



## ORIGINAL RESEARCH ARTICLE

# Interacting effects of the serotonin transporter gene and neuroticism in smoking practices and nicotine dependence

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Individual differences in propensity to nicotine dependence appear to be mediated, in part, by genetic factors.<sup>1</sup> The serotonin transporter gene has a functional polymorphism (5-HTTLPR) which modulates gene transcription and reuptake.<sup>2,3</sup> A possible role in nicotine dependence is suggested by a link between 5-HTTLPR and neuroticism,<sup>4</sup> a personality trait which has been related to smoking practices.<sup>5</sup> In a cross-sectional study of 185 smokers, we utilized multiple linear regression modeling to examine the interacting effects of the 5-HTTLPR and neuroticism on smoking practices and nicotine dependence. Genotype was classified according to the presence or absence of the short (*s*) allele vs the long (*l*) allele of 5-HTTLPR (ie, *s/s* or *s/l* vs *l/l*). Models controlled for gender, age, race, and alcohol use. The 5-HTTLPR by neuroticism interaction effect was statistically significant in the models of nicotine intake ( $P = 0.05$ ), nicotine dependence ( $P = 0.001$ ), and smoking motivations (smoking to reduce negative mood ( $P = 0.01$ ); smoking for stimulation ( $P = 0.01$ )). The results suggested that neuroticism was positively associated with these smoking practices among smokers with 5-HTTLPR *S* genotypes (*s/s* or *s/l*), but not among smokers with the *L* genotype (*l/l*). The 5-HTTLPR may modify the effects of neuroticism on smoking motivations and nicotine dependence. Assessment of 5-HTTLPR genotype and neuroticism may help to identify smokers who are more responsive to psychotropic medications, such as selective serotonin reuptake inhibitors (SSRIs), which are being used in smoking cessation treatment. *Molecular Psychiatry* (2000) 5, 189–192.

Individual differences in propensity to nicotine dependence are mediated in part by genetic factors.<sup>1</sup> Previous studies have linked smoking behavior with genes involved in the regulation of the neurotransmitter dopamine.<sup>6,7</sup> This is biologically plausible, because the reinforcing properties of nicotine have been attributed to its effects on dopamine transmission and stimulation of the reward pathways.<sup>8</sup> Another candidate gene for smoking behavior encodes for the serotonin transporter which regulates synaptic reuptake in the brain.<sup>2</sup> Gene transcription has been reported to be modulated by a polymorphism (5-HTTLPR), resulting

in a long allele (*l* = long) vs a short allele (*s* = short).<sup>3</sup> The *s* allele is associated with reduced transcription and has been linked with neuroticism, an anxiety-related personality trait.<sup>4</sup> However, the association with personality has not been supported in subsequent studies.<sup>9,10</sup> Although we did not find evidence for a main effect of 5-HTTLPR on smoking status in a previous analysis,<sup>11</sup> Hu and colleagues<sup>12</sup> have provided evidence that the 5-HTTLPR modifies the association of neuroticism with smoking status. In the present report, we sought to replicate and extend the findings by Hu *et al*<sup>12</sup> examining the interacting effects of the 5-HTTLPR and neuroticism on smoking practices and nicotine dependence in an independent sample of smokers.

The study sample included 185 smokers of an average age of  $45 \pm 11$  years. Forty-six percent were male and 54% were female. Eighty-five percent were Caucasian and 15% were African American. The average smoking rate was 22 cigarettes/day. Twenty percent ( $n = 37$ ) of subjects had *s/s* genotypes, 46.5% ( $n = 86$ ) had *l/s* genotypes, and 33.5% ( $n = 62$ ) had *l/l* genotypes. Thus, the allele frequencies were 43% and 57% for the *s* and *l* alleles, respectively. These frequencies are consistent with previous reports.<sup>4,9</sup>

Student *t*-tests were used to compare smokers in the two 5-HTTLPR groups (*s/s* or *s/l* genotypes vs *l/l* genotype) in terms of continuous scores for the neuroticism measure and for smoking outcomes. There were no significant main effects of 5-HTTLPR for neuroticism ( $T = 0.15$ ,  $P = 0.88$ ), smoking motivations (smoking to reduce negative mood ( $T = 0.22$ ,  $P = 0.82$ ); smoking to increase stimulation ( $T = 0.48$ ,  $P = 0.63$ )), nicotine intake (No. of cigarettes/day  $\times$  nicotine level reported for the type of cigarette smoked by the subject ( $T = 1.3$ ,  $P = 0.23$ )), or nicotine dependence.

