Nicotinic Acetylcholine Receptor β2 Subunit (CHRNB2) Gene and Short-Term Ability to Quit Smoking in Response to Nicotine Patch

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Abstract

Genes coding for nicotinic acetylcholine receptors may influence response to nicotine replacement therapy for smoking cessation. We examined the association of a 3′ untranslated region polymorphism (rs2072661) in the nicotinic acetylcholine receptor β2 subunit (CHRNB2) gene with quitting success in response to nicotine versus placebo patch during a short-term test of patch effects. In a within-subjects cross-over design, smokers of European descent (n = 156) received 21 mg nicotine and placebo patch in counter-balanced order, during two separate 5-day simulated quit attempts, each preceded by a week of ad libitum smoking. Abstinence was assessed daily by CO < 5 ppm. Smokers with the CHRNB2 GG genotype had more days of abstinence during the nicotine versus placebo patch week compared with those with the AG or AA genotypes (P < 0.01). Moreover, nicotine patch increased the probability of quitting on the target quit day, quitting anytime during the patch week, and avoiding relapse among those with the GG genotype but not the AA/AG genotypes, although the nicotine × genotype interaction was significant only for quitting on the target quit day (P = 0.05). Regardless of patch condition, quitting on the target quit day was more likely in those with the GG genotype versus AA/AG genotypes (P < 0.05). Genetic associations were not observed for craving or withdrawal responses to nicotine versus placebo patch. These findings are consistent with previous evidence of association of this variant with smoking cessation and suggest that polymorphisms in the nicotinic acetylcholine receptor β2 subunit gene may influence therapeutic responsiveness to cessation medications. (Cancer Epidemiol Biomarkers Prev 2009; 18(10):2608–12)

Introduction

Pharmacogenetics research in nicotine dependence holds future promise for optimizing pharmacotherapy outcomes by tailoring the choice and dose of medications based on individuals’ genetic profiles (1, 2). Results of such research also suggest directions for exploring the mechanisms through which genetic factors influence therapeutic response in clinical trials, as well as for understanding individual differences in severity of nicotine dependence (3).

Genes that regulate the structure or function of neuronal nicotinic acetylcholine receptors are of particular interest in pharmacogenetic studies because the actions of nicotine in the brain underlie virtually all of the psychoactive effects of cigarette smoking. In particular, α4β2 nicotinic acetylcholine receptors expressed on dopaminergic neurons in the ventral tegmental area play a key role in the reinforcing effects of nicotine (4) and are the target of nicotine dependence medications such as varenicline (5). Several studies (6-9) found no associations between various measures of nicotine dependence and a few single-nucleotide polymorphisms in the nicotinic acetylcholine receptor β2 subunit gene (CHRNB2), although another found a gene-gene interaction between CHRNB2 and CHRNA4 (10). Yet, a recent pharmacogenetic investigation (11) examining >1,200 single-nucleotide polymorphisms in a pathway-based analysis of nicotinic acetylcholine receptor and dopamine-related genes found a highly significant association of a 3′ untranslated region single-nucleotide polymorphism (rs2072661) in CHRNB2 with smoking cessation in a bupropion therapy trial. Specifically, the GG genotype was associated with a greater likelihood of quitting overall and a more favorable response to bupropion versus placebo compared with smokers with the minor allele (that is, AA or AG). Although the function of this variant is unknown, these data suggest that CHRNB2 variation may play a significant role in a smoker’s ability to quit.

We examined the association of this CHRNB2 single-nucleotide polymorphism (rs2072661) with ability to quit during a week of nicotine versus placebo patch use in a simulated quit trial. Genetic influences on therapeutic response to nicotine patch are potentially very important because nicotine patch is the most widely used medication for smoking cessation, with at least 25% of all smokers having tried it (12). A unique feature of this study was...
the within-subjects cross-over design in which all subjects received nicotine and placebo patch in different study phases so that they could serve as their own controls.

