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## Gender Stratified Gene and Gene–Treatment Interactions in Smoking Cessation

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### Abstract

We conducted gender-stratified analyses on a systems-based candidate gene study of 53 regions involved in nicotinic response and the brain-reward pathway in two randomized clinical trials of smoking cessation treatments (placebo, bupropion, transdermal and nasal spray nicotine replacement therapy). We adjusted *P*-values for multiple correlated tests, and used a Bonferroni corrected  $\alpha$ -level of  $5 \times 10^{-4}$  to determine system-wide significance. Four SNPs (rs12021667, rs12027267, rs6702335, rs12039988;  $r^2 > 0.98$ ) in erythrocyte membrane protein band 4.1 (*EPB41*) had a significant male-specific marginal association with smoking abstinence (OR=0.5; 95% CI 0.3–0.6) at end of treatment (adjusted  $P < 6 \times 10^{-5}$ ). rs806365 in cannabinoid receptor 1 (*CNRI*) had a significant male-specific gene-treatment interaction at 6-month follow-up (adjusted  $P = 3.9 \times 10^{-5}$ ); within males using nasal spray, rs806365 was associated with a decrease in odds of abstinence (OR=0.04; 95% CI 0.01–0.2). While the role of *CNRI* in substance abuse has been well studied, we report *EPB41* for the first time in the nicotine literature.

### Keywords

Genetic association studies; heterogeneity; smoking cessation

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## INTRODUCTION

Nicotine, the addictive component of cigarettes, plays a primary role in entrapping individuals in a cycle of tobacco dependence. It acts swiftly on central nervous system receptors and stimulates the release of neurotransmitters (e.g., dopamine). These neurotransmitters are involved in the brain reward pathway, resulting in pleasurable arousal and enhanced mood.<sup>1, 2</sup> Persistent exposure to nicotine results in the development of tolerance to its effects and a need for increased intake to achieve the same level of reward.<sup>1-3</sup> Subsequent withdrawal symptoms (e.g., irritability, hunger, anxiety) in the absence of nicotine and their severity are tied to the extent of dependence.<sup>3-5</sup>

Earlier efforts to elucidate the variability between women and men in smoking behavior and these effects focused on smoking frequency and inhalation differences.<sup>6, 7</sup> These data suggest that men smoke with greater frequency and inhale more deeply. However, among regular to heavy smokers, there were no gender differences in cigarette volume and inhalation patterns.<sup>7</sup> Among smokers, nicotine blood levels after smoking one cigarette did not differ between women and men.<sup>8</sup>

Gender-specific characteristics in smoking determinants and cessation have also been characterized. Women reported greater withdrawal symptoms, including higher levels of negative affect (e.g., anxiety, nausea, depression, neuroticism) than men.<sup>9, 10</sup> Smoking behavior in women was more strongly reinforced by external social and situational cues, and concern about the “consequences” of smoking cessation (e.g., weight gain, social isolation).<sup>9, 11</sup> In contrast, smoking behavior in men was reinforced more by nicotine dosage<sup>12</sup>, and pharmacological treatments were more effective for men than women.<sup>10, 12, 13</sup> Nicotine replacement therapies (e.g., nicotine patch, gum, spray) have been shown to be efficacious regardless of gender, increasing abstinence rates almost two-fold.<sup>14</sup> However, given the greater sensitivity to the effects of nicotine in men, along with lower relapse rates following nicotine replacement therapies and bupropion,<sup>11, 15</sup> we would anticipate a stronger pharmacogenetic effect in men.

These gender differences in response to nicotine may have a physiological basis. In a study of the effects of sex hormones on nicotine metabolism, Benowitz et al showed that females, especially those taking oral contraceptives, metabolized nicotine more quickly than men.<sup>16</sup> Estrogen upregulates CYP2A6 activity<sup>17</sup> and glucuronidation activity,<sup>18</sup> two drug metabolizing activities essential for nicotine metabolism.<sup>19</sup>

Animal models focusing on gender differences in response to nicotine showed that female rodents reached a threshold tolerance with less nicotine due to greater nicotine sensitivity, and subsequently, did not discriminate as well as male rodents between varying levels of nicotine.<sup>20-22</sup> This differential response to nicotine arises from the regulation of dopamine through ovarian, not testicular, hormones,<sup>13</sup> with a greater increase in dopamine concentration in female rodents in response to nicotine.<sup>21</sup> Moreover, female mice were less sensitive to the pain and anxiety reducing effects of nicotine, attributable to female sex

hormones (progesterone, estradiol) and not male sex hormones (testosterone) acting as nicotinic receptor antagonists.<sup>23</sup>

These differences motivated a gender-stratified analysis to investigate genes with potentially distinct roles in addiction specific to males and females. We utilized the same phenotype and genotype data from two comparable pharmacogenetic trials of smoking cessation treatment.<sup>24, 25</sup> As previously reported, a number of genetic variants were identified as predictors of cessation and/or therapeutic response in these studies, including the -141C *Ins/Del* and C957T in the dopamine D2 receptor gene (*DRD2*),<sup>25</sup> *CYP2A6* and *CYP2B6*,<sup>26-31</sup> a VNTR and SNPs in *SLC6A3*,<sup>24, 32, 33</sup> and a SNP (rs2072661) within the 3' UTR of the nicotinic acetylcholine receptor (nAChR)  $\beta$ 2 subunit gene (*CHRNA2*).<sup>24</sup> We present gender-stratified marginal effects and gender-stratified gene-treatment interactions across both clinical trials for 1,198 SNPs in 53 gene regions, in order to identify variants of interest within each gender. SNPs with interesting effects within each gender were also assessed for differences between genders.

## MATERIALS AND METHODS

### Study sample and design

We analyzed subjects enrolled in two randomized clinical trials conducted by the University of Pennsylvania Transdisciplinary Tobacco Use Research Center.<sup>24, 25</sup> The first study (Bupropion) was a double-blind randomized clinical trial comparing the efficacy of bupropion to placebo. This study population and SNPs within the candidate genes presented here were previously investigated for marginal SNP and SNP-treatment effects.<sup>27</sup> The second study (NRT) was an open-label randomized clinical trial comparing transdermal (patch) nicotine replacement therapy (NRT) to nicotine nasal spray NRT. The studies had similar designs, making subjects comparable for analysis. We limited our analyses to individuals who self-identified as white non-Hispanic race/ethnicity (n=411 and 378, respectively) to avoid the potential confounding and heterogeneity of effect estimates arising from differential linkage disequilibrium across ethnic groups.

In both studies, potential participants were smokers  $\geq$  18 years old who reported smoking 10 cigarettes per day over the prior 12 months. Through a medical and psychiatric screening, slightly different exclusion criteria were applied in each study and are detailed elsewhere.<sup>25</sup> Treatment included 7 group behavioral counseling sessions plus study medication. Participants were instructed to quit smoking on their target quit date (TQD) which occurred 1–2 weeks after pre-quit counseling. Participants in the Bupropion study initiated treatment during the first week of the study period. Those in the NRT study began treatment at TQD. In both studies we focused on smoking abstinence at two endpoints: (1) End of treatment (EOT) assessed 8 weeks post-TQD and (2) 6-months post-TQD. For both endpoints, those self-reporting smoking abstinence for the 7 days prior to assessment were biochemically verified using saliva cotinine concentrations measured by gas-liquid chromatography.<sup>34</sup> Subjects who self-reported abstinence over that prior week and had cotinine levels  $\leq$  15 ng/ml were classified as abstinent.<sup>24, 25</sup>

A candidate gene study was carried out as part of the Pharmacogenetics of Nicotine Addiction Treatment Consortium. We investigated previously genotyped SNPs within 53 genes involved in nicotine metabolism and the brain-reward pathway including nicotinic acetylcholine receptors, and dopamine candidate genes (see Supplemental Tables for complete list of genes and SNPs). Selection of the 1,528 SNPs has been described previously.<sup>24, 25, 35, 36</sup>

Among the 1,528 total SNPs, we excluded 41 SNPs with a call rate of zero and 57 SNPs with MAFs < 0.01, leaving 1,198 SNPs (118 with putative functional evidence, 1080 to capture underlying LD structure) within the 53 gene regions for association analysis and 232 ancestry informative markers (AIMs). Analysis of these AIMs using STRUCTURE<sup>37</sup> showed negligible admixture in our self-identified white non-Hispanic study population, confirming their genetic homogeneity. Self-reported gender was verified through 41 SNPs located within X-chromosome genes. We estimated heterozygosity and excluded four individuals who self-reported male but were heterozygous for more than 30% of the X-chromosome SNPs. A complete description of the quality control procedures has been previously published.<sup>24</sup>

