was concentrated mainly on dopamine system. However, the neurochemical homeostasis includes the close relationship between dopamine and serotonergic system.

Polesskaya and Sokolov [2002] developed a method to determine the 5-HT2A receptor mRNA molecules transcribed in gene expression. From heterozygous (C/T) subjects they determined the 5-HT2A receptor mRNA molecules transcribed from the T or C allele. This method did not involve the comparison of different individuals, making it possible to avoid effects of variation in demographic and tissue sampling. They demonstrated that the expression of allele C was ~20% lower than the expression of the T allele.

Studies shown that nicotine on tests of reinforcement and behavioral sensitization are primarily mediated through the mesolimbic dopamine system [Berke and Hyman, 2000]. These neurons are modulated by serotonergic system via 5-HT2A receptors [Milan, 2000]. In this case, the decrease in 5-HT2A receptors associated with C allele could be explaining the possible association with persistence in tobacco addition.

Additional studies could be conducted to clarify some open questions that this study does not answer. For this reason, the study described here could be considering a preliminary results and presented several limitations. One such limitation is population stratification related. Although the analysis were conducted in an admixture population, it is possible that ethnic admixture could bias the study results. However, recent analyses suggest that the extent of bias attributable to population stratification is minimal [Wacholder et al., 2000]. However, in order to rule out population stratification and to validate the present findings, family studies of 5-HT2A and other candidate genes are warranted.

Substance abuse is complex and involves multiple genetic and environmental risk factors. Colhoun et al. [2003] commented that inability to replicate many results has led to increasing skepticism about the value of simple association studies for detection of genetic variants contributing to common complex traits, and suggests that most important factors underlying incapacity to replicate these associations are publication bias, failure to attribute the results to chance, and inadequate sample sizes. However, to conduct the large studies for one gene polymorphism or without previous report could be expensive and spent much time. In this context, despite the limitations, the present study together with other investigations published by Ishikawa et al. [1999], Sullivan et al. [2001], Lerman et al. [2001], corroborate the possible role of serotonergic genes in smoking behavior.

The importance of the findings described here is related with a smoking cessation treatment, mainly if we consider the high number of CC subjects that never try to stop smoking before the interview. For the instance, complementary studies using pharmacogenetic approach could be performed.

### TABLE II. Genotype and Allele Numbers and Frequencies in Subjects Between Current Smokers and Never + Former Smokers

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Never + former smokers (n = 432)</th>
<th>Current smokers (n = 193)</th>
<th>OR*</th>
<th>95% IC</th>
<th>OR*</th>
<th>95% IC</th>
</tr>
</thead>
<tbody>
<tr>
<td>TT (%)</td>
<td>95 (22.0)</td>
<td>30 (15.5)</td>
<td>6.825^b</td>
<td>0.03</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC (%)</td>
<td>63 (14.6)</td>
<td>42 (21.8)</td>
<td>3.59^c</td>
<td>0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TC (%)</td>
<td>274 (63.4)</td>
<td>121 (62.7)</td>
<td>4.42^c</td>
<td>0.03</td>
<td></td>
<td></td>
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</table>

^aChi-square likelihood ratio test was used to compare the genotype frequencies.

^bDegrees of freedom (df) = 4.

^cdf = 2.

### TABLE III. Odds Ratio of T102C Polymorphism and Smoking Status

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Current smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>1.57</td>
</tr>
<tr>
<td>TT</td>
<td>0.89</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Alleles</th>
<th>Current smokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.29</td>
</tr>
<tr>
<td>T</td>
<td>0.8</td>
</tr>
</tbody>
</table>

^*Odds ratio and 95% CI (confidence intervals) were calculated by multiple logistic regression analysis.
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REFERENCES


