

Short Communication

The Role of the Serotonin Transporter Gene in Cigarette Smoking

Caryn Lerman,¹ Peter G. Shields, Janet Audrain, David Main, Brian Cobb, Neal R. Boyd, and Neil Caporaso

Cancer Genetics Program, Lombardi Cancer Center, Georgetown University Medical Center, Washington, D. C. 20007-4104 [C. L., J. A., D. M.]; Molecular Epidemiology Section, Laboratory of Human Carcinogenesis, Division of Basic Sciences, National Cancer Institute, Bethesda, Maryland [P. G. S., B. C.]; Division of Population Science, Fox Chase Cancer Center, Philadelphia, Pennsylvania [N. R. B.]; and Pharmacogenetics Section, Genetic Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, Maryland [N. C.]

Abstract

Data from twin studies have suggested that cigarette smoking has a significant heritable component. The serotonin transporter gene (*5-HTT*) is a plausible candidate gene for smoking predisposition because of its association with psychological traits relevant to smoking behavior. The present investigation evaluated the associations of smoking practices and smoking cessation with a common polymorphism in the upstream regulatory region of *5-HTT* that is manifested as either an inserted (long) variant or a deleted (short) variant. The short variant of the polymorphism is associated with reduced transcription of the gene promoter and diminished uptake. A case-control study design (268 smokers and 230 controls) was used to evaluate the associations of *5-HTT* genotype with smoking status. Case series analysis of smokers was used to evaluate the role of *5-HTT* in age at smoking initiation, previous quitting history, current smoking rate, and 12-month quit rate following a minimal-contact smoking cessation program. There were no significant differences in the distribution of *5-HTT* genotypes in smokers as compared with nonsmokers in either Caucasians or African Americans, nor was the *5-HTT* genotype associated with the smoking outcome variables. However, the results did reveal significant racial differences in the distribution of *5-HTT* genotypes: Caucasians were significantly more likely to carry the short variant of the *5-HTT* gene than were African Americans ($P = 0.005$). These findings suggest that the *5-HTT* gene may not play a significant role in cigarette smoking practices.

Introduction

Cigarette smoking is the greatest preventable cause of cancer mortality (1), yet approximately 26% of adults in the United

States continue to smoke (2). Evidence from twin studies (3) indicates that smoking has a significant heritable component. Previously, we reported on the results of a smoking case-control study that examined associations of smoking practices with polymorphic genes important in dopamine transmission. These studies were based upon evidence supporting the role of dopamine in the brain's reward mechanism (4) and suggesting that nicotine stimulates dopamine transmission (5). We found preliminary support for an association of the dopamine transporter (*SLC6A3*) gene with the likelihood of smoking, age at smoking initiation, and previous quitting history.² However, the tyrosine hydroxylase gene was not associated with any smoking outcomes (6).

In this study, we examined associations of a serotonin transporter (*5-HTT*) gene with smoking practices. The serotonin transporter gene is located on chromosome 17q11.2 (7), and gene transcription has been reported to be modulated by a polymorphism in its regulatory region (8). The polymorphism is a 44-bp deletion or insertion, in which the inserted variant (long) versus the deleted variant (short) occurs in 57 and 43% of Caucasians, respectively (9). The short variant is associated with reduced transcription, resulting in diminished *5-HTT* uptake (8).

The *5-HTT* gene is a plausible candidate gene for smoking predisposition because of its role in psychological traits relevant to smoking behavior. The *5-HTT* polymorphism has been linked with anxiety-related personality traits (9) and with depression (10, 11); however, the former finding was not replicated in a recent analysis (12). Both anxiety and depression have been linked with nicotine dependence (13, 14). Further, preliminary clinical data suggest that serotonin reuptake inhibitors, such as fluoxetine hydrochloride, may promote smoking cessation (15, 16). Of interest, smokers who are more nicotine dependent responded better to fluoxetine treatment than less dependent smokers (16). Because the short variant of *5-HTT* has been associated with reduced uptake (*i.e.*, more available serotonin), we predicted that the presence of *5-HTT* short alleles would be protective for smoking (*i.e.*, associated with a lower likelihood of being a smoker).

In the analysis reported here, we used a case-control study design to evaluate the association of smoking practices with the *5-HTT* polymorphism. A case-series analysis of smokers was performed to examine associations of *5-HTT* with age at smoking initiation, previous quitting history, current smoking rate, and 12-month cessation rates following a minimal-contact smoking cessation treatment program.