Imputation was carried out on selected gene regions using the haplotypes from the European populations (EUR) from the August release of the 1000 Genomes Project<sup>38</sup> and the program IMPUTE2.<sup>39, 40</sup> Ten regions with multiple associated SNPs or *a priori* evidence of association (*EPB41*, *CHRNA2*, *CHRNA3;CHRNA6* region, the *ANKK1;DRD2* region, *CHRNA7*, the chromosome 15 nAChR *CHRNA5;CHRNA3;CHRNA4* region, *CHRNA4*, *MAPK1*) were targeted and expanded up- and downstream 50 kb to encompass potential SNPs in LD with those lying in respective regions. Using the 245 SNPs across those regions, we imputed an additional 7957 SNPs. We excluded 1716 imputed SNPs with imputation certainty scores < 0.9. Based on best genotype calls, we excluded 4232 imputed SNPs with an observed MAF < 0.01, and an additional 7 with a *P*-value < 0.0001 from an exact test of Hardy-Weinberg proportions, leaving 2002 imputed SNPs for additional analysis. LocusZoom was used to plot *P*-values from the analysis of these expanded regions.<sup>41</sup>

## Statistical analysis

**SNP association**—To estimate gender-stratified SNP associations at EOT and 6-month follow up, we pooled both studies, and used logistic regression to estimate odds ratios for marginal SNP effects and SNP × treatment interaction effects. For each SNP we tested either an additive or dominant genetic model obtained from previously reported analyses.<sup>24</sup> The most common genotype served as the referent. We adjusted for study and treatment when estimating marginal effects, and used dummy variables indicating treatment assignment to assess within treatment effects of bupropion, patch, or spray vs. placebo. For all models we adjusted for age and the Fagerström Test for Nicotine Dependence.<sup>42</sup> For gender-stratified marginal SNP effects we performed a 1-df LRT within each gender to identify significant SNPs. For gender-specific gene-treatment interaction effects, we performed a 3-df LRT of gene-treatment interaction terms for each gender analysis. We did not test the treatment-

specific effects within each gender. Analyses were performed using the R Statistical Program.<sup>43</sup>

**Correcting for correlated tests within a gene and determining system-level significance**—Marginal 1-df LRT *P*-values were adjusted to account for the correlation and the number of tests performed across the SNPs within a gene region.<sup>24, 44</sup> The 3-df LRT *P*-values for the gene-treatment interaction were adjusted using an extension of the approach from Conneely and Boehnke<sup>44</sup> in which observed test statistics were compared to their asymptotic distribution through numerical integration. We used the correlation of individual contributions to score statistics for the 3-df tests to approximate the correlation structure. This approach performs similarly to computationally intensive permutation tests.<sup>45</sup> *P*-values are reported for each SNP adjusted for the number of correlated SNPs within each gene region. These adjusted *P*-values achieved significance within a gene region (i.e., region-wide significance) at an  $\alpha$ -level of 0.05. Overall significance was determined using an additional Bonferroni correction across the 53 gene regions and two genders. This gives a system-wide  $\alpha$ -level of  $0.05/(53 \times 2) = 5 \times 10^{-4}$  to determine the significance of adjusted *P*-values in four independent tests: marginal effects at EOT, marginal effects at 6-month follow-up, interaction effects at EOT, and interaction effects at 6-month follow-up. As a conservative benchmark, study-wise significance across the 53 independent gene regions, two outcomes of interest, two genders, and two sets of hypotheses tested (gender-stratified marginal tests and gender-stratified SNP-treatment interactions) yields a  $\alpha$ -level of  $0.05/(53 \times 2 \times 2 \times 2) = 1.1 \times 10^{-4}$ .

## RESULTS

Our analyses were restricted to subjects that self-identified and were confirmed European ancestry (Table 1). Fagerström Test for Nicotine Dependence (FTND) scores for women (5.1, SD=2.2) were lower compared with men (5.6, SD=2.1). Across all treatments, at EOT, 26% of women were abstinent compared with 34% of men (OR=0.7, 95% CI 0.5–0.9). At 6-month follow-up, 19% of women were abstinent compared with 23% of men (OR=0.82, 95% CI 0.6–1.2). However, within the spray treatment arm, a slightly higher proportion of women remained abstinent (OR=1.1, 95% CI 0.5–2.3).

### Associations with abstinence at EOT and 6-month follow-up

Gender stratified SNP associations are reported in Table 2. Gender stratified SNP  $\times$  treatment associations at EOT and 6-month follow-up are reported in Tables 3 and 4, respectively. Results are presented for males and females for all SNPs with adjusted *P*-values  $< 0.05$  for either gender-stratified analysis.

**Marginal associations**—Within males at EOT, marginal associations achieving system-wide significance were observed for four SNPs (rs6702335, rs12021667, rs12027267, rs12039988;  $r^2 > 0.98$ ) in the erythrocyte membrane protein band 4.1 gene (*EPB41*) ( $P < 6 \times 10^{-5}$ ). Two of these SNPs (rs6702335, rs12027267) had adjusted *P*-values that approached system-wide significance with abstinence at 6-month follow-up ( $P = 8.0 \times 10^{-4}$ ). Within males, these four SNPs were associated with a decrease in odds of abstinence at EOT

(e.g.,  $OR_{rs6702335} = 0.46$ , 95% CI 0.32–0.64). Similar effects were observed at 6-month follow-up for those three SNPs at or approaching system-wide significance ( $OR_{rs6702335} = 0.46$ , 95% CI 0.31–0.67). Within females at EOT and 6-month follow-up, these SNPs had no effect, and all four had region-wide significant SNP  $\times$  gender interactions (adjusted  $P$  for test of heterogeneity  $< 0.05$ ).

Abstinence rates within males at EOT and 6-month follow-up for each rs6702335 genotype are shown in Figure 1a. At EOT, 41% of males with two major alleles (WT-AA) were abstinent, but only 20% of heterozygotes and 15% of those with two minor alleles (Variant-GG) remained abstinent ( $OR = 0.4$ , 95% CI 0.2–0.6;  $OR = 0.25$ , 95% CI 0.1–0.5; respectively). At 6-month follow-up, 33% of males with two major alleles were abstinent, but only 19% of heterozygotes and 10% of those with two minor alleles remained abstinent ( $OR = 0.4$ , 95% CI 0.3–0.8;  $OR = 0.2$ , 95% CI 0.1–0.5; respectively).

Imputation using data from the 1000 Genomes Project lends support to the association between smoking cessation at EOT and *EPB41* within males (Supplemental Figure 1). For *EPB41*, along with the four genotyped SNPs showing association (rs6702335, rs12021667, rs12027267, rs12039988;  $r^2 > 0.98$ ), nine other imputed non-coding SNPs (rs4654328, rs2930833, rs35013556, rs7546832, rs34474391, rs10429865, rs12734106, rs35423185, rs35579088) in LD with our strongest signal (rs6702335,  $r^2 > 0.80$ ) were strongly associated with smoking abstinence. Two other SNPs lying between *OPRD1* and *EPB41* (rs61783628, rs1485471) were also strongly associated with smoking abstinence and in moderate LD with rs6702335 ( $0.6 < r^2 < 0.80$ ). The association between rs6702335 and cessation at EOT remained the strongest. At 6-month follow-up, associations for imputed SNPs within *EPB41* were similar to those at EOT.

Four SNPs (rs7123797, rs4938012, rs17115439, rs4938015;  $r^2 > 0.95$ ) in ankyrin repeat and kinase domain containing 1 (*ANKKI1*), a gene immediately proximal of *DRD2* on chromosome 11q,<sup>46</sup> achieved region-wide significance in association with abstinence at EOT within females ( $OR = 0.5$ , 95% CI 0.3–0.7, adjusted  $P < 0.05$ ). There was no significant SNP  $\times$  gender interaction among these four SNPs (adjusted  $P$  for test of heterogeneity  $> 0.05$ ).

Several SNPs located in or adjacent to nAChR subunit genes had marginal effects achieving region-wide significance. Within males, rs578776<sup>47</sup> in the *CHRNA5-CHRNA3-CHRNA4* gene cluster was associated with abstinence at EOT. Within females, rs4809549 in the nAChR  $\alpha 4$  subunit gene (*CHRNA4*) was associated with abstinence at EOT, and SNPs in *ADAR*, immediately distal to *CHRNA2* (rs3766927, rs1127314, rs2131902;  $r^2 = 0.94$ ) were associated with abstinence at 6-month follow-up. There was no significant SNP  $\times$  gender interaction among these SNPs (adjusted  $P$  for test of heterogeneity  $> 0.05$ ).

