Lack of Association of 5-HTTLPR Genotype with Smoking Cessation in a Nicotine Replacement Therapy Randomized Trial

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Introduction

Cigarette smoking is the leading preventable cause of death worldwide, accounting for at least 30% of all cancer deaths and over three quarters (87%) of lung cancer deaths in developed countries; however, despite progress made in the treatment of tobacco dependence, available Food and Drug Administration–approved treatments are effective for only a fraction of smokers. The wide individual variation in therapeutic response has prompted a growing interest in the study of the role of inherited factors in the efficacy of alternate pharmacotherapies (1). To date, two pharmacogenetic trials of NRT have been conducted. Based on the neurobiology of reward (2, 3), pharmacogenetic analyses have focused on genes in the dopamine pathway (4–6) and the opioid pathway (7).

Other promising candidate genes for studies of smoking cessation pharmacogenetics exist. A functional polymorphism in the promoter region of the serotonin transporter (5-HTT) gene has been identified (5-HTTLPR) and is known to be associated with altered serotonin activity, with the short (S) form of this polymorphism being associated with reduced transcriptional efficiency of the 5-HTT promoter compared with the long (L) form, thereby decreasing serotonin transporter expression and serotonin uptake (8), while a recent positron emission tomography study also showed an association of this polymorphism with 5-HT1A binding in healthy volunteers (9).

A recent meta-analysis of case-control genetic association studies of smoking behaviors (10) noted that the 5-HTT gene showed evidence of association with smoking cessation, in a comparison of current smokers with ex-smokers, with possession of one or more copies of the S allele associated with a reduced likelihood of cessation. It is possible that the S allele influences smoking cessation via increased anxiety-related withdrawal symptomatology, given evidence for an association of this polymorphism with anxiety-related traits (11).

However, no study has yet investigated the association of 5-HTTLPR genotype with smoking cessation in an explicitly designed study of smoking cessation or investigated possible genotype × treatment interaction effects.

We predicted that possession of one or more copies of the S allele of the 5-HTTLPR polymorphism would be associated with reduced likelihood of successful cessation. We also explored the possibility that NRT delivered via nasal spray might be more effective than NRT delivered via transdermal patch in smokers with one or more copies of the S allele, given that ad lib nasal spray delivery might be better suited to the relief of acute anxiety-related withdrawal symptomatology.

Materials and Methods

Three hundred and ninety-seven smokers of European ancestry, recruited by advertisements in local media in Philadelphia and Washington DC from February 2000 to April 2003, participated in this study. The trial was an open-label randomized clinical trial of transdermal patch versus nasal spray nicotine replacement therapy for smoking cessation. The University of Pennsylvania and Georgetown University Institutional Review Boards approved all study procedures, and all participants provided written, informed consent. All participants provided samples of whole blood for subsequent genotyping and cotinine analysis. To assess smoking status, telephone interviews were conducted at the end of treatment and at 6-month follow-up. Participants who reported completed abstinence for the previous 7 days were required to complete an in-person visit for biochemical verification of abstinence. Participants were genotyped for the 5-HTTLPR using primers as described by Heils et al. (8). The study is described in detail elsewhere (7).

Sustained abstinence, at end of treatment and 6-month follow-up, was the primary outcome measure. Self-reported abstinence at end of treatment and 6-month follow-up was confirmed by exhaled carbon monoxide monitoring (<10 ppm). Participants lost to follow-up were assumed to have relapsed to smoking (12) and coded as such in outcome analyses (i.e., intent to treat analyses). Separate models of outcome at end of treatment and 6-month follow-up were generated within a logistic regression framework because pharmacotherapy was available only during the treatment phase. Age, sex, and nicotine dependence score were entered in the first step, treatment group (transdermal patch and nasal spray) in the second step, and 5-HTTLPR genotype (LL, SL, and SS) and a genotype × treatment group interaction term in
the third step. For comparisons involving 5-HTTLPR genotype, LL was the reference group. An α level of 0.05 was maintained throughout.

The sample was adequate to detect small effects (Cohen’s d = 0.3) corresponding to an odds ratio of 1.9 for the main effect of genotype. Power calculations were done using Power and Sample Size Software (NCSS, Kaysville UT).

**Results**

Genotype data were missing on four participants so that the final sample for analysis consisted of 393 smokers (53% male). The mean age of participants was 46 years 7 months (SD, 11 years 5 months; range, 20-78 years). 5-HTTLPR genotype frequencies by treatment group and abstinence at both end of treatment and 6-month follow-up are presented in Table 1. Genotype frequencies did not deviate significantly from Hardy-Weinberg equilibrium (P = 0.11).