Subjects and Methods

Subjects. Smokers ($n = 268$) who reported smoking at least 5 cigarettes/day for at least 1 year were recruited through varied

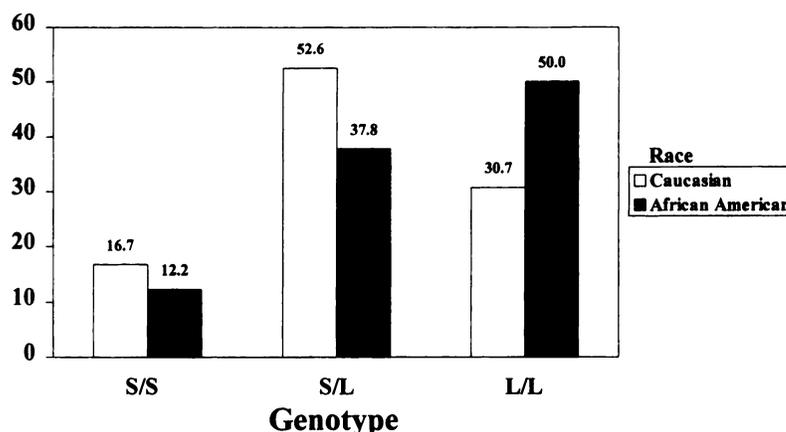
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¹ To whom requests for reprints should be addressed, at Georgetown University Medical Center, 2233 Wisconsin Avenue NW, Suite 535, Washington, DC 20007-4104.

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Fig. 1. Prevalence of 5-HTT genotypes by race. $\chi^2(2)$, 10.6; P , 0.005; S, short variant of 5-HTT; L, long variant.



newspaper advertisements and flyers in the metropolitan Washington, D. C., and Philadelphia areas for a free smoking cessation program. Nonsmoking controls ($n = 230$) who reported having smoked fewer than 100 cigarettes in their lifetimes were recruited through similar mechanisms. Exclusion criteria were as follows: under age 18, a personal history of cancer, undergoing treatment for drug or alcohol addiction, or presence of a psychiatric disorder that precluded informed consent.

Procedures. During a visit to the clinic, subjects completed an informed consent form reviewing the study procedures, including genotyping, and completed questionnaires assessing demographics and smoking history variables (age at smoking initiation, longest prior quitting period, and current smoking rate). Subjects then received a single, minimal contact (1 h) of behavioral smoking cessation counseling and self-help materials (17). They were followed for 1 year after the counseling to assess self reports of quitting. The quitting outcome measure was a 30-day period prevalence of smoking 12 months after smoking cessation treatment. All subjects donated blood for genetic analysis.

Oligonucleotide primers flanking the 5-HTTLPR sequence (5'ggcgtgcccgtctgaattgc and 5'-gagggactgagctggacaaccac; Ref. 7) from the 5-HTT gene 5'-flanking regulatory region generating 484 or 528 bp were amplified by PCR using 50 ng of genomic DNA, 2.5 mM deoxyribonucleotides, 0.1 μ g of primers, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, and 1 unit of Taq DNA polymerase. Cycling conditions included melting at 95°C (5 min), 35 cycles of 95°C for 30 s, annealing at 62°C for 45 s, and an extension at 72°C for 1 min. A final extension at 72°C for 4 min completed the PCR. The amplified product was resolved by agarose gel electrophoresis (1.5%). The assay was validated by confirming polymorphic Mendelian inheritance patterns in seven human family cell lines ($n = 134$) encompassing three generations each (data not shown; National Institute of General Medical Sciences, Human Genetic Mutant Cell Repository, Coriell Institute, Camden, NJ). Twenty % of the samples were repeated for quality control.

χ^2 tests were performed to evaluate associations of 5-HTT genotypes with ethnicity and with categorical smoking outcome variables (*i.e.*, smoker *versus* control; among smokers only: age at smoking initiation and 1-year abstinence). Fisher's exact test was used for the abstinence variable due to small cell sizes. One-way ANOVAs were performed to examine associations of 5-HTT genotype with continuous outcomes in smokers (*i.e.*, longest prior quit period and number of cigarettes/day).

Results

The study sample included 280 (56%) females and 218 (44%) males. Of the 268 smokers, 221 (84%) were Caucasian and 47 (16%) were African American. Of the 230 nonsmoking controls, 203 (88%) were Caucasian and 27 (12%) were African American. The average age of study participants was 43.8 \pm 11.5 years; 89% of participants had education beyond high school. Among the smokers, the average smoking level was 21.8 cigarettes/day.