**Gene  $\times$  treatment interactions**—Within males at 6-month follow-up, a gene  $\times$  treatment interaction achieving system-wide significance was observed for rs806365 in the cannabinoid receptor 1 gene (*CNRI*) (adjusted  $P = 3.9 \times 10^{-5}$ ; Table 4). The odds ratio of rs806365 within the placebo arm was 1.06 (0.30–3.75) with the odds of abstinence increased more than two-fold in the bupropion and patch arms ( $OR = 2.05$ , 95% CI 0.62–6.78;



OR=2.41, 95% 0.74–7.84; respectively). Most notably, however, was the 25-fold decrease in odds of abstinence within the spray arm (OR=0.04, 95% CI 0.01–0.21). Estimates of effect were consistent at EOT for rs806365 within males, with an adjusted interaction *P*-value near system-wide significance (*P*=0.001).

Gene × treatment interaction analysis also identified rs806369 within *CNR1*. Within males, rs806369 had adjusted interaction *P*-values that reached region-wide significance at EOT and 6-month follow-up (adjusted *P*s=0.007). These interactions were driven mostly by decreases in odds within the spray arm (OR=0.30, 95% CI 0.11–0.79; OR=0.14, 95% CI 0.03–0.63, respectively). For both cessation endpoints, rs806365 and rs806369 ( $r^2=0.5$ ) had region-wide significant SNP × treatment × gender interactions (adjusted *P* for test of heterogeneity < 0.05). Their effects within females were in the opposite direction as males with increased odds of abstinence within the spray arm for both cessation endpoints. Imputation of SNPs 50kb up- and downstream of *CNR1* with 1000 Genomes Project data did not identify other SNPs with stronger SNP × treatment interactions, or SNPs in LD with our strongest signal in this region (rs806365).

Treatment stratified abstinence rates within males for rs806365 carriers and non-carriers are in Figure 1b. For both study endpoints within the bupropion and patch arms, males with at least one minor allele (Carrier-TT, TC) had a roughly two-fold increase in odds of abstinence. Within the placebo arm, those with at least one minor allele had slightly increased odds of abstinence at EOT, but this disappeared at 6-month follow-up. However, overall abstinence rates decreased in these three treatment arms from EOT to 6-month follow-up. Within the spray arm, the proportion of males abstinent remained relatively consistent for males with two major alleles (WT-CC) (54% vs. 46%); but the proportion of abstinent males with at least one minor allele decreased (17% to 3%).

Three SNPs in *MAPK1* (rs9610375, rs1063311, rs2266966) achieved region-wide significant treatment interactions within females at EOT. Within males at 6-month follow-up, another five SNPs in *MAPK1* (rs743409, rs1892848, rs3788332, rs5999550, rs11704205) achieved region-wide significance for SNP × treatment and SNP × treatment × gender interactions (adjusted *P* for test of heterogeneity < 0.05).

A number of nAChR SNPs achieved region-wide significance for gender-stratified gene × treatment interaction effects, including two that have been published previously (rs3743075,<sup>47</sup> rs2072661<sup>24</sup>). Also, within males at 6-month follow-up, rs684513<sup>48</sup> (chromosome 15 nAChR region) had an adjusted gene × treatment interaction *P*-value that approached system-wide significance (*P*=0.0007). Within females, rs950776 had significant gene × treatment interactions at EOT and 6-month follow-up (adjusted *P* 0.02). Other previously reported SNPs within the nAChR chromosome 15 region (rs1051730, rs1317286, rs578776)<sup>4, 47, 49</sup> had null marginal and interaction effects. While two other previously reported SNPs (rs12914385, rs16969968)<sup>47, 49</sup> were not genotyped in this study, rs16969968 is in high LD ( $r^2>0.95$ ) with rs1051730 and rs1317286.

## DISCUSSION

Reported gender differences in smoking behavior and cessation motivated this gender-stratified analysis in two pharmacogenetic smoking cessation trials. Previous research identified SNPs and genes within the two separate clinical trials comprising this study.<sup>24–28, 30–32, 50</sup> This analysis builds upon those study results by pooling both studies to look within genders across all four treatments across 53 candidate gene regions. Instead of focusing on estimated heterogeneity between genders, our aim was to identify SNPs with effects specific to males and/or females.

Gender-stratified SNP marginal effects revealed a male-specific association between a region of high LD within *EPB41* and smoking abstinence at EOT and 6-month follow-up. Within males, we identified four non-coding SNPs in *EPB41* (rs6702335, rs12021667, rs12027267, rs12039988) in strong LD ( $r^2 > 0.98$ ). rs12021667 lies in the 5'-UTR, rs12039988 in the 3'-UTR, and rs12027267 and rs6702335 both in intronic regions. These SNPs achieved system-level significance in association with smoking abstinence at EOT (adjusted  $P < 6 \times 10^{-5}$ ). At 6-month follow-up, rs6702335 and rs12027267 approached system-level significance ( $P = 8.0 \times 10^{-4}$ ) while rs12021667 and rs12039988 achieved region-level significance (adjusted  $P$ s = 0.002 and 0.005, respectively). In males, these SNPs were associated with a more than two-fold decrease in odds of abstinence at both cessation endpoints.

Erythrocyte membrane protein band 4.1 (*EPB41*), known as protein 4.1R, is critical for red blood cell morphology and membrane function.<sup>51–54</sup> This protein and its homologues have not been associated with nicotine dependence and smoking, but they were shown to stabilize the localization of dopamine receptors to the plasma membrane,<sup>55</sup> and protein 4.1R had a specific organizational role in the arrangement of postsynaptic molecules.<sup>54</sup> Our data suggests that genetic variation in *EPB41* has gender-specific effects on smoking abstinence, potentially mediated through differential effects on the localization or function of dopamine receptors and further downstream effects on the brain reward pathway.

*EPB41* is adjacent to delta opioid receptor 1 (*OPRD1*), which is part of the family of opioid receptor genes associated with substance abuse.<sup>56</sup> Using HapMap SNP genotypes from the CEPH population (Release #28, NCBI Build 36)<sup>36</sup> and Haploview,<sup>57</sup> none of the SNPs in *EPB41* that achieved system-wide significance are in LD with any SNPs in *OPRD1* ( $r^2 < 0.35$ ). However, a SNP in *EPB41* achieving region-wide significance (rs16837840) is in moderate LD ( $r^2 = 0.6$ ) with a SNP in *OPRD1* (rs12404612). After imputation of *EPB41*, rs12404612 (certainty score  $> 0.9$ ) had male-specific marginal associations with abstinence at EOT and 6-month follow-up ( $0.01 < P = 0.02$  and  $0.03$ , respectively). Interestingly, other imputed SNPs in *OPRD1* with imputation certainty scores  $< 0.9$  showed strong associations with smoking abstinence.

Gender-stratified SNP  $\times$  treatment interaction analyses revealed a male-specific association between smoking abstinence and two SNPs in *CNRI* in weak LD (rs806365, rs806369,  $r^2 = 0.5$ ). rs806365 lies in the 3' flanking region of *CNRI*, while due to multiple *CNRI* transcript variants,<sup>58</sup> rs806369 lies either in an intron or the 5' flanking region. For



abstinence at 6-month follow-up, the male-specific interaction between rs806365 and treatment achieved system-level significance (adjusted interaction LRT  $P=3.9\times 10^{-5}$ ). The effect of this SNP was most prominent in males within the spray arm, where it was associated with a 25-fold decrease in odds of abstinence. Effects at EOT were similar, where rs806365 was associated with a nearly 6-fold decrease in abstinence odds for males in the spray arm (adjusted interaction LRT  $P=0.001$ ). Males with two major alleles in the spray arm had the highest abstinence rates, and this remained relatively unchanged from EOT to 6-month follow-up; males with at least one minor allele had the lowest abstinence rates across all males.

The relationship between cannabinoid receptor 1 (*CNRI*) and addictive behaviors has been well-characterized.<sup>58–61</sup> Along with its primary role in mediating the effects of marijuana,<sup>58</sup> it modulates dopamine release in response to other substances.<sup>62</sup> The *CNRI* antagonist, rimonabant, was effective in suppressing smoking relapse and attenuating reward seeking behaviors during abstinence.<sup>59, 60</sup> There was also a female-specific association between SNPs and haplotypes in *CNRI* and both nicotine dependence and smoking initiation.<sup>63</sup> Although there is no overlap of SNPs investigated in that study with our current investigation, both point to gender-specific associations of smoking behaviors with *CNRI*. Our analysis suggests that gene associations are not only gender-specific but treatment- and gender-specific. Of the treatments administered, the nasal spray most closely mimics the effects of smoking, introducing a sharp, rapid increase in nicotine levels.<sup>64</sup> Since males are more responsive to nicotine dosage and pharmacological reinforcement by nicotine, they may be more sensitive to the effects of the spray. Within the spray arm, males with two major alleles for rs806365 and rs806369 had higher abstinence rates than those with at least one minor allele. Opposite and variable effects of these SNPs in females may be attributed to gender differences in the pharmacological effects of nicotine.<sup>12, 65</sup>

It is worth highlighting that for both marginal and interaction effects, no SNPs achieving region- or system-wide significance within one gender stratum achieved significance within the other gender stratum. For marginal effects, there was no overlap in gene regions between genders, with male-specific significant SNPs in *EPB41*, and female-specific SNPs in *ANKK1* and  $\alpha 4$  and  $\beta 2$  nAChR gene subunits. Across both abstinence outcomes, gene  $\times$  treatment interactions identified associations across women and men in the nAChR subunit regions and *MAPK1*. This consistency in gene regions adds evidence of their putative roles in nicotine dependence and smoking behavior.<sup>47, 49, 66–71</sup> These regions, along with the others without overlap, have shown prior evidence of association with nicotine dependence and smoking cessation, and require further study to validate gender-specific effects.