As an initial test of hypotheses about the interacting effects of personality and 5-HTTLPR on smoking variables, we computed Pearson correlations for the association of neuroticism scores with these smoking variables, stratified by 5-HTTLPR genotype (*s/s* or *s/l* vs *l/l*). As predicted, among smokers with 5-HTTLPR *S* genotypes, neuroticism was significantly correlated

**Table 1** Pearson correlations of neuroticism with smoking practices by 5-HTTLPR genotype

Smoking variable	<i>S</i> <sup>a</sup> genotype <i>S/S</i> or <i>S/L</i> ( <i>n</i> = 123)		<i>L</i> <sup>a</sup> genotype <i>L/L</i> ( <i>n</i> = 62)	
	<i>r</i>	<i>P</i>	<i>r</i>	<i>P</i>
Smoking for stimulation	0.34	0.0001	-0.00	0.95
Smoking for negative mood	0.45	0.0001	0.17	0.20
Nicotine intake	0.19	0.04	-0.17	0.20
Nicotine dependence	0.30	0.0008	-0.12	0.35

<sup>a</sup>*S* denotes short genotype; *L* denotes long genotype.

(using the Bonferroni correction) with smoking for stimulation, smoking to reduce negative mood, and nicotine dependence. There were no significant correlations among smokers with the 5-HTTLPR *L* genotype (Table 1).

The main and interacting effects of 5-HTTLPR and neuroticism on the smoking outcomes were then tested in multiple linear regression equations controlling for race, gender, age, and alcohol use. As shown in Table 2, statistically significant interaction effects were found in the models of smoking for stimulation (*P* = 0.01), smoking to reduce negative mood (*P* = 0.01), nicotine dependence (*P* = 0.001), and nicotine intake (*P* = 0.05). The significant interaction effects suggest that the association between neuroticism and these smoking outcomes depends on smokers' 5-HTTLPR genotypes.

To address the possibility of confounding by ethnicity, the above models were rerun excluding the 26 African Americans. Despite reduced power, the interaction effects were still observed for smoking for stimulation (*P* = 0.06), smoking to reduce negative affect (*P* = 0.03), nicotine dependence (*P* = 0.004), and nicotine intake (*P* = 0.02).

Our findings suggest that neuroticism is linked to

heightened self-medication smoking (smoking for stimulation purposes and to reduce negative affect) and to nicotine dependence only in the subgroup of smokers with 5-HTTLPR *S* genotypes. This result validates the gene-personality interaction effect found by Hu and colleagues,<sup>12</sup> and extends their work by showing a similar interacting effect of 5-HTTLPR and neuroticism on smoking motivations, nicotine dependence, and nicotine intake in an independent study sample. The approach used in our study complements the within-family approach used by Hu *et al*,<sup>12</sup> however, both approaches have limitations in the study of complex behavioral phenotypes; therefore, these results should be replicated in other studies.<sup>13,14</sup> Furthermore, since we did not collect data on the ethnicity of our subjects within groups of Caucasians and African Americans, we cannot rule out the possibility of population stratification. It should also be noted that the recruitment of study subjects responding to newspaper advertisements for smoking cessation might generate a sample that is not representative of the general population of smokers. However, our study results are generalizable to smokers seeking treatment, the population of interest in smoking cessation research.

**Table 2** Linear regression models of smoking variables

Independent variables	Dependent variables							
	Smoking for stimulation		Smoking to reduce negative mood		Nicotine intake		Nicotine dependence	
	<i>F</i> <sup>a</sup>	<i>P</i> value <sup>b</sup>	<i>F</i> <sup>a</sup>	<i>P</i> value <sup>b</sup>	<i>F</i> <sup>a</sup>	<i>P</i> value <sup>b</sup>	<i>F</i> <sup>a</sup>	<i>P</i> value <sup>b</sup>
Age	0.9	0.33	0.8	0.37	1.5	0.22	0.7	0.39
Gender	0.2	0.62	16.5	0.0001	0.1	0.73	0.0	0.86
Race	3.7	0.06	0.0	0.86	0.3	0.58	0.3	0.58
Alcohol	0.2	0.66	1.5	0.22	0.3	0.57	1.7	0.20
5-HTTLPR	7.3	0.008	5.7	0.02	3.5	0.06	12.3	0.0006
Neuroticism	11.3	0.001	15.4	0.0001	4.3	0.04	13.8	0.0003
(5-HTTLPR <sup>a</sup> neuroticism)	6.4	<b>0.01</b>	6.4	<b>0.01</b>	4.0	<b>0.05</b>	10.5	<b>0.001</b>
<i>P</i> value, model		0.007		0.0001		0.57		0.01
<i>R</i> <sup>2</sup> , model		0.10		0.22		0.03		0.10

<sup>a</sup>*F* value in final model.

<sup>b</sup>*P* value in final model.