The main effect of 5-HTTLPR genotype was not associated with abstinence at either end of treatment (SL, P = 0.51; SS, P = 0.74) or 6-month follow-up (SL, P = 0.23; SS, P = 13) and there was no evidence for a genotype × treatment interaction effect (Table 2). All other effects were nonsignificant although the effect of nicotine dependence score on abstinence at 6-month follow-up approached statistical significance (P = 0.07). The full logistic regression models for abstinence at both end of treatment and 6-month follow-up are presented in Table 2.

**Discussion**

These results provide no support for an association between 5-HTTLPR genotype and smoking cessation in response to nicotine replacement therapy. Our study was adequately powered to detect an odds ratio of 1.9 for the main effect of genotype. The therapeutic action of NRT is considered to operate via a reduction in the acute nicotine withdrawal symptoms, on which our hypotheses about genotype may be associated with smoking cessation, if the small effect sizes observed in our data will be shown to be statistically significant in a future large-scale pharmacogenetic trial. Although our data do not give any reason to believe that 5-HTTLPR genotype is associated with smoking cessation, if the small effect sizes observed in our data are real, a sample of n = 7,200 would be required to achieve 80% power to show statistical significance for both the main effect of genotype and the genotype × treatment interaction. It is also possible that 5-HTTLPR genotype may be associated with response to other pharmacologic treatments for smoking cessation, in particular those with an antidepressant action, including bupropion and nortriptyline, as well as selective serotonin reuptake inhibitors. Future human behavioral pharmacology studies that test genotype and medication effects on intermediate phenotype measures (also known as endophenotypes, which may be biological more proximal to genetic antecedents of interest) may also be informative.

**Acknowledgments**

We thank Pharmacia (Helsingborg, Sweden) for nicotine nasal spray.

<table>
<thead>
<tr>
<th>Variable</th>
<th>End of treatment</th>
<th>6-month follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Age (y)</td>
<td>1.00 (0.98-1.02)</td>
<td>0.924</td>
</tr>
<tr>
<td>Sex</td>
<td>0.73 (0.47-1.12)</td>
<td>0.154</td>
</tr>
<tr>
<td>Nicotine dependence</td>
<td>0.93 (0.84-1.02)</td>
<td>0.138</td>
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</table>

**Table 2. Logistic regression models of abstinence at end of treatment and 6-month follow-up**

### Table 1. 5-HTTLPR genotype frequencies by treatment and abstinence at end of treatment and 6-month follow-up

<table>
<thead>
<tr>
<th>5-HTTLPR genotype</th>
<th>SS</th>
<th>SL</th>
<th>LL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patch (n = 31)</td>
<td>Spray (n = 34)</td>
<td>Combined (n = 65)</td>
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<tr>
<td>End of treatment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succeeded</td>
<td>11 (35%)</td>
<td>9 (26%)</td>
<td>20 (31%)</td>
</tr>
<tr>
<td>Failed</td>
<td>20 (65%)</td>
<td>25 (74%)</td>
<td>45 (69%)</td>
</tr>
<tr>
<td>6 months</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Succeeded</td>
<td>8 (26%)</td>
<td>6 (18%)</td>
<td>14 (21%)</td>
</tr>
<tr>
<td>Failed</td>
<td>23 (74%)</td>
<td>28 (82%)</td>
<td>51 (79%)</td>
</tr>
</tbody>
</table>

**Abbreviations:** OR, odds ratio; 95% CI, 95% confidence interval.

*Reference group is LL.*

Nonetheless, the lack of an association at the end of the treatment phase or at 6-month follow-up suggests that this polymorphism is unlikely to play a major role in smoking cessation or response to nicotine replacement therapy. Whereas the direction of the main effect observed at the end of treatment phase was broadly consistent with our a priori hypothesis, with lowest overall cessation in the SS group, this was reversed at 6-month follow-up. Moreover, the direction of the interaction effect was opposite to that predicted by our a priori hypothesis, with the greatest relative benefit of patch over nasal spray observed in the SS group.

It remains a possibility that the effect sizes that we observed will be shown to be statistically significant in a future large-scale pharmacogenetic trial. Although our data do not give any reason to believe that 5-HTTLPR genotype is associated with smoking cessation, if the small effect sizes observed in our data are real, a sample of n = 7,200 would be required to achieve 80% power to show statistical significance for both the main effect of genotype and the genotype × treatment interaction. It is also possible that 5-HTTLPR genotype may be associated with response to other pharmacologic treatments for smoking cessation, in particular those with an antidepressant action, including bupropion and nortriptyline, as well as selective serotonin reuptake inhibitors. Future human behavioral pharmacology studies that test genotype and medication effects on intermediate phenotype measures (also known as endophenotypes, which may be biological more proximal to genetic antecedents of interest) may also be informative.
References
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