The use of 1000 Genomes Project data for imputation helped to broaden the areas for potential follow-up for the specific putatively causal variant within our top gene regions. Additionally, we imputed within the previously reported region of chromosome 15, a region linked to smoking dependence<sup>72</sup> and other smoking related diseases.<sup>49, 73–75</sup> For this region, imputation identified two gene regions proximal of the chromosome 15  $\alpha 5$ - $\alpha 3$ - $\beta 4$  nAChR complex associated with smoking abstinence (Supplemental Figure 2). *AGPHD1* and *PSMA4* have imputed SNPs in LD ( $r^2>0.6$ ) with our strongest signal in the nAChR gene region (rs684513) that had comparable or more significant SNP  $\times$  treatment interactions on

smoking abstinence at 6-month follow-up within males ( $1 \times 10^{-5} < P < 5 \times 10^{-5}$ ). An intronic SNP in *AGPHDI*, rs12441354, had the strongest association ( $P=1.3 \times 10^{-5}$ ).

Strengths and limitations of this study have been described previously,<sup>24, 25</sup> but we highlight a few here. We did not have to address potential biases in determining smoking abstinence since information was collected from individuals in a prospective manner, and those claiming to be abstinent were subjected to biochemical verification. Similar study designs between studies suggest that subjects are comparable. However, differential responses in abstinence rates across genders and treatments could have arisen from a key difference in exclusion criteria.<sup>25</sup> Only participants in the NRT study were excluded for drug or alcohol dependence or any subsequent treatment. Subjects in the Bupropion study may have used other substances to compensate for any adverse effects from smoking cessation.

In our analysis, we performed an adjustment of *P*-values that accounted for multiple correlated tests within respective gene regions. A Bonferroni-corrected *P*-value was then applied across the 53 gene regions and two genders. This correction was less stringent than a uniform Bonferroni-correction across all SNPs, while still accounting for independent gene regions and both genders. Thus, a SNP was significant within a respective region and gender at a *P*-value  $< 0.05$ , while system-wide significance was set at an  $\alpha$ -level of  $0.05 / (53 \times 2) = 5 \times 10^{-4}$ .

While our determination of significance was conservative, the results require independent replication. Identified SNPs with the strongest evidence of association have not been replicated in other studies, and the region with strongest marginal SNP effects, *EPB41*, has not been associated with nicotine dependence or smoking previously. Confirmation from other studies, especially gender-specific results, would lend support to our findings. However, our study has identified a novel gene region, *EPB41*, which may be associated with smoking cessation, along with gene regions in *CNRI* that may be targeted to further elucidate the etiology of gender differences in smoking behaviors.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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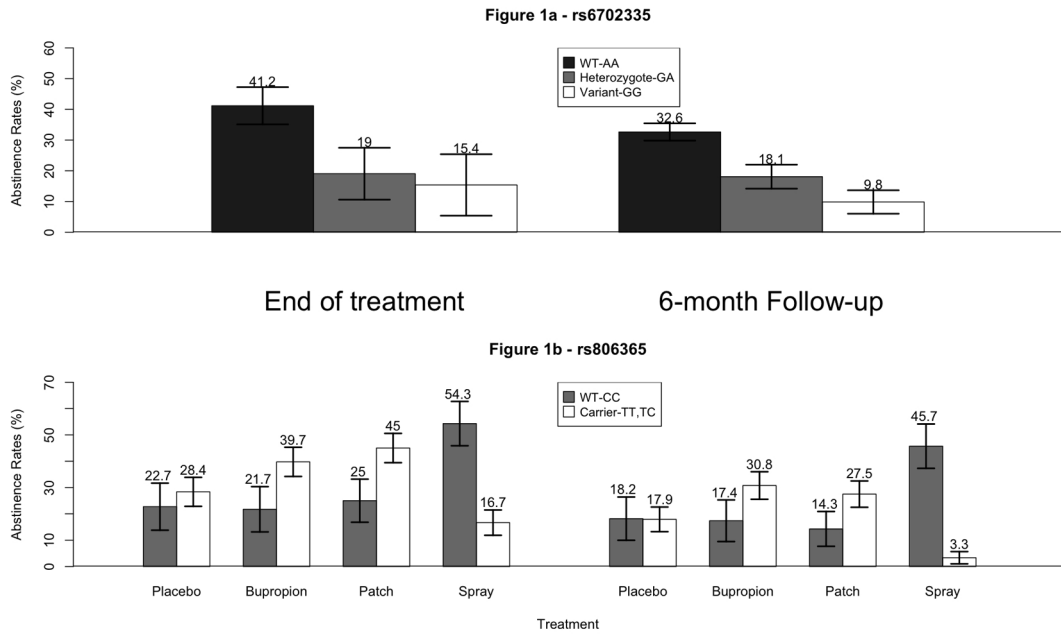
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**Figure 1.**

**Table 1**

## Study Characteristics

	<b>Male N (%)</b>	<b>Female N (%)</b>
	<b>386</b>	<b>403</b>
Age	46.1 ± 10.8	44.8 ± 12.1
Treatment		
Placebo	88 (23%)	106 (26%)
Bupropion	97 (25%)	120 (30%)
Patch	107 (28%)	81 (20%)
Spray	94 (24%)	96 (24%)
FTND <sup>a</sup>		
0	2 (1%)	6 (1%)
1	13 (3%)	22 (5%)
2	18 (5%)	28 (7%)
3	32 (8%)	37 (9%)
4	54 (14%)	55 (14%)
5	63 (16%)	70 (17%)
6	62 (16%)	69 (17%)
7	68 (18%)	58 (14%)
8	41 (11%)	40 (10%)
9	26 (7%)	14 (3%)
10	7 (2%)	4 (1%)
Mean	5.6 ± 2.1	5.1 ± 2.2
End of treatment - Abstinent		
All treatments	130 (34%)	105 (26%)
Placebo	24 (27%)	18 (17%)
Bupropion	35 (36%)	35 (29%)
Patch	42 (39%)	25 (31%)
Spray	29 (31%)	27 (28%)
6-month Follow-up - Abstinent		
All treatments	87 (23%)	78 (19%)
Placebo	16 (18%)	18 (17%)
Bupropion	27 (28%)	29 (24%)
Patch	26 (24%)	11 (14%)
Spray	18 (19%)	20 (21%)

All subjects were confirmed European ancestry.

