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.

Cragging and Withdrawal. Cragging 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 Scale2 using the following six items: depressed mood/sad, irritable/angry/frustrated, anxious/nervous, difficult concentrating, restless/impatient, and drowsiness. Items on each measure were averaged to get total craving and total withdrawal.

Procedures. Procedural details for this study have been reported elsewhere (13). Briefly, this study involved a 4-wk cross-over design, with 2 2-wk phases. Each phase consisted of ad libitum smoking during the first week, followed by attempting to quit while using a patch during the second week. Following week 2, subjects proceeded to the next phase, starting with the resumption of ad libitum smoking during week 3. The phases differed only in the patch condition during the second week, nicotine (NicoDerm CQ 21 mg) versus placebo patches matched in size and appearance (1-800-Patches, Inc.). Order of nicotine and placebo patch across phases was counter balanced between subjects. The main study also manipulated another between-subjects factor, monetary reinforcement versus no reinforcement, for abstinence on each day of patch use (13), which was included as a covariate in the current analyses.

During the introductory session before week 1, all subjects, regardless of current quit interest, agreed that they would try to quit during the two patch weeks (weeks 2 and 4). Subjects were then randomized to either the abstinence reinforcement or the no reinforcement condition, stratified by quit interest group and sex. The reinforcement condition involved paying subjects $12 each day during the patch week that they had quit for 24 h, defined as CO < 5 ppm and self-report of no smoking at all in the last 24 h. Subjects in the no reinforcement condition received no money for abstinence but otherwise were treated the same as those in the reinforcement condition.

Participants came to the clinic 3 days per week during each ad libitum smoking week (e.g., Monday, Wednesday, Friday) and all 5 weekdays (Monday to Friday) during each patch week. Daily assessments included CO, withdrawal, and craving. Only abstinence on Tuesday to Friday of each patch week (that is, range of 0-4 days per week) was included in data analyses because patch use did not begin until Monday morning, just a few hours before the Monday clinic visit. This study was conducted in accordance with the principles of the Helsinki Declaration and approved by the University of Pittsburgh Institutional Review Board.

Genotyping. Taqman 5’ nuclease PCR primers and probes for alleles of a 3’ untranslated region polymorphism (rs2072661) in the nicotinic acetylcholine receptor 2 subunit (CHRN2) gene were obtained as Assay-On-Demand (C_15946677_10) from Applied Biosystems. Each probe consisted of an oligonucleotide with a fluorescent 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 denaturatation 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 fluorescence (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 CHRN2 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.
and abstinence reinforcement condition were covariates in the ANOVA because of their influence on abstinence (13). We hypothesized an interaction of *CHRNB2* genotype by patch condition, showing differential success in quitting due to nicotine versus placebo patch as a function of genotype. We also used nonparametric tests to determine the main effects of nicotine versus placebo patch (Wilcoxon signed ranks) and of genotype (χ²) and the interaction of nicotine × genotype (Mann-Whitney U test) on the (a) ability to quit on the target quit date of each patch week (that is, meeting abstinence criteria on the first full day of abstinence assessment, Tuesday), (b) ability to quit at all during each patch week (that is, meeting abstinence criteria on at least one day that week), and (c) ability to avoid relapse during the patch week after initiating abstinence (that is, no relapse at any point before the end of the week). In exploratory analyses of craving and withdrawal, we used repeated measures linear mixed effects models with restricted or residual maximum likelihood (REML) estimation to determine effects of nicotine patch, genotype, and day. All models assumed a compound symmetrical covariance structure between repeated measurements.

**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 were 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]. As shown in Fig. 1, days quit due to the nicotine versus placebo patch were greater among smokers with the GG genotype compared with those with AG or AA genotypes. No main effects were observed for genotype or patch condition, both F(1,152) < 1.

Nicotine (versus placebo) patch significantly increased ability to quit on the target quit date, ability to quit any time during the patch week, and ability to avoid relapse throughout the week among those who initiated quitting (Z's of 2.14, 2.92, and 2.36, respectively; P's < 0.05, 0.005, and 0.05, respectively). As shown in Fig. 2, these effects of nicotine patch were significant for the GG but not AG or AA genotypes, although the interaction of genotype × nicotine was significant only for ability to quit on the target quit date (Z = 2.11; P < 0.05). Regardless of patch condition, those with the GG genotype were more likely than those with AG or AA genotypes to quit on the target quit date (that is, main effect of genotype; χ²(1) = 5.91; P < 0.05), but there were no main effects of genotype for the other two outcomes.

**CHRNB2 Genotype, Craving, and Withdrawal.** Because nicotine through smoking would confound responses to patch condition, the exploratory analyses of craving and withdrawal included only responses from abstinent days, limiting the amount of data (and power) available for analysis. Nicotine versus placebo patch decreased Questionnaire of Smoking Urges craving, 29.8 ± 2.6 versus 37.1 ± 2.7 [F(1,328) = 25.81; P < 0.001], but not withdrawal [F(1,325) < 1]. The effects of nicotine did not differ by *CHRNB2* genotype because the interaction of genotype × nicotine was not significant for either craving [F(1,328) < 1] or for withdrawal [F(1,325) = 1.64; P > 0.20]. There was also no main effect of genotype on craving or withdrawal, both F(1,83) < 1.

**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 × 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 × 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

\begin{figure}[h]
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\includegraphics[width=\textwidth]{figure1.png}
\caption{Days abstinent during nicotine versus patch weeks (range, 0-4) by \textit{CHRNB2} genotype groups. The difference due to nicotine versus placebo was significant in GG (n = 96) but not in AA/AG (n = 60).}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure2.png}
\caption{Percentage of quitting on the target quit day, quitting any day during the patch week, and avoiding relapse if able to quit during nicotine versus placebo patch weeks by \textit{CHRNB2} genotype groups. The main effect of nicotine patch was significant for all three outcomes. The main effect of genotype and the interaction of genotype × nicotine were significant only for quitting on the target quit day.}
\end{figure}
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 absent 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 CHRNβ2. 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 CHRNβ2 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 Glaxo-SmithKline, Pfizer, AstraZeneca, and Novartis; and K. Perkins is a consultant/advisory board member of GlaxoSmithKline.

Acknowledgments
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We thank John Scott, Sarah Coddington, Roy Chengappa, Robert Ferrell, Riju Ray, Paul Wileyto, Daniel Heitjan, and Don Baldwin for their assistance.

References
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