Short Communication

Association between Serotonin Transporter Gene Polymorphism and Smoking among Japanese Males

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Abstract

The serotoninergic system may be involved in smoking behavior because nicotine increases brain serotonin secretion, nicotine withdrawal decreases serotonin levels, and a selective serotonin reuptake inhibitor antagonizes the response to nicotine. Compared with the *L* allele, the *S* allele of the polymorphism in the upstream regulatory region of the serotonin transporter gene is associated with decreased transcription efficiency of the *5-HTT* gene promoter. We examined this polymorphism in a Japanese population consisting of 387 males from two different areas in Japan. The *L* allele was observed significantly more often in smokers (21%) than in nonsmokers (lifetime nonsmokers + ex-smokers, 14%; $P = 0.005$). The presence of the *L* allele (the *L/L* + *L/S* genotypes) was also significantly increased in smokers (37%) compared with that in nonsmokers (24%; $P = 0.003$). The present study suggests that individuals with the *S/S* genotype are less inclined to smoke and/or can more easily stop smoking than others, supporting a role of the serotoninergic system in smoking behavior.

Introduction

Cigarette smoking is the greatest known preventable cause of cancer and coronary heart disease. Family and twin studies have suggested that hereditary factors are involved in the initiation and continuance of tobacco use (1). The concordance rates for smoking, not smoking, and quitting smoking have been reported higher for monozygotic twins than for dizygotic twins (2). People smoke cigarettes habitually to maintain nicotine levels in the body, and nicotine plays a role in stimulating brain reward mechanisms via central neuronal dopaminergic pathways. An association between the dopamine D4 receptor gene exon III polymorphism and smoking was recently reported in African-Americans (3). The serotoninergic system may also be implicated in habitual smoking because nicotine increases brain serotonin secretion and nicotine withdrawal has the opposite effect (4, 5).

The serotonin transporter gene (*5-HTT*) is a plausible candidate for smoking predisposition because a polymorphism in the 5′ flanking region of the *5-HTT* gene is associated with its transcriptional efficacy (6, 7). There are two common alleles, a 44-bp insertion (*L* allele) or deletion (*S* allele). The *S* allele is associated with decreased transcriptional activity compared with the *L* allele. Serotonin transporter activity has been shown to be decreased *in vivo* in the *S/S* genotype in comparison to activity in the *L/S* and *L/L* genotypes. Because the selective serotonin reuptake inhibitor fluoxetine antagonizes the ability of nicotine to evoke hyppocampal noradrenaline release *in vitro* (8), it is expected that the *S* allele would protect against habitual smoking and/or promote successful smoking cessation.

In this study, we looked for an association between the *5-HTT* polymorphism and smoking behavior in two Japanese populations. Taking into account gender differences in the prevalence of smoking (9, 10), we examined only male subjects.

Materials and Methods

Subjects. Among 496 Japanese men who visited medical checkup clinics in Aomori or Tsuchiura annually, 387 (78%) men who completed an informed consent form and a questionnaire assessing demographics and smoking history variables (age at smoking initiation, average number of cigarettes smoked in the last week, total days of quit attempts, and number of quit attempts lasting 24 h) were the subjects in this study. Aomori and Tsuchiura are 650 and 50 km north of Tokyo, respectively. The subjects ranged in age from 37–59 years (mean, 46.8; $n = 148$) in Aomori and 46–65 years (mean 52.6; $n = 239$) in Tsuchiura. The subjects were divided into three groups based on their smoking behavior: (*a*) those who had never smoked (lifetime nonsmokers, $n = 82$); (*b*) those who had smoked and had successfully stopped smoking (ex-smokers, $n = 103$); and (*c*) those who had and continued to smoke at the time of the study (smokers, $n = 202$). The shortest smoking-cessation period of the ex-smokers was 4 months. The smokers included subjects who fit the criteria irrespective of whether or not they had ever attempted to quit smoking. Exclusion criteria were as follows: a personal history of cancer, coronary heart disease, or liver diseases, or undergoing treatment for drug or alcohol addiction. There was no significant difference in age and alcohol consumption (average weight of absolute ethanol consumed/week) among the smoking groups. The subjects' medication histories were not evaluated in this study.

Procedures. Genomic DNAs were prepared from peripheral whole blood cells by phenol extraction. Genotyping was carried out according to the method of Heils *et al.* (6, 7).