As shown in Fig. 1, significant racial differences in the distribution of 5-HTT genotypes were found ($\chi^2 = 10.6$; $P = 0.005$). Caucasians were significantly more likely to carry the short variant of the 5-HTT gene than were African Americans. Therefore, analyses of the associations of genotype with smoking practices were stratified by race.

The prevalence of 5-HTT genotypes by smoking groups is presented in Table 1. There were no significant differences in the distribution of genotypes in smokers *versus* nonsmokers in either Caucasians or African Americans. Among smokers, we used χ^2 tests of associations of age at smoking initiation (<16 *versus* ≥ 16 years) and the 12-month posttreatment quit rates with 5-HTT genotypes; no associations were found in Caucasians or African Americans. The 5-HTT genotype was not associated significantly with the longest prior quitting period (in days) in either Caucasian smokers ($n = 221$; $F = 0.14$, $P = 0.89$) or African American smokers ($n = 47$; $F = 0.43$, $P = 0.65$). Nor was 5-HTT genotype related significantly to current smoking (number of cigarettes/day) in Caucasians ($F = 0.41$; $P = 0.66$) or African Americans ($F = 0.44$; $P = 0.64$).

Discussion

The present case-control study was the first to evaluate whether or not smoking was associated with a polymorphism in the serotonin transporter (5-HTT) gene in Caucasians and African Americans. Previous evidence linking this polymorphism with anxiety (9) and supporting the potential benefits of serotonin reuptake inhibitors in smoking cessation (15) suggested that this gene may be a plausible candidate for predisposition to nicotine dependence. In this study, we found no evidence for associations of 5-HTT with current smoking, smoking history, or cessation rates in either racial group. It should be noted, however, that subjects recruited through newspaper advertisements may not be representative of smokers and nonsmokers in the population. Nevertheless, these findings suggest that the serotonin transporter gene polymorphism studied here

Table 1 Prevalence of 5-HTT genotypes within smoking groups by race

Group	Caucasian			African American		
	S/S ^a	S/L	L/L	S/S	S/L	L/L
Smokers, n (%)	43 (19.4)	108 (48.9)	70 (31.7)	5 (10.6)	18 (38.3)	24 (51.1)
Controls, n (%)	28 (13.8)	115 (56.6)	60 (29.6)	4 (14.8)	10 (37.0)	13 (48.2)
χ^2	3.40			0.28		
P	0.18			0.87		
Smokers, starting age <16 yr	17 (22.7)	38 (50.7)	20 (26.6)	1 (7.7)	5 (38.5)	7 (53.8)
Smokers, starting age \geq 16 yr	26 (17.9)	69 (47.6)	50 (34.5)	4 (11.8)	13 (38.2)	17 (50.0)
χ^2	1.61			0.17		
P	0.45			0.92		
Smokers, quit at 1-yr follow-up	6 (22.2)	12 (44.5)	9 (33.3)	0 (0)	2 (50.0)	2 (50.0)
Smokers, smoking at 1-yr follow-up	27 (18.6)	73 (50.3)	45 (31.0)	1 (4.4)	9 (39.1)	13 (56.5)
P ^b	0.85			1.00		

^a S, short form; L, long form.

^b Fisher's exact test.

is not a major determinant of cigarette smoking practices. Further investigation of other polymorphic serotonergic genes, such as those regulating postsynaptic receptor function, are needed to fully evaluate the role of serotonin transmission in smoking behavior.

As in our previous studies of dopaminergic genes, we found evidence for significant racial variation in genotype frequencies. In the present study, Caucasians were significantly more likely than African Americans to carry the short variant of 5-HTT, which has been associated with anxiety-related traits (9). However, the sample size of African Americans in our study was small. Previously, we found racial differences in the frequencies of the dopamine D2 receptor (*DRD2*), and dopamine transporter (*SLC6A3*) genes.² In both cases, African Americans were significantly more likely to have genotypes associated with reduced dopamine transmission. Evaluation of racial differences in the frequency of genes governing neurotransmitter function may enhance our understanding of genetic contributions to race differences in smoking practices (18).

To fully elucidate the influence of genetic factors in cigarette smoking, it will be necessary to examine the interplay of the genes involved in synthesis, release, and receptor function for a variety of neurotransmitters. Examination of genetic factors in nicotine metabolism may also be fruitful. A better understanding of these pharmacogenetic mechanisms can lead to the development of improved prevention and treatment strategies tailored to the needs of individual smokers.

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