Materials and Methods

Participants. Participants (n = 156) were those of European descent from a study aimed primarily at assessing characteristics, including current quit interest, of those better able to quit smoking while using nicotine versus placebo patch during a week of patch use (13, 14). Only those of European descent were included in this genetic analysis to reduce the potential for confounding by ancestry. Those eligible were required to be between the ages of 18 and 65 and to smoke at least 10 cigarettes per day for at least the past 2 y. Mean (SD) sample characteristics were as follows: 29.0 (11.0) y of age, 17.4 (5.1) cigarettes per day, and Fagerstrom Test of Nicotine Dependence (15) score of 4.6 (1.4), indicating moderate dependence. Participants were recruited from the surrounding community. Advertisements indicated that smokers who were or were not interested in quitting soon were eligible.

Current quit interest or motivation to make a permanent quit attempt soon was a focus of the primary study (13) and served as a covariate in analyses (see Data Analyses). “High” quit interest labeled those who wanted to quit permanently within the next 1 mo (n = 64), whereas “low” quit interest labeled those who had no intention of quitting in the next 6 mo (n = 92). Those stating an intention to quit between 1 and 6 mo were excluded from participation, whereas those interested in quitting immediately were referred to treatment programs elsewhere and not included in the study because of the need for smoking resumption between cross-over patch conditions.

Craving and Withdrawal. Craving and withdrawal were assessed at every visit. The 11-item Questionnaire of Smoking Urges brief (16) assessed craving for cigarettes. Nicotine withdrawal was assessed by the Minnesota Nicotine Withdrawal Scale using the following six items: depressed mood/sad, irritable/angry/frustrated, anxious/nervous, difficulty concentrating, restless/impatient, and drowsiness. Items on each measure were protected using different reporter dyes for each probe (6FAM reporter dye, a nonfluorescent quencher, and minor groove binder. Allele-specific cleavage of probes was detected using different reporter dyes for each probe (6FAM and VIC fluorophores for the each allele) with separate wavelength maxima. PCR amplifications were set up in a 384-well plate format in total volume of 5 μL, containing 2.5 μL 2× universal master mix, 0.25 μL 1× primer and probe from Applied Biosystems, and 2.25 μL of DNA at a concentration of 5 ng/μL. Controls representing each genotype for each variant and a no template (water) control were included in each 384-well plate. PCR was done in ABI 7900 HT Sequence Detection System (Applied Biosystems). After an enzyme activation step for 10 min at 95°C, 60 two-step cycles were done, 15-s denaturation at 95°C, followed by 1 min annealing/extension at 60°C for all variants. After PCR endpoint fluorescence levels of 6FAM and VIC were measured automatically in each well using the SDS 2.1 manufacturer’s custom software (Applied Biosystems). Allelic discrimination results were then graphed on a scatter plot contrasting reporter dye florescence (that is, allele A versus allele G). As in previous work (11), smokers were classified based on the presence (AA or AG) versus absence (GG) of the minor allele.

Data Analyses. No significant main or interaction effects were found for sex or patch order in preliminary ANOVAs, so subsequent analyses collapsed across sex and patch order. The primary analysis was a repeated-measures ANOVA of days quit per patch week (range, 0-4), with CHRNA2 genotype (GG versus AG/AA) as the between-subjects factor and patch treatment (nicotine, placebo) as the within-subjects factor. Current quit interest

2 Available at www/uvm.edu/~hbpl.
Table 1. Mean (SE) demographics and smoking history characteristics for each CHRNB2 genotype group

