Varenicline for smoking cessation: nausea severity and variation in nicotinic receptor genes

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This study evaluated association between common and rare sequence variants in 10 nicotinic acetylcholine receptor subunit genes and the severity of nausea 21 days after initiating the standard, Food and Drug Administration-approved varenicline regimen for smoking cessation. A total of 397 participants from a randomized clinical effectiveness trial with complete clinical and DNA resequencing data were included in the analysis (mean age = 49.2 years; 68.0% female). Evidence for significant association between common sequence variants in CHRNB2 and nausea severity was obtained after adjusting for age, gender and correlated tests (all $P_{\text{ACT}} < 0.05$). Individuals with the minor allele of CHRNB2 variants experienced less nausea than did those without the minor allele, consistent with previously reported findings for CHRNB2 and the occurrence of nausea and dizziness as a consequence of first smoking attempt in adolescents, and with the known neurophysiology of nausea. As nausea is the most common reason for discontinuance of varenicline, further pharmacogenetic investigations are warranted.

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Introduction

Varenicline tartrate (Chantix, Pfizer, New York, NY, USA) was developed as a partial agonist at the $\alpha_4\beta_2$ nicotinic acetylcholine receptor (nAChR)¹,² and was approved by the Food and Drug Administration for smoking cessation in May 2006 following a series of phase 3 randomized clinical trials. Use of varenicline is associated with a significantly increased pooled risk ratio for quitting of 2.33 over placebo at six months.³ In addition, varenicline has been shown to function as a partial and full agonist at $\alpha_3\beta_4$ and $\alpha_7$ nAChRs, respectively, and as a partial agonist at $\alpha_3\beta_2$- and $\alpha_6$-containing receptors although with lower efficacy.⁴

The most common adverse drug reaction reported by people taking varenicline is nausea and its occurrence is dose related. In an analysis of randomized clinical trials, 30–40% of participants receiving varenicline reported mild-to-moderate levels of nausea and, relative to placebo, were 3.25 times more likely to report any nausea.³ In an ongoing analysis of adverse events within a cohort of more than 2500 patients prescribed varenicline in a nonclinical trial setting in the United Kingdom,⁶ nausea/vomiting was the most frequent suspected adverse drug reaction among the 51% of patients reporting an adverse drug reaction and was the most frequent (35%) clinical reason given for discontinuation. In a randomized, double-blind, placebo-controlled trial of varenicline for smoking cessation in smokers with stable cardiovascular disease ($n = 714$),⁷ nausea was the
most commonly reported adverse drug reaction by varenicline users, a significantly higher rate than for placebo (29.5 versus 8.6%). Participants randomized to take varenicline were also significantly more likely than those on placebo to discontinue treatment due to adverse events (9.6 versus 4.3%).

\[ nAChRs \] have a critical role in current models of nausea both at the central (through their regulatory role in neurotransmitter pathways) and peripheral levels (through their role in gastric motility).\(^5\)\(^6\) Although we are unaware of any published studies of genetic variation in relation to varenicline-related nausea, recent evidence suggests a role for variation in \( \beta_2 nAChR \) subunit in the experience of nausea and dizziness as an immediate reaction to first initiation of smoking in young adults\(^10\) and with withdrawal severity following treatment with behavioral counseling and placebo medication in a randomized trial of bupropion.\(^11\) Nausea, regardless of etiology, results in diminished quality of life for the individual patient and could result in reduced rates of adherence and/or premature termination of pharmacotherapy and less likelihood of positive clinical outcomes in a variety of therapeutic areas including the treatment of nicotine dependence.\(^12\)

We have recently reported a resequencing scan of 10 \( nAChR \) subunit genes for common and rare variants and their association with pretreatment levels of nicotine dependence in participants in a randomized behavioral effectiveness trial involving varenicline.\(^13\) The present analysis describes (a) the prevalence and severity of nausea 21 days following the initiation of the Food and Drug Administration-approved regimen involving varenicline for smoking cessation; (b) pretreatment predictors of 21-day nausea severity; (c) the relation between 21-day nausea severity and discontinuation of the medication, non-adherence and point-prevalent smoking at 12 weeks); and (d) association analyses of common and rare variants of the \( CHRNA2–7 \) and \( CHRNA1–4 \) \( nAChR \) subunit genes with nausea severity at 21 days subsequent to the use of varenicline.

**Subjects and methods**

**Population**

Current smokers (\( \geq 10 \) cigarettes per day over the past year, \( N = 1202 \)) were recruited from members of Group Health, a consumer-governed non-profit health care organization that serves more than 600,000 residents of Washington and Idaho, for participation in a randomized behavioral intervention combined with varenicline tartrate (marketed as Chantix by Pfizer).