<sup>a</sup>Fagerström test for nicotine dependence

**Table 2**  
Gender stratified marginal single nucleotide polymorphism (SNP) results for abstinence rates

SNP	Gene Region	Chromosome	MAF	Male			Female			
				SNP OR (95% CI)	Observed <i>P</i>	Adjusted <i>P</i> <sup>a</sup>	MAF	SNP OR (95% CI)	Observed <i>P</i>	Adjusted <i>P</i> <sup>a</sup>
<i>Significant SNPs within Males</i>										
<i>End of treatment</i>										
rs6702335 <sup>b</sup>	EPB4I	1	0.39	0.46 (0.32–0.64)	2.30 × 10 <sup>-6</sup>	3.27 × 10 <sup>-5</sup>	0.39	0.93 (0.67–1.30)	6.67 × 10 <sup>-1</sup>	1
rs12021667 <sup>b</sup>	EPB4I	1	0.4	0.45 (0.32–0.64)	2.01 × 10 <sup>-6</sup>	3.65 × 10 <sup>-5</sup>	0.39	0.96 (0.69–1.34)	8.15 × 10 <sup>-1</sup>	1
rs12027267 <sup>b</sup>	EPB4I	1	0.4	0.46 (0.33–0.64)	2.38 × 10 <sup>-6</sup>	4.04 × 10 <sup>-5</sup>	0.39	0.95 (0.68–1.33)	7.62 × 10 <sup>-1</sup>	1
rs12039988 <sup>b</sup>	EPB4I	1	0.39	0.47 (0.33–0.65)	3.40 × 10 <sup>-6</sup>	5.77 × 10 <sup>-5</sup>	0.39	1.00 (0.72–1.40)	9.97 × 10 <sup>-1</sup>	9.97 × 10 <sup>-1</sup>
rs203278 <sup>b</sup>	EPB4I	1	0.35	2.27 (1.44–3.60)	3.30 × 10 <sup>-4</sup>	5.57 × 10 <sup>-3</sup>	0.34	0.79 (0.50–1.25)	3.20 × 10 <sup>-1</sup>	9.74 × 10 <sup>-1</sup>
rs150089	EPB4I	1	0.32	1.71 (1.23–2.39)	1.48 × 10 <sup>-3</sup>	2.20 × 10 <sup>-2</sup>	0.31	0.86 (0.61–1.23)	4.09 × 10 <sup>-1</sup>	9.91 × 10 <sup>-1</sup>
rs2985322	EPB4I	1	0.32	1.71 (1.23–2.39)	1.48 × 10 <sup>-3</sup>	2.22 × 10 <sup>-2</sup>	0.31	0.87 (0.61–1.24)	4.38 × 10 <sup>-1</sup>	9.94 × 10 <sup>-1</sup>
rs578776	CHRNA3;CHRNA5	15	0.24	1.75 (1.21–2.54)	2.86 × 10 <sup>-3</sup>	2.85 × 10 <sup>-2</sup>	0.21	0.94 (0.63–1.39)	7.47 × 10 <sup>-1</sup>	9.99 × 10 <sup>-1</sup>
rs4654390	EPB4I	1	0.5	0.62 (0.45–0.85)	2.48 × 10 <sup>-3</sup>	3.50 × 10 <sup>-2</sup>	0.49	0.98 (0.72–1.34)	8.97 × 10 <sup>-1</sup>	1
rs10915216	EPB4I	1	0.5	0.62 (0.45–0.85)	2.68 × 10 <sup>-3</sup>	3.72 × 10 <sup>-2</sup>	0.49	0.97 (0.71–1.33)	8.70 × 10 <sup>-1</sup>	1
<i>6-month Follow-up</i>										
rs6702335 <sup>b</sup>	EPB4I	1	0.39	0.46 (0.31–0.67)	3.44 × 10 <sup>-5</sup>	6.97 × 10 <sup>-4</sup>	0.39	1.11 (0.77–1.59)	5.87 × 10 <sup>-1</sup>	1
rs12027267 <sup>b</sup>	EPB4I	1	0.4	0.46 (0.31–0.68)	3.89 × 10 <sup>-5</sup>	7.95 × 10 <sup>-4</sup>	0.39	1.17 (0.81–1.69)	3.97 × 10 <sup>-1</sup>	9.91 × 10 <sup>-1</sup>
rs12021667 <sup>b</sup>	EPB4I	1	0.4	0.48 (0.32–0.70)	8.25 × 10 <sup>-5</sup>	1.61 × 10 <sup>-3</sup>	0.39	1.16 (0.80–1.66)	4.37 × 10 <sup>-1</sup>	9.96 × 10 <sup>-1</sup>
rs12039988 <sup>b</sup>	EPB4I	1	0.39	0.40 (0.24–0.66)	2.89 × 10 <sup>-4</sup>	5.26 × 10 <sup>-3</sup>	0.39	1.53 (0.89–2.63)	1.20 × 10 <sup>-1</sup>	7.83 × 10 <sup>-1</sup>
rs2238687	FOSB	19	0.14	2.17 (1.33–3.55)	2.42 × 10 <sup>-3</sup>	1.48 × 10 <sup>-2</sup>	0.14	1.57 (0.99–2.49)	6.30 × 10 <sup>-2</sup>	2.62 × 10 <sup>-1</sup>
rs11101694	CALY	10	0.13	0.42 (0.21–0.85)	1.01 × 10 <sup>-2</sup>	3.74 × 10 <sup>-2</sup>	0.12	0.84 (0.44–1.59)	5.89 × 10 <sup>-1</sup>	5.89 × 10 <sup>-1</sup>
rs16837840	EPB4I	1	0.11	2.40 (1.38–4.17)	2.46 × 10 <sup>-3</sup>	3.78 × 10 <sup>-2</sup>	0.1	0.70 (0.35–1.41)	3.01 × 10 <sup>-1</sup>	9.71 × 10 <sup>-1</sup>

*Significant SNPs within Females*

<i>End of treatment</i>										
rs4809549	CHRNA4	20	0.47	1.00 (0.74–1.35)	9.96 × 10 <sup>-1</sup>	9.96 × 10 <sup>-1</sup>	0.48	0.59 (0.42–0.83)	1.99 × 10 <sup>-3</sup>	1.69 × 10 <sup>-2</sup>
rs7123797	ANKK1	11	0.34	0.95 (0.62–1.46)	8.23 × 10 <sup>-1</sup>	1	0.34	0.46 (0.29–0.73)	8.58 × 10 <sup>-4</sup>	1.86 × 10 <sup>-2</sup>
rs4938012	ANKK1	11	0.33	0.97 (0.63–1.48)	8.74 × 10 <sup>-1</sup>	1	0.33	0.47 (0.29–0.74)	1.09 × 10 <sup>-3</sup>	2.33 × 10 <sup>-2</sup>

SNP	Gene Region	Chromosome	MAF	Male			Female				
				SNP OR (95% CI)	Observed <i>P</i>	Adjusted <i>P</i> <sup>a</sup>	MAF	SNP OR (95% CI)	Observed <i>P</i>	Adjusted <i>P</i> <sup>a</sup>	
rs17115439	ANKK1	11	0.33	0.94 (0.61–1.44)	7.67 × 10 <sup>-1</sup>	1	0.33	0.47 (0.30–0.75)	1.45 × 10 <sup>-3</sup>	3.02 × 10 <sup>-2</sup>	
rs4938015	ANKK1	11	0.33	0.94 (0.61–1.44)	7.67 × 10 <sup>-1</sup>	1	0.33	0.47 (0.30–0.75)	1.49 × 10 <sup>-3</sup>	3.10 × 10 <sup>-2</sup>	
<b>6-month Follow-up</b>											
rs3766927	CHRNA2	1	0.31	0.86 (0.53–1.39)	5.30 × 10 <sup>-1</sup>	7.77 × 10 <sup>-1</sup>	0.29	0.45 (0.26–0.76)	2.24 × 10 <sup>-3</sup>	1.92 × 10 <sup>-2</sup>	
rs1127314	CJRN2	1	0.31	0.88 (0.62–1.27)	4.99 × 10 <sup>-1</sup>	7.99 × 10 <sup>-1</sup>	0.29	0.53 (0.34–0.82)	2.48 × 10 <sup>-3</sup>	2.07 × 10 <sup>-2</sup>	
rs2834600	CLIC6	21	0.13	0.89 (0.51–1.57)	6.93 × 10 <sup>-1</sup>	9.87 × 10 <sup>-1</sup>	0.14	2.29 (1.34–3.91)	2.86 × 10 <sup>-3</sup>	3.08 × 10 <sup>-2</sup>	
rs17759598	MAPK1	22	0.17	0.87 (0.55–1.39)	5.67 × 10 <sup>-1</sup>	9.82 × 10 <sup>-1</sup>	0.14	0.44 (0.23–0.83)	5.60 × 10 <sup>-3</sup>	3.71 × 10 <sup>-2</sup>	
rs2131902	CHRNA2	1	0.32	0.82 (0.50–1.33)	4.20 × 10 <sup>-1</sup>	8.24 × 10 <sup>-1</sup>	0.3	0.49 (0.29–0.82)	5.66 × 10 <sup>-3</sup>	4.33 × 10 <sup>-2</sup>	

All models were adjusted for age, FTND, and treatment.

SNPs above the dashed line have adjusted *P*-values < 0.05 within males. SNPs below the dashed line have adjusted *P*-values < 0.05 within females.

<sup>a</sup> *P*-values adjusted for the number of correlated tests within respective gene regions.

<sup>b</sup> Adjusted SNP × Gender Interaction *P*-value < 0.05.