Group comparisons of allele and genotype frequencies were made using Fisher's exact test. Genotypes were divided

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Table 1 Genotype and allele numbers and frequencies in subjects according to smoking history

	Aomori subjects (n = 148)				Tsuchiura subjects (n = 239)				Total subjects (n = 387)			
	Lifetime nonsmoker	Ex-smoker	Nonsmoker[a]	Smoker	Lifetime nonsmoker	Ex-smoker	Nonsmoker[a]	Smoker	Lifetime nonsmoker	Ex-smoker	Nonsmoker[a]	Smoker
Genotype (n)	(40)	(19)	(59)	(89)	(42)	(84)	(126)	(113)	(82)	(103)	(185)	(202)
L/L	1	0	1	4	2	3	5	5	3	3	6	9
S/L	9	2	11	27	9	18	27	39	18	20	38	66
S/S	30	17	47	58	31	63	94	69	61	80	141	127
Presence of *L* allele (%)	25%	11%	20%	35%	26%	25%	25%	39%	26%	22%	24%	37%
Allele (n)	(80)	(38)	(118)	(178)	(84)	(168)	(252)	(226)	(164)	(206)	(370)	(404)
L	11	2	13	35	13	24	37	49	24	26	50	84
S	69	36	105	143	71	144	215	177	140	180	320	320
L (%)	14%	5%	11%	20%	15%	14%	15%	22%	15%	13%	14%	21%
OR (95% CI)[b], P[c]												
L/L + L/S vs S/S	OR = 2.1 (1.0–4.3), P = 0.04				OR = 1.9 (1.1–3.2), P = 0.02				OR = 1.9 (1.2–2.9), P = 0.003			
L vs S	OR = 2.0 (1.0–3.8), P = 0.03				OR = 1.6 (1.0–2.6), P = 0.03				OR = 1.7 (1.1–2.5), P = 0.005			

[a] Nonsmokers are lifetime nonsmokers and ex-smokers.
[b] Nonsmoker *versus* smoker.
[c] Fisher's exact test.

Table 2 Characteristics of smokers according to serotonin transporter genotypes

	S/S (n = 127) Mean (SD)	*S/L* (n = 66) Mean (SD)	*L/L* (n = 9) Mean (SD)	ANCOVA F(P)[a]
Age at smoking initiation[b]	21.1 (2.5)	19.6 (2.3)	19.7 (0.5)	0.5 (0.50)
Cigarettes/day in the last week	27.4 (10.6)	34.0 (14.5)	33.3 (10.0)	15.0 (0.0002)
Total days of quit attempts	516 (1575)	254 (414)	16 (22)	3.6 (0.06)
Number of quit attempts lasting 24 h	1.6 (3.7)	1.1 (2.0)	0.7 (0.5)	2.9 (0.09)

[a] P for *L/L+L/S versus S/S*, adjusted by age and average weight of absolute ethanol consumed/week.
[b] Age at smoking initiation may be underreported because smoking by persons under age 20 is prohibited by law in Japan.

into the *S/S* and *S/L + L/L* genotypes because whole blood serotonin levels were significantly lower in individuals with the *S/S* genotypes than in those with other genotypes (11) and because the *L/L* genotype is uncommon among the Japanese (12). The relationship between smoking status and genotype was shown by ORs.[2] When examining continuous variables (age at smoking initiation, number of cigarettes/day in the last week, total days of quit attempts, number of quit attempts lasting 24 h) in smokers, an ANCOVA with age and average weight of absolute ethanol consumed/week as covariate was performed to test for the *S/S* and *S/L + L/L* genotypic group differences. Deviations of genotype distribution from Hardy-Weinberg equilibrium were assessed by χ^2 tests. Criterion for statistical significance was set at $P < 0.05$ (two-sided).

Results

The *L* allele frequency was observed slightly more frequently in the Tsuchiura subjects (0.18) than in the Aomori subjects (0.16), but the difference was not significant (P = 0.30). The genotype distributions did not deviate significantly from Hardy-Weinberg expectations (P = 0.50 in Aomori and P = 0.32 in Tsuchiura). The *L* allele and the *L/L + L/S* genotypes were found significantly more often in smokers than in nonsmokers (lifetime nonsmokers + ex-smokers) among the Tsuchiura subjects [OR = 1.6 (95% CI, 1.0–2.6), P = 0.03, and OR = 1.9 (95% CI, 1.1–3.2), P = 0.02, respectively]. Similar significant differences in allelic and genotypic frequencies were observed in the Aomori subjects [OR = 2.1 (95% CI, 1.0–3.8), P = 0.03, and OR = 2.1 (95% CI, 1.0–4.3), P = 0.04, respectively]. Among all subjects, the *L* allele and the *L/L + L/S* genotypes were found significantly more often in smokers than in nonsmokers [OR = 1.7 (95% CI, 1.1–2.5), P = 0.005, and OR = 1.9 (95% CI, 1.2–2.9), P = 0.003, respectively; Table 1] or than in ex-smokers [OR = 1.8 (95% CI, 1.1–2.5, P = 0.008, and OR = 2.1 (95% CI, 1.2–3.5), P = 0.006, respectively]. The smoking variables were analyzed only in the smokers (Table 2). The number of cigarettes/day was significantly lower in the smokers homozygous for the *S* allele than in those with the *L* allele (P = 0.0002, ANCOVA with age and average weight of absolute ethanol consumed/week as covariate). Age at smoking initiation, total days of quit attempts, and number of quit attempts lasting 24 h were not associated significantly with the polymorphism.