Recruitment, treatment and assessment methods for the COMprehensive Medication Program And Support Services (COMPASS) study, sponsored by the National Cancer Institute (R01 CA071358), have been described.\(^12\)\(^14\)\(^16\) Briefly, volunteers were screened for exclusions and after the completion of a baseline telephone interview were randomized to one of three modes of delivery of behavioral treatment: telephone-based, web-based or a combined telephone/web-based intervention. Participants were prescribed a standard 12-week course of varenicline and were instructed to take it according to recommended guidelines\(^17\) starting at 1 week before the quit date. Telephone follow-up interviews were conducted by non-intervention study staff at 21 days, 12 weeks and at 6 months after the target quit date. All recruitment, consent, screening and data collection methods were reviewed and approved by the Institutional Review Boards of SRI International, Group Health and Free & Clear.

**The measurement of pretreatment characteristics, adherence and clinical outcome**

Pretreatment measures included age, gender, years of formal education, cigarettes smoked per day and the Fagerström Test for Nicotine Dependence.\(^18\) At each of the follow-up interviews, participants were asked if they had taken any varenicline (yes/no), if they were still taking varenicline (yes/no)—and if they had stopped taking the medication, whether it was because of the side effects (yes/no), the proportion of varenicline pills typically taken during the prescribed 12 week interval (1 = none; 2 = very few; 3 = about one-half; 4 = most; 5 = all) and the number of days the prescribed pills had been taken. During each of the follow-up interviews, participants were asked if they had smoked a cigarette, even a puff, in the last 7 days. Quit outcomes did not differ based on modality (phone, web and combined) of behavioral counseling.\(^16\)

**The measurement of nausea**

During the interview at 21 days, participants were asked if they had experienced any nausea in the last month. Those participants who indicated they had experienced nausea were asked to rate its severity on a five-point scale as follows: 1 = very mild, 2 = mild, 3 = moderate, 4 = severe and 5 = very severe, whereas participants who indicated that they had not experienced any nausea were given a severity rating of 0 = none.

**Biospecimen collection and DNA extraction**

COMPASS participants were invited by telephone to provide a saliva sample for DNA extraction for a National Institute of Drug Abuse-sponsored study being conducted by the Pharmacogenetics of Nicotine Addiction and Treatment consortium (http://www.pharmgkb.org/contributors/pgrn/pn_at_profile.jsp). Complete details of saliva sample collection and processing can be found in Nishita et al.\(^19\)

**Sequence variant discovery**

The sequence variant data available for association analyses of identified common and rare variants and 21-day nausea severity is described elsewhere.\(^14\) In that study, a recently developed (454) and a traditional (Sanger) method of resequencing\(^20\)\(^21\) were used to identify both common and rare sequence variation at ten \( nAChR \) subunit genes from DNA provided by COMPASS participants who self-identified as non-Hispanic White, had never used varenicline.
previously and who had complete questionnaire data on smoking behaviors.

Association analyses
Following a review of the association between pretreatment characteristics and 21-day nausea severity ratings, common variants (defined as having a minor allele frequency (MAF) $\geq 5\%$) were analyzed separately for association with nausea severity either controlling for or residualizing for age, age$^2$ (adjusting for nonlinear effects of age), and gender using linear regression model with both additive and dominant genotype models. Let $Y_i$ be the nausea severity for the $i$-th individual, age$_i$ be the person’s age, age$_i$$^2$ be the square of age, gender$_i$ be an indicator for male gender, single-nucleotide polymorphism (SNP) be either an indicator for a dominant genotype or a variable taking values of 0, 1 or 2 for an additive model, and $e_i$ be an independent normally distributed error term. The following model was fit:

$$Y_i = b_0 + b_1 \times \text{age}_i + b_2 \times \text{age}_i^2 + b_3 \times \text{gender}_i + b_4 \times \text{SNP}_i + e_i$$  \hspace{1cm} (1)

The statistical significance for the additive or dominant model was obtained by testing $H_0: b_4 = 0$. When using an analysis approach that did not allow for covariates (that is, the tests for association of multiple rare, common and rare, or common variants simultaneously, described below), we fit the following model:

$$Y_i = b_0 + b_1 \times \text{age}_i + b_2 \times \text{age}_i^2 + b_3 \times \text{gender}_i + e_i$$  \hspace{1cm} (2)

and formed the residualized nausea ratings (denoted $Z_i$) as:

$$Z_i = Y_i - b_0' - b_1' \times \text{age}_i - b_2' \times \text{age}_i^2 - b_3' \times \text{gender}_i$$  \hspace{1cm} (3)

where the $b$’s are the estimated coefficients from regression (2) and used the $Z_i$ in the analyses. Neither pretreatment cigarettes smoked per day nor the Fagerström Test for Nicotine Dependence score were significantly associated with 21-day nausea severity. The significance of regression models was reported for each SNP and with adjustment for correlated tests ($P_{\text{ACT}}$)$^{22}$ and via permutation testing.