**Table 3**

Gender stratified SNP × treatment interaction results at end of treatment

SNP	Gene Region	Gender	MAF	Placebo		Bupropion		Patch		Spray		Test of Interaction <sup>d</sup>	
				SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	Observed P	Adjusted P		
<i>Significant SNPs within Males</i>													
rs806365b	<i>CNR1</i>	Male	0.46	1.38 (0.44–4.30)	2.31 (0.77–6.96)	3.07 (1.11–8.47)	0.17 (0.07–0.45)	7.01 × 10 <sup>-5</sup>	1.05 × 10 <sup>-3</sup>				
		Female	0.44	0.40 (0.14–1.13)	0.64 (0.28–1.49)	0.67 (0.25–1.84)	1.82 (0.63–5.24)	2.15 × 10 <sup>-1</sup>	7.70 × 10 <sup>-1</sup>				
rs806369b	<i>CNR1</i>	Male	0.31	3.53 (1.23–10.09)	1.33 (0.57–3.10)	3.11 (1.33–7.27)	0.30 (0.11–0.79)	5.56 × 10 <sup>-4</sup>	7.08 × 10 <sup>-3</sup>				
		Female	0.3	0.35 (0.11–1.06)	0.87 (0.39–1.93)	0.68 (0.26–1.80)	1.83 (0.73–4.57)	1.29 × 10 <sup>-1</sup>	6.27 × 10 <sup>-1</sup>				
rs10107450	<i>CHRNA6</i>	Male	0.23	2.11 (0.81–5.49)	2.43 (1.02–5.80)	0.34 (0.14–0.80)	0.62 (0.25–1.53)	3.09 × 10 <sup>-3</sup>	9.66 × 10 <sup>-3</sup>				
		Female	0.22	0.69 (0.23–2.03)	0.87 (0.39–1.95)	0.72 (0.27–1.92)	0.62 (0.23–1.69)	9.61 × 10 <sup>-1</sup>	9.61 × 10 <sup>-1</sup>				
rs7017612	<i>CHRNA6:CHRNA3</i>	Male	0.21	0.91 (0.33–2.47)	2.27 (0.96–5.34)	0.24 (0.10–0.59)	0.92 (0.37–2.28)	3.94 × 10 <sup>-3</sup>	1.16 × 10 <sup>-2</sup>				
		Female	0.18	0.68 (0.22–2.13)	0.61 (0.25–1.47)	0.80 (0.29–2.21)	0.43 (0.14–1.29)	8.66 × 10 <sup>-1</sup>	9.70 × 10 <sup>-1</sup>				
rs878887	<i>CRHR1</i>	Male	0.22	2.25 (1.07–4.74)	2.03 (0.98–4.23)	0.40 (0.19–0.85)	0.68 (0.31–1.50)	1.24 × 10 <sup>-3</sup>	1.31 × 10 <sup>-2</sup>				
		Female	0.2	0.30 (0.09–1.07)	1.07 (0.47–2.44)	0.57 (0.23–1.39)	0.84 (0.37–1.94)	3.40 × 10 <sup>-1</sup>	5.82 × 10 <sup>-1</sup>				
rs2072661	<i>CHRNA2</i>	Male	0.26	0.26 (0.09–0.74)	0.33 (0.13–0.80)	1.30 (0.59–2.85)	2.14 (0.87–5.24)	2.00 × 10 <sup>-3</sup>	1.68 × 10 <sup>-2</sup>				
		Female	0.22	0.71 (0.23–2.20)	0.54 (0.22–1.29)	1.35 (0.51–3.57)	0.37 (0.14–1.00)	3.02 × 10 <sup>-1</sup>	7.64 × 10 <sup>-1</sup>				
rs2304297	<i>CHRNA6</i>	Male	0.24	2.11 (0.81–5.49)	2.30 (0.97–5.45)	0.44 (0.20–1.01)	0.59 (0.24–1.48)	1.17 × 10 <sup>-2</sup>	2.86 × 10 <sup>-2</sup>				
		Female	0.22	0.88 (0.31–2.52)	0.96 (0.43–2.14)	0.72 (0.27–1.92)	0.55 (0.20–1.48)	8.42 × 10 <sup>-1</sup>	9.60 × 10 <sup>-1</sup>				
rs11575553	<i>DDC</i>	Male	0.1	2.27 (0.74–6.94)	15.33 (3.18–74.04)	0.67 (0.22–2.11)	0.88 (0.32–2.42)	1.74 × 10 <sup>-3</sup>	2.89 × 10 <sup>-2</sup>				
		Female	0.11	0.32 (0.07–1.50)	1.59 (0.61–4.16)	1.31 (0.42–4.12)	0.71 (0.20–2.46)	2.68 × 10 <sup>-1</sup>	9.28 × 10 <sup>-1</sup>				
rs2267366	<i>PRKCABP</i>	Male	0.13	1.17 (0.43–3.15)	0.56 (0.23–1.36)	0.53 (0.21–1.38)	4.39 (1.61–11.94)	5.38 × 10 <sup>-1</sup>	2.93 × 10 <sup>-2</sup>				
		Female	0.16	1.39 (0.57–3.42)	0.85 (0.41–1.77)	0.36 (0.08–1.57)	1.25 (0.54–2.92)	3.41 × 10 <sup>-1</sup>	7.06 × 10 <sup>-1</sup>				
rs1876828	<i>CRHR1</i>	Male	0.22	2.19 (1.01–4.74)	2.07 (0.98–4.38)	0.45 (0.21–0.96)	0.63 (0.26–1.54)	3.34 × 10 <sup>-3</sup>	3.20 × 10 <sup>-2</sup>				
		Female	0.21	0.30 (0.09–1.08)	0.89 (0.38–2.08)	0.49 (0.19–1.28)	0.90 (0.39–2.09)	3.90 × 10 <sup>-1</sup>	6.18 × 10 <sup>-1</sup>				

SNP	Gene Region	Gender	MAF	Placebo		Bupropion		Patch		Spray		Test of Interaction <sup>a</sup>					
				SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	Observed P	Adjusted P						
rs356165	SNCA	Male	0.38	0.86 (0.30-2.45)	0.95 (0.39-2.30)	2.50 (1.03-6.06)	0.22 (0.08-0.61)	4.54 × 10 <sup>-3</sup>	4.27 × 10 <sup>-2</sup>	Female	0.41	0.93 (0.29-2.95)	2.38 (0.87-6.54)	1.21 (0.42-3.52)	0.88 (0.34-2.28)	4.88 × 10 <sup>-1</sup>	9.75 × 10 <sup>-1</sup>
rs17690326	CRHR1	Male	0.22	2.02 (0.96-4.23)	1.58 (0.78-3.19)	0.41 (0.19-0.85)	0.67 (0.31-1.47)	5.53 × 10 <sup>-3</sup>	4.84 × 10 <sup>-2</sup>	Female	0.2	0.30 (0.08-1.05)	1.09 (0.48-2.49)	0.48,0 (20-1.18)	0.85 (0.37-1.94)	2.61 × 10 <sup>-1</sup>	4.78 × 10 <sup>-1</sup>
<b>Significant SNPs within Females</b>																	
rs950776	CHRNA4	Male	0.33	1.65 (0.64-4.24)	1.37 (0.56-3.31)	0.69 (0.31-1.53)	0.55 (0.23-1.35)	2.62 × 10 <sup>-1</sup>	7.68 × 10 <sup>-1</sup>	Female	0.35	1.71 (0.58-5.02)	0.38 (0.17-0.87)	0.49 (0.18-1.30)	3.61 (1.34-9.74)	1.52 × 10 <sup>-3</sup>	1.09 × 10 <sup>-2</sup>
rs871058	CHRNA5	Male	0.33	1.21 (0.47-3.11)	0.89 (0.37-2.12)	0.69 (0.31-1.53)	0.41 (0.17-1.01)	4.07 × 10 <sup>-1</sup>	9.17 × 10 <sup>-2</sup>	Female	0.37	1.31 (0.45-3.86)	0.24 (0.10-0.55)	0.59 (0.22-1.56)	2.44 (0.95-6.28)	1.78 × 10 <sup>-3</sup>	1.24 × 10 <sup>-1</sup>
rs514743	CHRNA3;CHRNA5	Male	0.35	1.00 (0.39-2.55)	1.16 (0.49-2.77)	0.49 (0.21-1.09)	0.51 (0.21-1.25)	3.80 × 10 <sup>-1</sup>	8.98 × 10 <sup>-1</sup>	Female	0.39	1.07 (0.36-3.15)	0.35 (0.15-0.81)	0.69 (0.26-1.83)	3.96 (1.40-11.23)	2.79 × 10 <sup>-3</sup>	1.87 × 10 <sup>-2</sup>
rs9610375	MAPK1	Male	0.49	0.78 (0.26-2.37)	0.87 (0.33-2.29)	0.76 (0.31-1.84)	0.59 (0.20-1.76)	9.65 × 10 <sup>-1</sup>	9.65 × 10 <sup>-1</sup>	Female	0.48	0.56 (0.19-1.64)	0.38 (0.16-0.88)	6.54 (1.38-30.93)	0.66 (0.25-1.71)	3.48 × 10 <sup>-3</sup>	2.01 × 10 <sup>-2</sup>
rs4275821	CHRNA5	Male	0.35	1.25 (0.48-3.24)	0.89 (0.37-2.12)	0.71 (0.32-1.57)	0.47 (0.19-1.16)	5.14 × 10 <sup>-1</sup>	9.67 × 10 <sup>-1</sup>	Female	0.38	1.10 (0.37-3.23)	0.27 (0.12-0.62)	0.54 (0.20-1.44)	2.58 (0.98-6.79)	3.49 × 10 <sup>-3</sup>	2.29 × 10 <sup>-2</sup>
18737865 <sup>b</sup>	COMT	Male	0.31	0.70 (0.34-1.44)	1.13 (0.55-2.31)	0.72 (0.38-1.33)	2.11 (1.11-4.01)	5.70 × 10 <sup>-2</sup>	4.22 × 10 <sup>-1</sup>	Female	0.28	1.25 (0.58-2.70)	1.86 (0.92-3.73)	0.64 (0.30-1.36)	0.28 (0.12-0.67)	3.23 × 10 <sup>-3</sup>	3.71 × 10 <sup>-2</sup>
rs3743075	CHRNA3	Male	0.35	1.27 (0.49-3.25)	1.09 (0.46-2.61)	0.52 (0.23-1.17)	0.51 (0.21-1.25)	3.37 × 10 <sup>-1</sup>	8.60 × 10 <sup>-1</sup>	Female	0.38	1.11 (0.37-3.26)	0.32 (0.14-0.73)	0.50 (0.19-1.34)	2.66 (1.01-7.02)	6.41 × 10 <sup>-3</sup>	3.92 × 10 <sup>-2</sup>
rs1063311	MAPK1	Male	0.46	0.83 (0.43-1.62)	0.84 (0.46-1.52)	1.37 (0.77-2.45)	0.73 (0.36-1.46)	4.82 × 10 <sup>-1</sup>	8.10 × 10 <sup>-1</sup>	Female	0.43	0.77 (0.38-1.56)	0.61 (0.34-1.09)	3.08 (1.36-6.98)	0.97 (0.53-1.80)	8.76 × 10 <sup>-3</sup>	4.44 × 10 <sup>-2</sup>
rs2266966	MAPK1	Male	0.46	0.83 (0.43-1.62)	0.80 (0.44-1.45)	1.38 (0.77-2.45)	0.73 (0.36-1.46)	4.56 × 10 <sup>-1</sup>	7.89 × 10 <sup>-1</sup>	Female	0.43	0.81 (0.40-1.62)	0.60 (0.33-1.07)	3.08 (1.36-6.98)	0.97 (0.53-1.80)	8.72 × 10 <sup>-3</sup>	4.44 × 10 <sup>-2</sup>

