

Brief Research Communication

Tryptophan Hydroxylase Gene Variant and Smoking Behavior

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Approximately 50% of the variance in smoking behavior is attributable to genetic factors. Genes in the serotonin system are plausible candidates because of serotonin's role in mood regulation. The present study examined the association of smoking behavior with a polymorphism in the *TPH* gene, which codes for a rate limiting enzyme in the biosynthesis of serotonin. A polymorphism in intron 7 has been linked with a variety of traits involving poor impulse control. Participants in this study were 249 Caucasian smokers and 202 nonsmokers recruited through newspaper advertisements. Smokers completed smoking history and nicotine dependence assessments. The overall frequencies of the A- and C-allele were 42% and 58%, respectively. There was no association of *TPH* alleles with smoking status. However, case series analysis indicated that individuals with the A/A genotype started smoking at age 15.6 years, compared with 17.3 years among smokers with other genotypes. This association was significant in a multivariate regression model controlling for age, education, body mass index (BMI), alcohol use, and medication use. This

finding is consistent with previous studies

relating the A-allele to impulsive behavior and suggests that it may predispose to early smoking initiation. Future family-based studies are needed to confirm this finding.

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Despite decades of intensive tobacco control efforts, about one-quarter of Americans continue to smoke. Even more disturbing is the fact that over 3,000 youth begin smoking every day and many of these will progress to regular smoking by adulthood [Pierce et al., 1989]. While social and environmental factors clearly play an important role in smoking initiation and persistence, there is also evidence for genetic susceptibility to tobacco use [Carmelli et al., 1992].

Initial studies of genetic influences on smoking behavior have focused on genes important in the regulation of the neurotransmitters dopamine and serotonin. While not yet investigated, several lines of evidence suggest that the tryptophan hydroxylase (*TPH*) gene may also play a role in smoking initiation and maintenance. The *TPH* gene codes for a primary enzyme in the biosynthesis of serotonin. Two polymorphisms in intron 7 have been identified and found to be in complete linkage disequilibrium, the A218C and the A779C [Nielsen et al., 1997]. There is initial evidence linking the more rare *TPH* 779A allele with behaviors related to poor impulse control, including suicide [Mann et al., 1997] and aggression [Manuck et al., 1999]. Genotypes containing the alternate C-allele have been related to a higher rate of suicide among impulsive alcoholics; however, in nonimpulsive offenders, suicide incidence was greater among those with A-genotypes [Nielsen et al., 1998]. The *TPH*

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TABLE I. Bivariate Analyses of TPH Genotype by Smoking History Variables

Smoking variables	Genotype			F statistic	P value
	A/A	A/C	C/C		
Age at smoking initiation (years)	15.6±2.6	17.1±3.8	17.4±3.7	3.8	0.02
Longest quit time (days)	388.9±569.8	283.2±654.0	434.3±1119.3	0.09	0.42
Nicotine dependence (FTND score)	4.9±2.6	5.6±2.4	5.1±2.4	1.5	0.22
Smoking rate (cigarettes/day)	22.6±12.2	23.8±10.3	21.9±9.0	0.8	0.45

variant has also been related to levels of cerebrospinal fluid 5-hydroxyindoleacetic acid, a serotonin metabolite [Nielsen et al., 1994]. Because of the association of tobacco and other substance use with poor impulse control [Wills et al., 1999], we hypothesized that TPH might play a role in smoking predisposition as well.

Participants were 249 smokers who reported smoking a minimum of five cigarettes per day for the past year and 202 nonsmokers who reported having smoked fewer than 100 cigarettes in their lifetime. Exclusion criteria included age under 18 years, current treatment for drug or alcohol dependence, or a personal cancer history. Recruitment was conducted through newspaper advertisements in the Washington, D.C., and Philadelphia areas. All subjects completed a written consent form and a set of self-report questionnaires, including demographic characteristics (age, gender, ethnicity, marital status, and education), psychological traits, and smoking history (smokers only). Smoking history variables included age at smoking initiation (“how old were you when you started smoking at least one cigarette a day?”), longest prior abstinence period, current smoking rate, and nicotine dependence. Nicotine dependence was measured using the Fagerstrom Test of Nicotine Dependence (FTND) [Heatherton et al., 1991]. Subjects were genotyped for the A779C TPH polymorphism using a method modified from Nielsen et al. [1997]. Chi-square tests of association, analysis of variance, and multiple regression were used to assess associations of genotype with smoking status and with continuous smoking history phenotypes.