For rare variants, gene-based association tests were performed by the cohort allelic sum test and by the weighted sum statistic.$^{23}$ Cohort allelic sum test was used to test for the association between nausea severity and counts of rare alleles, which were based on two fixed thresholds (MAF <1% and <5%). The weighted sum statistic was used to test for association between nausea severity and weighted counts of rare variants (defined as MAF <5%), with an inverse relation between weights and the frequency of minor alleles. Both tests were applied only to rare variants under the assumption that rare variants are more likely to be deleterious than common ones.$^{24}$ Linear regression coefficients, $P$-values from likelihood ratio tests and empirical $P$-values from permutation testing were reported.

Multivariate distance-based matrix regression was also used to test associations of common and rare (MAF <5%) variants with nausea severity, with either identical by state allele sharing across individuals and variants in each gene, or with allele sharing weighted by the Lynch–Ritland calculation, with 100,000 permutations. The latter approach gives more weight to rare variants.$^{25–27}$ When multivariate distance-based matrix regression analyses with both common and rare variants identified significant association, two post hoc tests were performed: common variants alone and rare variants alone. Pairwise linkage disequilibrium values $D^*$ and $r^2$ were calculated for three common CHRNA2 SNPs from the COMPASS sequence data using Haploview.$^{28}$ For the nAChr subunit genes that are clustered in the genome (CHRNA3 and CHRNA6 at chr8p11, and CHRNA5, CHRNA3 and CHRNA4 at chr15q25.1), cohort allelic sum test, weighted sum statistic and multivariate distance-based matrix regression association analyses were performed to evaluate variants available in these genes as gene regions.

Results
Comparison of individuals analyzed versus those not analyzed
Table 1 provides descriptive information for the COMPASS participants in the base analysis sample and those not in the base association analysis sample. Those in the base analysis sample were self-identified non-Hispanic White had genotypes with 90% or higher call rates, and reported having taken varenicline at the 21 day interview ($n = 397$). Compared with the remaining 805 COMPASS participants (81.3% of whom self-identified as non-Hispanic White), the participants comprising the base analysis sample were significantly older and more likely to have reported 7-day non-smoking at the 21 day and 12 week follow-ups. There were no significant differences between the two groups with respect to average level of reported nausea severity at 21 days. The proportion of participants who reported having stopped taking varenicline because of side effects was also not significantly different between the two groups at either the 21 day or 12-week follow-up periods.

Association of nausea severity with clinical outcomes
Among the 397 participants in the analysis sample, 58.7% ($n = 233$) reported experiencing any nausea at the 21 day follow-up. Of these individuals, 66.8% were no longer taking varenicline at the 12 week follow-up. The average 21-day nausea severity rating was 1.6 ($\pm 1.6$), with 34.3% of participants rating severity as moderate or higher. A higher 21-day nausea rating was associated significantly with a smaller proportion of pills typically taken during the 12 week treatment ($r = -0.18$, $P < 0.001$) and fewer number of days on which the varenicline was taken ($r = -0.14$, $P = 0.005$). The 21-day nausea rating was significantly associated with increased likelihood of discontinuing varenicline by 12 weeks (odds ratio $= 1.24$, 95% confidence interval: 1.08–1.42; $P = 0.002$), with increased likelihood of stopping due to side effects at 12 weeks (odds ratio $= 1.58$, 95% confidence interval: 1.34–1.86; $P < 0.001$), and of having smoked (7-day point prevalence smoking) at the 12-week follow-up (odds ratio $= 1.20$, 95% confidence interval: 1.05–1.37; $P = 0.008$).
Pretreatment correlates of nausea at 21 days
Age, gender, years of formal schooling, Fagerström Test for Nicotine Dependence score and cigarettes smoked per day at the pretreatment assessment were examined as potential correlates of the 21-day nausea severity rating. Females rated the severity of nausea higher than did males, (1.9 verses 1.1, \( t(302) = 5.16, P < 0.0001 \)), whereas age was negatively associated (\( r = -0.13, P = 0.007 \)) with the nausea rating. Age and years of smoking were correlated at 0.20 (\( P < 0.001 \)). Alone, years of smoking was not a statistically significant predictor of nausea at 21 days (\( P = 0.399 \)). When age and years of smoking were both used as predictors of nausea, age remained statistically significant (\( P = 0.010 \)), whereas years of smoking did not (\( P = 0.756 \)). Nonsignificant associations between pretreatment number of cigarettes smoked per day (\( r = -0.08, P = 0.108 \)), the Fagerström Test for Nicotine Dependence score (\( r = 0.00, P = 0.991 \)), and years of formal schooling (\( r = 0.09, P = 0.620 \)) and the 21-day nausea severity rating were observed. Age was therefore selected for inclusion in the subsequent analysis of genetic correlates of nausea.