All models were adjusted for age and FTND.



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SNPs above the dashed line have adjusted  $P$ -values  $< 0.05$  within males, SNPs below the dashed line have adjusted  $P$ -values  $< 0.05$  within females.

<sup>a</sup> Observed  $P$ -values were obtained using a 3-df test of the gene-treatment interaction effect.  $P$ -values were adjusted for the correlation of individual contributions to score statistics for the 3-df tests within respective gene regions.

<sup>b</sup> Adjusted SNP  $\times$  Treatment  $\times$  Gender Interaction  $P$ -value  $< 0.05$ .

Table 4

Gender stratified SNP × treatment interaction results at 6-month follow-up

SNP	Gene Region	Gender	MAF	Placebo		Bupropion		Patch		Spray		Test of Interaction <sup>a</sup>	
				SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	Observed P	Adjusted P		
<i>Significant SNPs within Males</i>													
rs806365 <sup>b</sup>	<i>CNR1</i>	Male	0.46	1.06 (0.30–3.75)	2.05 (0.62–6.78)	2.41 (0.74–7.84)	0.04 (0.01–0.21)	3.76 × 10 <sup>-6</sup>	3.86 × 10 <sup>-5</sup>				
		Female	0.44	0.30 (0.11–0.87)	0.80 (0.33–1.92)	0.53 (0.14–1.95)	1.32 (0.43–4.12)	2.66 × 10 <sup>-1</sup>	7.34 × 10 <sup>-1</sup>				
rs684513	<i>CHRNA5</i>	Male	0.17	1.56 (0.51–4.74)	0.57 (0.19–1.74)	4.00 (1.53–10.42)	0.11 (0.02–0.50)	7.74 × 10 <sup>-5</sup>	6.98 × 10 <sup>-4</sup>				
		Female	0.15	1.56 (0.51–4.72)	1.84 (0.71–4.73)	1.19 (0.31–4.50)	1.59 (0.55–4.59)	9.64 × 10 <sup>-1</sup>	9.64 × 10 <sup>-1</sup>				
rs4887069	<i>CHRNA3;CHRNA4</i>	Male	0.2	1.83 (0.72–4.61)	0.62 (0.22–1.72)	2.63 (1.24–5.55)	0.17 (0.04–0.61)	2.83 × 10 <sup>-4</sup>	2.30 × 10 <sup>-3</sup>				
		Female	0.17	1.22 (0.46–3.27)	0.91 (0.39–2.15)	1.51 (0.46–5.01)	0.69 (0.29–1.66)	7.19 × 10 <sup>-1</sup>	9.73 × 10 <sup>-1</sup>				
rs938682	<i>CHRNA3</i>	Male	0.2	2.10 (0.79–5.57)	0.62 (0.23–1.72)	2.24 (1.07–4.67)	0.17 (0.04–0.61)	5.43 × 10 <sup>-4</sup>	4.20 × 10 <sup>-3</sup>				
		Female	0.16	1.26 (0.47–3.36)	1.33 (0.58–3.06)	1.08 (0.31–3.79)	0.75 (0.32–1.78)	7.88 × 10 <sup>-1</sup>	9.48 × 10 <sup>-1</sup>				
rs637137	<i>CHRNA5</i>	Male	0.2	1.85 (0.70–4.93)	0.67 (0.24–1.86)	2.38 (1.14–4.97)	0.17 (0.04–0.61)	6.30 × 10 <sup>-4</sup>	4.81 × 10 <sup>-3</sup>				
		Female	0.16	1.54 (0.60–3.98)	1.33 (0.58–3.06)	1.17 (0.31–4.43)	0.73 (0.32–1.71)	6.51 × 10 <sup>-1</sup>	9.76 × 10 <sup>-1</sup>				
rs3743078	<i>CHRNA3;CHRNA5</i>	Male	0.21	1.97 (0.81–4.81)	0.71 (0.27–1.87)	2.24 (1.07–4.67)	0.17 (0.04–0.61)	6.79 × 10 <sup>-4</sup>	5.13 × 10 <sup>-3</sup>				
		Female	0.16	1.13 (0.42–3.03)	1.33 (0.58–3.06)	1.17 (0.31–4.43)	0.75 (0.32–1.78)	8.07 × 10 <sup>-1</sup>	9.59 × 10 <sup>-1</sup>				
rs667282	<i>CHRNA5</i>	Male	0.2	1.85 (0.69–4.92)	0.67 (0.24–1.86)	2.32 (1.11–4.86)	0.17 (0.04–0.61)	7.10 × 10 <sup>-4</sup>	5.30 × 10 <sup>-3</sup>				
		Female	0.16	1.54 (0.60–3.98)	1.33 (0.58–3.06)	1.17 (0.31–4.43)	0.73 (0.32–1.71)	6.51 × 10 <sup>-1</sup>	9.76 × 10 <sup>-1</sup>				
rs11954565	<i>DRD1</i>	Male	0.34	2.77 (0.81–9.47)	1.43 (0.57–3.59)	0.59 (0.23–1.48)	0.09 (0.02–0.43)	5.83 × 10 <sup>-4</sup>	6.66 × 10 <sup>-3</sup>				
		Female	0.36	3.12 (0.84–11.64)	1.76 (0.73–4.23)	1.20 (0.32–4.54)	1.11 (0.41–3.01)	6.08 × 10 <sup>-1</sup>	9.42 × 10 <sup>-1</sup>				
rs806369 <sup>b</sup>	<i>CNR1</i>	Male	0.31	2.16 (0.97–4.79)	1.31 (0.64–2.71)	1.68 (0.82–3.42)	0.14 (0.03–0.63)	7.65 × 10 <sup>-4</sup>	6.80 × 10 <sup>-3</sup>				
		Female	0.3	0.55 (0.23–1.32)	0.80 (0.43–1.49)	0.45 (0.13–1.52)	1.42 (0.63–3.22)	3.14 × 10 <sup>-1</sup>	7.88 × 10 <sup>-1</sup>				
rs6495309	<i>CHRNA3;CHRNA4</i>	Male	0.19	2.19 (0.83–5.81)	0.67 (0.24–1.86)	1.98 (0.87–4.53)	0.17 (0.05–0.61)	1.37 × 10 <sup>-3</sup>	9.26 × 10 <sup>-3</sup>				
		Female	0.15	1.45 (0.54–3.86)	0.96 (0.40–2.31)	0.81 (0.21–3.16)	0.80 (0.33–1.92)	8.33 × 10 <sup>-1</sup>	9.55 × 10 <sup>-1</sup>				