<table>
<thead>
<tr>
<th>CHRNB2 genotype groups</th>
<th>AA/ AG (n = 60)</th>
<th>GG (n = 96)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M</td>
<td>SE</td>
</tr>
<tr>
<td>Demographics</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age, y</td>
<td>29.3</td>
<td>1.5</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>58.3</td>
<td>4.7</td>
</tr>
<tr>
<td>BMI</td>
<td>25.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Smoking history</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cigarettes per day</td>
<td>17.3</td>
<td>0.8</td>
</tr>
<tr>
<td>Fagerstrom Test of Nicotine Dependence (0-10)</td>
<td>4.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Years smoking</td>
<td>12.2</td>
<td>1.4</td>
</tr>
<tr>
<td>Earlier quit attempts</td>
<td>1.5</td>
<td>0.2</td>
</tr>
<tr>
<td>Longest duration of earlier quit attempt, wk</td>
<td>10.3</td>
<td>3.6</td>
</tr>
</tbody>
</table>

NOTE: Groups did not differ on any characteristics. Abbreviations: M, mean; BMI, body mass index.

Results

Ninety-six subjects (61.5%) were homozygous for the G allele (that is, GG genotype), whereas 60 (38.5%) were homozygous for the A allele (AA; n = 12; 7.7%) or were heterozygous (AG; n = 48; 30.8%). The allele frequencies in Hardy-Weinberg equilibrium ($P = 0.24$). The demographic and smoking history characteristics of the GG and AG/AA genotype groups are presented in Table 1. The groups did not differ significantly on any of these characteristics.

**CHRNB2 Genotype, Nicotine Patch, and Ability to Quit Smoking.** The distribution of the number of quit days (0-4) for nicotine and placebo patch weeks is presented for each CHRNB2 genotype in Table 2. A significant CHRNB2 genotype × patch condition interaction effect was found for days quit during the patch weeks $F(1,152) = 7.64; P < 0.01$.

Table 2. Distribution of the number of quit days per patch week (range, 0-4) by CHRNB2 genotype

<table>
<thead>
<tr>
<th>Days quit</th>
<th>AA/ AG (n = 60)</th>
<th>Nicotine patch</th>
<th>Placebo patch</th>
<th>Nicotine patch</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>37 (61.7)</td>
<td>33 (55.0)</td>
<td>53 (55.2)</td>
<td>41 (42.7)</td>
</tr>
<tr>
<td>1</td>
<td>3 (5.0)</td>
<td>9 (15.0)</td>
<td>12 (12.5)</td>
<td>12 (12.5)</td>
</tr>
<tr>
<td>2</td>
<td>1 (1.7)</td>
<td>3 (5.0)</td>
<td>8 (8.3)</td>
<td>6 (6.3)</td>
</tr>
<tr>
<td>3</td>
<td>10 (16.7)</td>
<td>6 (10.0)</td>
<td>9 (9.4)</td>
<td>11 (11.5)</td>
</tr>
<tr>
<td>4</td>
<td>9 (15.0)</td>
<td>9 (15.0)</td>
<td>14 (14.6)</td>
<td>26 (27.1)</td>
</tr>
</tbody>
</table>

Discussion

This study provides evidence for association of a common 3’ untranslated region single-nucleotide polymorphism in the β2 subunit nicotinic acetylcholine receptor gene with...
responsiveness to nicotine patch effects on abstinence in a 1-week simulated quit attempt. Specifically, those with the \textit{CHRNB2} GG genotype quit on more days with the nicotine versus placebo patch compared with those with the AA or AG genotypes. In addition, nicotine patch increased the probability of quitting on the target quit day, quitting anytime during the patch week, and avoiding relapse during the week after initiating abstinence among those with the GG genotype but not AA/AG genotypes, although the interaction of nicotine $\times$ genotype was significant only for quitting on the target quit day. Therefore, our results suggest a rather specific benefit of nicotine patch in those with the GG genotype, that of enhancing their ability to initiate abstinence on the scheduled target quit day.