Common and rare variant association analyses
A total of 45 common variants were tested for association with nausea severity at 21 days using two transmission models (Table 2). Significant \((P < 0.05)\) unadjusted associations were found with \( CHRN B2 \) (rs2072660, \( \beta = -0.428; \) rs2072661, \( \beta = -0.443; \) rs4292956, \( \beta = -0.542 \)) and \( CHRN B1 \) (rs2302764, \( \beta = 0.337 \)). Permutation analysis resulted in nearly identical significance values. The three \( CHRN B2 \) variants are found within the \( CHRN B2 \) 3' untranslated region within 224 basepairs of each other. \( D^2 \) and \( r^2 \) values are 0.96 and 0.92 between rs2072660 and rs2072661, and 0.97 and 0.21 between these two SNPs and rs4292956. After adjustment for multiple correlated tests within each gene, significant associations remained between three \( CHRN B2 \) variants and the 21-day nausea severity rating: rs2072660 (\( P_{\text{ACT}, \text{additive}} = 0.013; \) \( P_{\text{ACT}, \text{dominant}} = 0.019 \)); rs2072661 (\( P_{\text{ACT}, \text{additive}} = 0.021; \) \( P_{\text{ACT}, \text{dominant}} = 0.016 \)); and rs4292956 (\( P_{\text{ACT}, \text{additive}} = 0.120; \) \( P_{\text{ACT}, \text{dominant}} = 0.045 \)). Individuals with one or two copies of the minor alleles of these \( CHRN B2 \) SNPs exhibited the following unit decreases in 21-day mean nausea severity relative to those without the minor allele: rs2072660, 0.44 (mean (s.d.) = 1.81 (1.53) versus 1.37 (1.51); \( P = 0.004 \)); rs2072660, 0.43 (1.80 (1.54) versus 1.37 (1.50); \( P = 0.006 \)); and, rs4292956, 0.54 (1.69 (1.55) versus 1.15 (1.33); \( P = 0.021 \)). No significant associations between rare variation in \( CHRN B2 \) and the 21-day nausea severity rating score were observed from either the cohort allelic sum test \((P > 0.07)\) or

### Table 1 COMPASS analysis sample versus remaining sample characteristics

| Baseline characteristic          | Analysis sample N = 397 | Remaining sample N = 805 | P-value |
|----------------------------------|------------------------|--------------------------|---------|
| **Demographics**                 |                        |                          |         |
| Age in years (M)                 | 49.2                   | 46.4                     | 0.001   |
| Gender (% female)                | 68.0                   | 66.3                     | 0.562   |
| Years of formal schooling (M)    | 14.2                   | 14.0                     | 0.064   |
| **Smoking history**              |                        |                          |         |
| Cigarettes per day (M)           | 20.2                   | 19.4                     | 0.129   |
| FTND (M)                         | 5.1                    | 4.9                      | 0.065   |
| **Status at 21 days**            | N = 397                | N = 621                  |         |
| Take any varenicline (% yes)     | 100.0                  | 94.8                     | 0.001   |
| 7-Day pp smoking (respondent; % not smoking) | 64.0     | 52.2                     | 0.002   |
| Nausea (ranking 0–5; M)          | 1.6                    | 1.5                      | 0.108   |
| Still taking varenicline (% yes) | 86.4                   | 80.6                     | 0.018   |
| Stopped taking varenicline       | N = 54                 | N = 114                  |         |
| Stopped due to side effects (% yes) | 53.7                  | 52.6                     | 0.897   |
| **Status at 12 weeks**           | N = 371                | N = 544                  |         |
| Take any varenicline (% yes)     | 99.5                   | 97.8                     | 0.043   |
| 7-Day pp smoking (respondent; % not smoking) | 64.0     | 53.0                     | 0.001   |
| Still taking varenicline (% yes) | 38.5                   | 35.2                     | 0.304   |
| Stopped taking varenicline       | N = 225                | N = 343                  |         |
| Stopped because of side effects (% yes) | 38.0                  | 39.9                     | 0.634   |