These two studies are the first to document an interaction between a genetic predisposition for smoking behavior and a personality trait. Neuroticism is a personality trait involving depression, anxiety, and reactivity to stress. Twin studies have suggested that this personality trait has a significant heritable component.<sup>15</sup> Although we did not find evidence for a main effect of 5-HTTLPR on neuroticism, Hu and colleagues<sup>12</sup> and previous investigations<sup>4</sup> have reported that neuroticism scores are higher in persons with the 5-HTTLPR *S* genotypes. Thus, one interpretation of these results is that smoking behavior may be most strongly linked with a genetically-based form of neuroticism. However, this conclusion is tentative in light of non-replications of an association between 5-HTTLPR and neuroticism.<sup>9,10</sup>

The relationship between neuroticism and smoking behavior has been widely supported.<sup>5</sup> Anxious persons tend to smoke more<sup>16</sup> and have more difficulty quitting smoking.<sup>17</sup> They are also more likely to use cigarettes to 'self-medicate' their anxiety and negative mood states.<sup>18</sup> These findings have prompted use of anti-anxiolytic drugs for smoking cessation.<sup>19</sup> Preliminary data suggest that selective serotonin reuptake inhibitors (SSRIs), such as fluoxetine, may reduce the risk of smoking relapse among a subset of depressed smokers.<sup>20</sup> This is particularly relevant to our study because SSRIs inhibit serotonin reuptake, a process modulated, in part, by the 5-HTTLPR. Taken together, the results of our study and that of Hu *et al*<sup>12</sup> suggest that SSRIs may be differentially effective in promoting cessation depending on both neuroticism and 5-HTTLPR genotypes. A recent study suggests that the efficacy of fluvoxamine, an SSRI, in reducing depression is diminished in patients with the 5-HTTLPR *s/s* genotype.<sup>21</sup> Prospective clinical trials could be conducted to test the hypothesis that 5-HTTLPR genotype modifies response to SSRIs used for smoking cessation. Ultimately, this genetics research could lead to the tailoring of pharmacologic smoking cessation treatments to smokers most likely to benefit.

## Methods

### Study population

Participants included 185 males and females who reported smoking at least five cigarettes/day for at least 1 year. Exclusion criteria were: under age 18; a personal history of cancer, undergoing treatment for drug or alcohol addiction; or presence of a psychiatric disorder which precluded informed consent.

### Procedures

Smokers were recruited through newspaper advertisements and flyers in the metropolitan Washington and Philadelphia areas for a free self-help smoking cessation program. During a visit to the clinic, they completed an informed consent form reviewing the study procedures including genotyping. They also completed self-report questionnaires assessing demographics, personality, and smoking practices.

### Measures

The *Eysenck Personality Inventory (EPI)* neuroticism subscale<sup>22</sup> was used to assess neuroticism, a personality trait involving heightened anxiety, depression, and reactivity to stress. The neuroticism subscale of the EPI has been shown to correlate with clinical measures of anxiety and depression.<sup>23</sup>

Smoking motivations were assessed using a modified version of the *Horn-Waingrow Reasons for Smoking (RFS) Scale*.<sup>24</sup> The stimulation smoking subscale measures smoking for stimulation purposes (eg, 'I get a definite lift and feel more alert when smoking'; range = 0–12). The negative mood reduction subscale measures smoking to relieve negative mood (eg, 'When I feel blue or want to take my mind off cares and worries, I smoke cigarettes'; range = 0–9). These subscales have been validated against self-monitored smoking data.<sup>25,26</sup>

*Nicotine intake* was computed as the cross-product of the number of cigarettes smoked per day and the nicotine level in the cigarette brand. A meta-analysis of studies comparing self-reported smoking behavior and biochemical measures of smoking suggests that most studies find self-reports of smoking are accurate (average sensitivity = 87.5%, average specificity = 89.2%).<sup>27</sup>

*Nicotine dependence* was assessed using the *Fagerstrom test for nicotine dependence (FTND)*, a highly reliable six-item self-report measure.<sup>28,29</sup> The FTND has been shown to correlate with plasma levels of nicotine and cotinine, and predicts withdrawal symptoms and smoking cessation.<sup>30</sup>

For the genetic analysis, oligonucleotide primers flanking the 5-HTTLPR (5'ggcgtgcccgtctgaattgc and 5'-gaggactgagctggacaaccac) from the 5-HTT gene 5'-flanking regulatory region generating 484 bp or 528 bp were amplified by PCR using 50 ng of genomic DNA, 2.5 mM deoxyribonucleotides, 0.1 µg of primers, 10 mM tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, and 1 U 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; NIGMS Human Genetic Mutant Cell Repository, Corell Institute, Camden, NJ, USA). Twenty percent of the total number of samples were repeated for quality control.

### Statistical analysis

The data analysis was conducted in three stages. First, student *t*-tests were used to assess the main effects of 5-HTTLPR genotype (*s/s* or *s/l* genotypes vs *l/l* genotype) on continuous measures of neuroticism and smoking practices. Second, Pearson correlations were computed to relate neuroticism with smoking practices in the two groups stratified by 5-HTTLPR genotype. A Bonferroni correction was applied to address the mul-

tiple comparisons (thus requiring  $P \leq 0.0125$  for statistical significance). Third, the main and interacting effects of 5-HTTLPR genotype and neuroticism on the smoking outcomes were assessed in multiple linear regression models which controlled for potential confounder variables (age, gender, race, and alcohol use).

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