The \textit{CHRNB2} association with ability to quit due to nicotine versus placebo patch compared with those with the AA or AG genotypes. In addition, nicotine patch increased the probability of quitting on the target quit day, quitting anytime during the patch week, and avoiding relapse during the week after initiating abstinence among those with the GG genotype but not AA/AG genotypes, although the interaction of nicotine $\times$ genotype was significant only for quitting on the target quit day. Therefore, our results suggest a rather specific benefit of nicotine patch in those with the GG genotype, that of enhancing their ability to initiate abstinence on the scheduled target quit day.

The \textit{CHRNB2} association with ability to quit due to nicotine versus placebo patch is consistent with the results of a previous pharmacogenetic study (11). Among participants in a randomized clinical trial of bupropion versus placebo, the odds of being abstinent at 6-month follow-up were significantly greater among smokers with the GG genotype than those with the AG or AA genotype, and there was a trend for greater responsiveness to bupropion in this group as well. Thus, genetic variation in \textit{CHRNB2} may be related to greater therapeutic response to cessation medications in general or to medications acting on a mechanism that is shared by nicotine patch and bupropion. Conti et al. (11) also found an overall greater odds of abstinence at the end of 12 weeks of treatment, regardless of medication condition, for the GG genotype versus AG/AA, a main effect of \textit{CHRNB2} genotype we also observed for quitting on the target quit day but not for quitting on any day or avoiding relapse once quit.

Although nicotine patch reduced craving, patch effects on craving and withdrawal did not reveal genotype differences in those responses to nicotine versus placebo patch that might help explain the greater quitting of those with the GG versus AG or AA genotypes. Yet, those analyses were exploratory in that they were limited to only those days when subjects were abstinent so that responses to nicotine through patch would not be confounded with effects due to nicotine through smoking. Restricting these analyses to abstinent subjects may also introduce self-selection bias because those unable to abstain may have experienced the most severe withdrawal. Possible genetic influences on craving and withdrawal responses to nicotine replacement therapy should be examined in larger samples during enforced periods of abstinence to reduce the self-selection bias (that is, the ability to quit each patch week) that may have obscured our results on craving and withdrawal. Moreover, smoking history and demographic characteristics did not differ between \textit{CHRNB2} genotypes (Table 1), consistent with previous studies (6-9) and suggesting that the greater ability of those with the GG genotype to quit with nicotine patch is not due to a difference in level of dependence or smoking behavior but rather to unique influences on the ability to quit.

These results are limited in several ways and the associations of \textit{CHRNB2} with quitting success on nicotine patch require replication. First, our sample of 156 was...
small for genetic analyses, although the within-subjects design greatly enhanced power by allowing subjects to act as their own controls (17). Moreover, power in the ANOVA was further augmented by our continuous dependent measure of abstinence of the number of days quit compared with the dichotomous dependent measure of abstinence versus relapsed typical in clinical trials. Second, in addition to the possible bias in the analysis of withdrawal and craving, as noted previously, our sample may have been biased due to self-selection of smokers interested in participating in a short-term simulated clinical trial rather than an actual clinical trial. Third, similarly, the genotype differences in quitting due to nicotine patch may have been specific to the procedures or short duration of this simulated trial, and our findings need to be confirmed in an actual clinical trial of nicotine versus placebo patch involving long-term follow-up, as in the bupropion trial reported by Conti et al. (11).

Further studies are needed to determine whether the single-nucleotide polymorphism in the present study has functional properties or is in linkage disequilibrium with unknown functional variants in CHRN2. Building on the results from Conti et al. (11) with bupropion and the current study with nicotine replacement therapy patch, such research should also examine associations of CHRN2 with clinical response to other formulations of nicotine replacement therapy and particularly to the other Food and Drug Administration–approved cessation medication, varenicline (Chantix), which is a partial agonist of α4β2 nicotine receptors (5).

Disclosure of Potential Conflicts of Interest

C. Lerman is a consultant/advisory board member of GlaxoSmithKline, Pfizer, AstraZeneca, and Novartis; and K. Perkins is a consultant/advisory board member of GlaxoSmithKline.

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References

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