Abbreviations: COMPASS, COmprehensive Medication Program And Support Services; FTND, Fagerström Test of Nicotine Dependence; pp, point prevalence.
weighted sum statistic ($P > 0.06$) tests (Table 3). Significant associations between common and rare variants combined and 21-day nausea severity were identified at CHRN82 by both the allele sharing ($P = 0.02$) and weighted allele sharing ($P = 0.01$) multivariate distance-based matrix regression tests (Table 4). Subsequent post hoc testing revealed that this association was because of the effects of common variants only (both tests, $P = 0.02$).

### Discussion

This analysis identified common variants in CHRN82 associated with nausea severity at 21 days of use of varenicline for smoking cessation. The presence of the minor allele in these variants is associated with reduced levels of reported nausea. The prevalence of the CHRN82 minor alleles ranges from 6.6 to 24.5% in this

| SNP ID     | A1 | A2 | Gene Type | Type | MAF  | $P_{Add}$ | $P_{Dom}$ |
|------------|----|----|-----------|------|------|-----------|-----------|
| rs2280781  | C  | T  | CHRN82    | 5' UTR | 0.101| 0.359     | 0.278     |
| rs4845378  | G  | T  | CHRN82    | Intron | 0.097| 0.259     | 0.259     |
| rs2072660  | T  | C  | CHRN82    | 3' UTR | 0.244| 0.004     | 0.006     |
| rs2072661  | G  | A  | CHRN82    | 3' UTR | 0.245| 0.006     | 0.005     |
| rs4292956  | C  | T  | CHRN82    | 3' UTR | 0.066| 0.056     | 0.021     |
| rs2472553  | G  | A  | CHRNA2    |        |      |           |           |
| rs13277254 | G  | A  | CHRN83    |        |      |           |           |
| rs13280301 | A  | G  | CHRN83    |        |      |           |           |
| rs13277524 | G  | T  | CHRN83    |        |      |           |           |
| rs6474413  | C  | T  | CHRN83    |        |      |           |           |
| rs4950     | G  | A  | CHRN83    | 5' UTR | 0.236| 0.901     | 0.855     |
| rs2304297  | G  | C  | CHRNA6    | 3' UTR | 0.244| 0.288     | 0.281     |
| rs71653603 | C  | T  | CHRNA7    | Synonymous | 0.060| 0.878     | 0.878     |
| rs569207   | C  | T  | CHRNA5    |        |      |           |           |
| rs16969968 | G  | A  | CHRNA5    |        |      |           |           |
| rs615470   | T  | C  | CHRNA5    | 3' UTR | 0.381| 0.912     | 0.462     |
| rs8192482  | C  | T  | CHRNA5    | 3' UTR | 0.368| 0.911     | 0.541     |
| rs564585   | A  | G  | CHRNA5    | 3' UTR | 0.237| 0.786     | 0.607     |
| rs12899226 | T  | G  | CHRNA3    | Down   | 0.052| 0.051     | 0.051     |
| rs660652   | A  | G  | CHRNA3    | 3' UTR | 0.383| 0.929     | 0.462     |
| rs472054   | A  | G  | CHRNA3    | 3' UTR | 0.379| 0.925     | 0.475     |
| rs578776   | A  | G  | CHRNA3    | 3' UTR | 0.247| 0.836     | 0.749     |
| rs1051730  | G  | A  | CHRNA3    | Synonymous | 0.367| 0.802     | 0.675     |
| rs3743075  | T  | C  | CHRNA3    | Synonymous | 0.378| 0.981     | 0.533     |
| rs3743074  | G  | A  | CHRNA3    | Intron  | 0.378| 0.969     | 0.587     |
| rs8040868  | T  | C  | CHRNA3    | Synonymous | 0.429| 0.376     | 0.491     |
| rs8192475  | C  | T  | CHRNA3    | Non-synonymous | 0.050| 0.362     | 0.362     |
| rs12914008 | G  | A  | CHRNA4    | Non-synonymous | 0.051| 0.208     | 0.208     |
| rs3813567  | G  | A  | CHRNA4    |        |      |           |           |
| rs2302765  | T  | C  | CHRNA1    | Intron  | 0.159| 0.182     | 0.142     |
| rs12452047 | A  | G  | CHRNA1    | Intron  | 0.166| 0.235     | 0.172     |
| rs7210231  | C  | A  | CHRNA1    | Intron  | 0.199| 0.268     | 0.242     |
| rs2302761  | C  | T  | CHRNA1    | Intron  | 0.202| 0.192     | 0.183     |
| rs2302763  | T  | C  | CHRNA1    | Intron  | 0.164| 0.462     | 0.394     |
| rs2302764  | T  | C  | CHRNA1    | 3' UTR | 0.160| 0.602     | 0.047     |
| rs3827020  | T  | C  | CHRNA4    | Intron  | 0.153| 0.160     | 0.271     |
| rs45442394 | G  | A  | CHRNA4    | Intron  | 0.066| 0.333     | 0.235     |
| rs1044397  | C  | T  | CHRNA4    | Synonymous | 0.460| 0.331     | 0.369     |
| rs1044396  | G  | A  | CHRNA4    | Synonymous | 0.458| 0.180     | 0.177     |
| rs229960   | A  | G  | CHRNA4    | Synonymous | 0.059| 0.627     | 0.681     |
| rs229959   | C  | A  | CHRNA4    | Synonymous | 0.122| 0.702     | 0.858     |
| rs1044394  | A  | G  | CHRNA4    | Synonymous | 0.071| 0.985     | 0.919     |
| rs6090384  | T  | C  | CHRNA4    | Intron  | 0.063| 0.752     | 0.814     |
| rs2273505  | C  | T  | CHRNA4    | Intron  | 0.066| 0.269     | 0.337     |
| rs2273506  | G  | A  | CHRNA4    |        |      |           |           |