All participants were Caucasian and about 56% were female. Almost two-thirds of participants had completed college. The average age was 44±14 years. Among smokers, the average smoking rate was 23±10 cigarettes/day. The overall frequencies of the A- and C-alleles of the TPH A779C polymorphism were 42% and 58%, respectively. Hardy-Weinberg conditions were met for both smokers and nonsmokers.

The frequency of the A-allele did not differ significantly between smokers and nonsmokers (43% vs. 40%, respectively; chi-square = 0.86; P = 0.35). The association of TPH genotypes (A/A, A/C, or C/C) with smoking status (smoker vs. nonsmoker) was also not significant (chi-square = 1.2; P = 0.53). Eighteen percent of smokers were A/A, 51% were A/C, and 31% were C/C. Among nonsmokers, 14% were A/A, 52% were A/C, and 34% were C/C.

The associations of TPH genotype with continuous smoking phenotypes are shown in Table I. TPH genotype was associated significantly with self-reported age at smoking initiation in a one-way analysis of variance. The ages at initiation were 15.6 years, 17.1 years, and 17.4 years for smokers with A/A, A/C, and C/C genotypes, respectively (F = 3.8; P = 0.02). TPH genotype was unrelated to previous quitting history, smoking rate, or nicotine dependence.

The association of genotype with age at smoking initiation was tested in a multivariate linear regression model. The model included demographic factors that were associated with the outcome in bivariate analyses (age and education), as well as a priori confounder variables (body mass index, psychotropic medication use, and alcohol use). As shown in Table II, TPH genotype remained significant in the model.

In this study, TPH allele frequencies and genotypes were not significantly related to smoking status, nicotine dependence, or quitting history. This is in contrast to the findings of Sullivan et al. [2001] showing that the A-allele was associated significantly with smoking initiation. Based on their results, we conducted a reanalysis of our data using comparable criteria for selection of study groups; however, the results were unchanged (data not shown).

We did, however, find a significant association between TPH genotype and age at initiation of regular smoking. Specifically, the presence of the A-allele was associated with an earlier age at smoking initiation. On average, individuals with A/A genotypes initiated regular smoking about 1.5 years earlier than those with other TPH genotypes. This result is consistent with several studies reporting associations of the A-allele

TABLE II. Linear Regression Model of Age at Smoking Initiation (n=249)

Variables	Beta	P value
Education	0.13	0.03
Age	0.26	<0.0001
BMI	-0.01	0.81
Alcohol	-0.01	0.93
Psychotropic medications	0.07	0.23
TPH (A/A vs. A/C)	0.21	0.01
TPH (A/A vs. C/C)	0.20	0.02
Total Model R ²	0.13	<0.0001

with conditions attributed to poor impulse control, such as suicide, and aggression [Mann et al., 1997; Manuck et al., 1999]. The present study is the first to suggest that *TPH* may be related to the age at which individuals progress to regular smoking behavior. In light of other research on this genotype, one interpretation for this finding is that individuals with *TPH* A-genotypes and poor impulse control may be prone to engage in risky behaviors, such as smoking, at an earlier age. Alternatively, such individuals may find the mood-altering effects of nicotine more reinforcing and therefore progress more rapidly to regular smoking.

The present results are preliminary and several limitations of this study must be considered. First, although the analyses were limited to Caucasians, 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 *TPH* and other candidate genes are warranted. A second consideration is that all participants had responded to newspaper advertisements and therefore may not be representative of smokers in the population. However, such individuals may be representative of persons seeking smoking treatment. Third, the retrospective self-report assessment of age at smoking initiation is subject to bias, and the association of genotype with this outcome may be due to chance alone.

Despite these limitations, the present study, together with the work of Sullivan et al. [2001], provides preliminary data on which to base future studies of the *TPH* gene and other genetic influences on smoking behavior. A better understanding of the role of serotonergic genes in smoking behavior may become valuable for individualizing behavioral and/or pharmacologic prevention and treatment strategies to those individuals who are most likely to benefit.

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