Discussion

This study suggests that the *L* allele or the *L/L+ L/S* genotypes is associated with smoking. This implies that the *S* allele or the *S/S* genotype is associated with nonsmoking and quitting smoking. A frequent concern in association studies is the possibility that results may be due to a hidden population stratification. Because the subjects we examined were Japanese, racial or ethnic stratification is not likely. However, unknown population stratification rather than the genetic polymorphism itself is a possible explanation. We attempted to minimize this by replicating the findings in two independent subject groups. A second potential problem is that the P of < 0.01 is a chance finding. This depends on *a priori* probability. Before this study, we hypothesized that if the *5-HTT* gene *S* allele is an anxiety-associated and/or harm avoidance-associated allele (7), individ-

[2] The abbreviations used are: OR, odds ratio; ANCOVA, analysis of covariance; CI, confidence interval.

uals with the *S* allele or the *S/S* genotype may be more concerned about smoking health hazards, consequently more likely to avoid smoking or succeed in quitting. Some studies replicated an association between the *S* allele and anxiety or harm avoidance (13–15), but not others (16–18). Many factors, such as scoring bias and different subject characteristics, may obscure an association between multifactorial quantitative personality traits and genotypes. However, both anxiety and depression, which are reported to be associated with the *S* allele, are linked to nicotine dependence. The frequency of a lifetime history of major depressive disorder is more than double in individuals who have smoked habitually than in nonsmokers (19). In this context, an association between the *S* allele and smoking, the opposite direction of the association observed in our study, is expected. Thus, both association directions are possible if the association between personality traits or depression and the *5-HTT* gene polymorphism is actual. We will be unable to evaluate an association between psychological traits and smoking behavior until an association between the *5-HTT* polymorphism and psychological traits is established.

We proposed another, a neurochemical, basis for the association before our present study. Nicotine increases brain serotonin secretion, and nicotine withdrawal has the opposite effect (4, 5), leading to the hypothesis that the appetite and mood disturbances associated with nicotine withdrawal may be mediated by diminished serotoninergic transmission. Fluoxetine treatment effectively prevents the increased food intake and weight gain in smokers who reduce their nicotine intake (20). Fluoxetine antagonizes the response to nicotine that evokes hyppocampal noradrenaline release in the rat (8). The *5-HTT S* allele is associated with lower transcriptional activity. Therefore, individuals with the *S* allele or *S/S* genotype might be at less risk for nicotine dependence and/or can more easily stop smoking than others. The results of the present study support this working hypothesis.

Recently, Lerman *et al.* (21) reported no association between the *5-HTT* polymorphism and cigarette smoking in Caucasians and African-Americans. They observed no significant difference in allele or genotype frequencies between smokers and nonsmoking controls. Smokers studied by Lerman *et al.* (21) were recruited through media advertising for a smoking cesstion program; these smokers may be eager to quit smoking but unable to quit on their own. Our subjects were recruited through an annual medical checkup and, therefore, were not selected by whether they had the will to quit smoking. Our subjects were males only, whereas more than half of the smokers studied by Lerman *et al.* (21) were females. The *L* allele frequency is known to be lower in Japanese (0.19) than in European Americans (0.60) and African-Americans (0.70; Ref. 12). We evaluated associations by the genotype classification (*L/L+L/S versus S/S*) because of the low number of *L/L* subjects in Japanese and because of one study looking at blood serotonin levels (11). However, this classification is not consistent with some previous epidemiological studies or with functional studies, suggesting that the presence of the S allele (*i.e., S/S + S/L versus L/L*) may be the important factor (6, 7). These differences in genotype grouping and recruitment methods limit the ability to compare the present study with the previous research. Because differences in racial genetic backgrounds, cultural backgrounds, subject recruitment methods, definition of smoking groups, age, and gender could be contributing factors, additional studies are needed to evaluate the association and to understand why some people smoke cigarettes and have difficulty quitting despite health hazard warnings. In particular, association between this polymorphism and success rates for persons using selective serotonin reuptake inhibitor pharmaceutical agents as adjuncts for smoking cessation is worth studying.

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