Abbreviations: A1, allele 1; A2, allele 2; MAF, minor allele frequency; nAChR, α4β2 nicotinic acetylcholine receptor; $P_{Add}$, $P$ of additive model; $P_{Dom}$, $P$ of dominant model; SNP, single-nucleotide polymorphism; UTR, untranslated region.

Note: SNPs with $P < 0.05$ are in bold.
treatment-seeking sample. For the rs2072660 minor allele (C), allele frequencies of 0.21, 0.23, 0.29 and 0.54 are observed in HapMap samples JPT, CEU, CHB and YRI, respectively, suggesting that ~50% of individuals with Caucasian and east Asian ancestry, and about 15% of individuals with west African ancestry are without the rs2072660 nausea-reducing genotypes observed in this study (rs2072661 and rs4292956 are not genotyped in as many HapMap samples but have lower MAF in those samples that have been genotyped).

Ehringer et al.\textsuperscript{10} reported a relation between one of the \textit{CHRN2} SNPs examined here (rs2072660) and feelings of dizziness or nausea (tobacco sensitivity) shortly after smoking initiation in 1068 young adults aged 17–21 years. The direction of the association noted by Ehringer et al.\textsuperscript{10} was the same as that seen here. That is, the minor allele of this SNP was associated with lower levels of sensitivity to tobacco to the first few cigarettes.

In additional studies of \textit{CHRN2} promoter and 3\textsuperscript{'} untranslated region variants, Ehringer et al.\textsuperscript{30} assessed

| Gene          | CAST\textsubscript{P} | \textit{P}\textsubscript{1}\textsuperscript{b} | \textit{P}\textsubscript{2}\textsuperscript{c} | CAST\textsubscript{P}\textsuperscript{d} | \textit{P}\textsubscript{1} | \textit{P}\textsubscript{2} | WSS\textsubscript{P} | \textit{P}\textsubscript{1} | \textit{P}\textsubscript{2} |
|---------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
| \textit{CHRN2} | 0.85                 | 0.07                 | 0.09                 | 0.85                 | 0.07                 | 0.07                 | 0.0040               | 0.07                 | 0.06                 |
| \textit{CHRN2} | –0.73                | 0.12                 | 0.11                 | –0.73                | 0.12                 | 0.09                 | –0.0004              | 0.62                 | 0.56                 |
| \textit{CHRN3} | –0.90                | 0.25                 | 0.29                 | –0.90                | 0.25                 | 0.21                 | –0.0040              | 0.25                 | 0.27                 |
| \textit{CHRN4} | 0.09                 | 0.93                 | 0.93                 | 0.09                 | 0.93                 | 0.93                 | 0.0004               | 0.93                 | 0.95                 |
| \textit{CHRN5} | 0.26                 | 0.81                 | 0.88                 | 0.26                 | 0.81                 | 0.80                 | 0.0010               | 0.35                 | 0.38                 |
| \textit{CHRN6} | –0.11                | 0.81                 | 0.76                 | –0.27                | 0.36                 | 0.37                 | 0.0002               | 0.87                 | 0.83                 |
| \textit{CHRN7} | –0.29                | 0.45                 | 0.53                 | –0.23                | 0.13                 | 0.15                 | –0.0003              | 0.67                 | 0.69                 |
| \textit{CHRN8} | –0.99                | 0.20                 | 0.23                 | –0.99                | 0.20                 | 0.17                 | –0.0003              | 0.71                 | 0.62                 |
| \textit{CHRN9} | –0.63                | 0.32                 | 0.45                 | –0.63                | 0.32                 | 0.34                 | –0.0002              | 0.84                 | 0.95                 |