SNP	Gene Region	Gender	MAF	Placebo		Bupropion		Patch		Spray		Test of Interaction <sup>d</sup>					
				SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	Observed P	Adjusted P						
rs934778	<i>POMC</i>	Male	0.32	1.73 (0.54–5.53)	2.35 (0.92–5.99)	0.21 (0.07–0.61)	0.85 (0.30–2.42)	2.85 × 10 <sup>-3</sup>	1.51 × 10 <sup>-2</sup>	Female	0.34	0.94 (0.34–2.60)	3.11 (1.20–8.01)	2.45 (0.60–10.09)	0.84 (0.31–2.29)	1.75 × 10 <sup>-1</sup>	4.35 × 10 <sup>-1</sup>
rs708905b	<i>FOSB</i>	Male	0.14	0.14 (0.02–1.17)	2.52 (0.91–7.02)	0.27 (0.07–0.98)	1.29 (0.42–3.93)	5.68 × 10 <sup>-3</sup>	2.54 × 10 <sup>-2</sup>	Female	0.13	0.72 (0.21–2.40)	0.72 (0.24–2.15)	3.14 (0.74–13.29)	0.37 (0.10–1.39)	1.91 × 10 <sup>-1</sup>	4.04 × 10 <sup>-1</sup>
rs743409b	<i>MAPK1</i>	Male	0.44	0.37 (0.15–0.94)	1.22 (0.64–2.35)	0.97 (0.52–1.80)	2.95 (1.33–6.54)	5.47 × 10 <sup>-3</sup>	2.55 × 10 <sup>-2</sup>	Female	0.47	2.10 (1.02–4.33)	0.81 (0.46–1.42)	1.57 (0.63–3.96)	1.05 (0.55–2.00)	1.84 × 10 <sup>-1</sup>	4.72 × 10 <sup>-1</sup>
rs2267366	<i>PRKCBP</i>	Male	0.13	0.61 (0.16–2.28)	0.53 (0.20–1.41)	0.73 (0.25–2.12)	5.32 (1.80–15.74)	6.33 × 10 <sup>-3</sup>	2.64 × 10 <sup>-2</sup>	Female	0.16	0.86 (0.33–2.29)	0.76 (0.34–1.70)	0.47 (0.07–3.33)	0.56 (0.19–1.63)	9.03 × 10 <sup>-1</sup>	9.03 × 10 <sup>-1</sup>
rs1892848b	<i>MAPK1</i>	Male	0.45	0.35 (0.14–0.90)	1.14 (0.60–2.18)	0.97 (0.52–1.81)	2.79 (1.23–6.33)	7.26 × 10 <sup>-3</sup>	3.24 × 10 <sup>-2</sup>	Female	0.47	2.29 (1.09–4.80)	0.79 (0.45–1.40)	1.57 (0.63–3.96)	1.05 (0.55–2.00)	1.27 × 10 <sup>-1</sup>	3.52 × 10 <sup>-1</sup>
rs3788332b	<i>MAPK1</i>	Male	0.45	0.35 (0.14–0.90)	1.14 (0.60–2.18)	0.97 (0.52–1.81)	2.79 (1.23–6.33)	7.26 × 10 <sup>-3</sup>	3.25 × 10 <sup>-2</sup>	Female	0.47	2.38 (1.12–5.02)	0.77 (0.43–1.37)	1.57 (0.63–3.92)	1.05 (0.55–2.00)	9.92 × 10 <sup>-2</sup>	2.88 × 10 <sup>-1</sup>
rs5999550b	<i>MAPK1</i>	Male	0.45	0.35 (0.14–0.90)	1.14 (0.60–2.18)	0.97 (0.52–1.81)	2.79 (1.23–6.33)	7.26 × 10 <sup>-3</sup>	3.25 × 10 <sup>-2</sup>	Female	0.47	2.29 (1.09–4.80)	0.79 (0.45–1.40)	1.57 (0.63–3.96)	1.05 (0.55–2.00)	1.27 × 10 <sup>-1</sup>	3.52 × 10 <sup>-1</sup>
rs11704205b	<i>MAPK1</i>	Male	0.45	0.35 (0.14–0.90)	1.14 (0.60–2.18)	0.97 (0.52–1.81)	2.79 (1.23–6.33)	7.26 × 10 <sup>-3</sup>	3.26 × 10 <sup>-2</sup>	Female	0.45	0.35 (0.14–0.90)	1.14 (0.60–2.18)	0.97 (0.52–1.81)	2.79 (1.23–6.33)	7.26 × 10 <sup>-3</sup>	3.26 × 10 <sup>-2</sup>
rs2797855	<i>DBH</i>	Male	0.4	1.91 (0.56–6.57)	0.17 (0.06–0.45)	0.41 (0.16–1.02)	1.32 (0.45–3.81)	4.14 × 10 <sup>-3</sup>	4.74 × 10 <sup>-2</sup>	Female	0.45	0.91 (0.29–2.84)	1.39 (0.57–3.40)	0.77 (0.20–2.94)	0.31 (0.11–0.87)	1.86 × 10 <sup>-1</sup>	7.44 × 10 <sup>-1</sup>
<b>Significant SNPs within Females</b>																	
rs10423854b	<i>FOSB</i>	Male	0.43	1.12 (0.35–3.61)	1.05 (0.41–2.72)	0.38 (0.15–0.96)	1.78 (0.57–5.54)	1.69 × 10 <sup>-1</sup>	4.26 × 10 <sup>-1</sup>	Female	0.41	0.19 (0.06–0.55)	0.48 (0.20–1.14)	6.33 (0.76–52.37)	1.14 (0.40–3.19)	2.65 × 10 <sup>-3</sup>	1.34 × 10 <sup>-2</sup>
rs950776	<i>CHRNA4</i>	Male	0.33	1.28 (0.43–3.82)	1.22 (0.47–3.16)	0.75 (0.30–1.85)	0.73 (0.26–2.06)	7.77 × 10 <sup>-1</sup>	8.67 × 10 <sup>-1</sup>	Female	0.35	3.29 (1.00–10.88)	0.41 (0.17–0.97)	0.38 (0.10–1.44)	2.46 (0.85–7.11)	3.42 × 10 <sup>-3</sup>	2.11 × 10 <sup>-2</sup>

SNP	Gene Region	Gender	MAF	Placebo		Bupropion		Patch		Spray		Test of Interaction <sup>d</sup>	
				SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	SNP OR (95% CI)	Observed <i>P</i>	Adjusted <i>P</i>		
rs7178270	CHRNA3:CHRNA4	Male	0.37	0.97 (0.33–2.88)	1.45 (0.53–3.94)	0.79 (0.31–1.97)	1.11 (0.39–3.14)	8.46 × 10 <sup>-1</sup>	9.29 × 10 <sup>-1</sup>				
		Female	0.42	2.83 (0.75–10.66)	0.31 (0.13–0.75)	0.25 (0.07–0.97)	1.64 (0.56–4.76)	4.24 × 10 <sup>3</sup>	2.56 × 10 <sup>-2</sup>				
rs7543174 <sup>b</sup>	CHRNA2	Male	0.14	0.31 (0.06–1.48)	2.20 (0.80–6.06)	1.53 (0.57–4.12)	1.71 (0.56–5.23)	1.37 × 10 <sup>-1</sup>	3.35 × 10 <sup>-1</sup>				
		Female	0.18	4.07 (1.41–11.75)	0.76 (0.30–1.93)	0.15 (0.02–1.22)	0.92 (0.31–2.69)	7.61 × 10 <sup>-3</sup>	3.14 × 10 <sup>-2</sup>				
rs7539745 <sup>b</sup>	CHRNA2	Male	0.13	0.61 (0.16–2.37)	3.10 (1.08–8.91)	1.37 (0.49–3.83)	1.81 (0.59–5.59)	2.80 × 10 <sup>-1</sup>	4.55 × 10 <sup>-1</sup>				
		Female	0.15	4.54 (1.56–13.25)	0.65 (0.24–1.79)	0.19 (0.02–1.54)	1.18 (0.40–3.49)	7.55 × 10 <sup>-3</sup>	3.17 × 10 <sup>-2</sup>				

All models were adjusted for age and FTND.

SNPs above the dashed line have adjusted *P*-values < 0.05 within males. SNPs below the dashed line have adjusted *P*-values < 0.05 within females.

<sup>a</sup> Observed *P*-values were obtained using a 3-df test of the gene-treatment interaction effect. *P*-values were adjusted for the correlation of individual contributions to score statistics for the 3-df tests within respective gene regions.

<sup>b</sup> Adjusted SNP × Treatment × Gender Interaction *P*-value < 0.05