Abbreviations: CAST\textsubscript{P}, cohort allelic sum test; MAF, minor allele frequency; nAChR, \textit{\alpha}4\beta2 nicotinic acetylcholine receptor; WSS\textsubscript{P}, weighted sum statistic.

\textsuperscript{a}MAF <1%.

\textsuperscript{b}\textit{P}\textsubscript{1} = \textit{P}-value from standard F-test.

\textsuperscript{c}\textit{P}\textsubscript{2} = \textit{P}-value from permutation testing.

\textsuperscript{d}MAF <5%.

\textsuperscript{e}Analysis of \textit{CHRN3} and \textit{CHRNA6} variants together.

\textsuperscript{f}Analysis of \textit{CHRNA5}, \textit{CHRNA3} and \textit{CHRN4} variants together.

Note: Genes with \textit{P} < 0.05 are in bold.

| Gene          | N, SNPs | MDMR allele sharing | MDMR weighted allele sharing |
|---------------|---------|---------------------|-----------------------------|
|               |         | Pseudo-F | \textit{P} | % Variation | Pseudo-F | \textit{P} | % Variation |
| \textit{CHRN2} | 24      | 4.70     | 0.02     | 0.012       | 5.58     | 0.01     | 0.014       |
| \textit{CHRN2} | 5       | 5.30     | 0.01     | 0.013       | 5.55     | 0.01     | 0.014       |
| \textit{CHRN2} | 19      | 1.21     | 0.42     | 0.003       | 0.56     | 0.44     | 0.001       |
| \textit{CHRNA2} | 11      | 0.32     | 0.68     | 0.008       | 1.34     | 0.26     | 0.003       |
| \textit{CHRNA3} | 12      | 0.16     | 0.77     | 0.004       | –0.01    | 0.92     | 0.000       |
| \textit{CHRNA6} | 3       | 1.00     | 0.32     | 0.003       | –0.90    | 0.56     | –0.002      |
| \textit{CHRN7} | 15      | 0.16     | 0.78     | 0.004       | 0.15     | 0.74     | 0.004       |
| \textit{CHRNA7} | 4       | –0.22    | 0.97     | –0.006      | –26.88   | 0.92     | –0.073      |
| \textit{CHRNA5} | 15      | 0.12     | 0.82     | 0.003       | 0.11     | 0.74     | 0.003       |
| \textit{CHRNA3} | 33      | –0.06    | 0.95     | –0.001      | 0.22     | 0.71     | 0.006       |
| \textit{CHRN4} | 15      | 0.72     | 0.52     | 0.002       | 0.16     | 0.78     | 0.004       |
| \textit{CHRNA4} | 63      | 0.08     | 0.90     | 0.002       | 0.07     | 0.83     | 0.002       |
| \textit{CHRNA1} | 25      | 1.81     | 0.18     | 0.005       | 2.24     | 0.11     | 0.006       |
| \textit{CHRNB2} | 31      | 0.69     | 0.53     | 0.002       | 20.67    | 0.18     | 0.050       |

Abbreviations: MDMR, multivariate distance-based matrix regression; nAChR, \textit{\alpha}4\beta2 nicotinic acetylcholine receptor; SNP, single-nucleotide polymorphism.

\textsuperscript{a}Post hoc MDMR test performed with common variants only.

\textsuperscript{b}Post hoc MDMR test performed with rare variants only.

\textsuperscript{c}Analysis of \textit{CHRN3} and \textit{CHRNA6} variants together.

\textsuperscript{d}Analysis of \textit{CHRNAS}, \textit{CHRNA3} and \textit{CHRN4} variants together.

Note: Genes with \textit{P} < 0.05 are in bold.
association with dizziness after the first few cigarettes in 1600 ever-smokers in the COGEND sample, and Hoft et al. \(^{31}\) assessed association with subjective physical effects (including dizziness and nausea) following cigarette smoking in a controlled laboratory environment in a sample of 316 adult daily smokers. Although Ehringer et al. \(^{32}\) did not observe association of CHRN22 SNPs with dizziness in the COGEND sample, Hoft et al. report association of a CHRN2 promoter variant (rs2072669) with physical effects. Significant association with sweating, heart pounding and nausea (three of six components of the physical effects score) were identified in post hoc analysis.

In contrast, Conti et al. \(^{11}\) reported rs2072660 and rs2072661 significantly associated with the likelihood of abstinence and the severity of withdrawal symptoms in a placebo-randomized trial of bupropion therapy for smoking cessation, with the minor alleles inversely associated with abstinence and positively associated with severity of withdrawal symptoms. Another investigation showed the major allele of rs2072660 to be associated with an increased number of days of abstinence following treatment with nicotine patch. \(^{33}\) Etter et al. \(^{33}\) on the other hand, found no association between variation in this SNP and nicotine dependence or smoking behavior. A number of other papers have also reported null associations between variation in CHRN22 and nicotine dependence. \(^{44-37}\) or smoking behaviors. \(^{38,39}\) The rare variant analyses at CHRN22 identified P-values ranging from 0.06 to 0.44. Thus, the possible contribution from rare variants at CHRN22 to 21-day nausea severity requires further study, for example, resequencing of additional samples and/or in silico assessment of rare variant function.

Possible mechanisms

Although animal models of nausea have been difficult to establish for a variety of reasons including a lack of definitive knowledge of neural circuitry for nausea in humans, \(^{40}\) conditioned taste aversion paradigms may be one potential model to study the aversive effects of drugs at high doses. Studies involving wild-type and CHRN22-knockout mice revealed that while nicotine produced conditioned taste aversion in both genotypes, the magnitude of the effect was less in the mutant mice, thereby implicating the CHRN22 subunit in the taste aversion effects of nicotine. \(^{41}\)

Nausea in humans can be generated peripherally by toxic materials within the lumen of the gut from which abdominal vagal afferents project to the dorsal brainstem via the nucleus tractus solitarius (a structure in the brainstem that receives inputs from visceral sensations including taste) and/or the area postrema (a structure in the medulla that controls nausea and vomiting). Accumulating data indicate that small intestinal (myenteric) neurons in the intestinal (enteric) nervous system possess not only somatodendritic nAChRs, which mediate cholinergic transmission between neurons, but also presynaptic nAChRs. Myenteric motor neurons express a large number of nAChR subunits including α3, α5, α7, β2 and β4, \(^{42}\) which comprise the nAChRs on which varenicline exerts action.

Nausea in humans can also be generated centrally as a consequence of the absorption of toxic materials (including drugs) with direct actions on the area postrema. \(^{43}\) It is possible that varenicline results in nausea as a consequence of its agonist effects on presynaptic z4- and z6-containing receptors involved in the regulation of dopamine release in the striatum. \(^{44}\) Although nausea and emesis have been observed in Parkinson’s patients taking dopaminergic agonists, \(^{45}\) the precise pathway by which this might occur is unknown. \(^{9}\) A recent paper describing the results of a randomized clinical trial of the potent z4β2 neuronal nicotinic agonist, ABT-594, in the context of the management of pain associated with diabetic peripheral neuropathy, \(^{44}\) found that treatment emergent adverse events (including nausea, dizziness and vomiting) were very high and three to four times more common than that seen in the placebo condition. These authors concluded that this profile is consistent with that seen for z4β2 agonists as a drug class and that the CHRN22 subunit, in particular, could partner with other z subunits to form a functional receptor that influences autonomic ganglia. Because nicotine has a high affinity for z4β2 receptors, it is interesting to note here that nausea and dizziness are also commonly reported following smoking of the first cigarette in naïve individuals who later become smokers. \(^{45-47}\)

Implications for the pharmacogenetic management of varenicline-related nausea

There is evidence that not completing approved cessation pharmacotherapy is associated with relapse to smoking. \(^{48}\) The present analysis revealed that the experience of nausea early in the recommended course of treatment with varenicline impacted negatively a number of indicators of adherence and outcome later in the course of treatment. These indicators include smaller proportion of varenicline pills taken, fewer total days taken the pills, increased chances of complete discontinuation, and an increased chance of relapse at 12 weeks. These results suggest that the early identification of risk for nausea and preemptive treatment could further maximize the clinical effectiveness of varenicline.

One possibility could be to provide an inexpensive test for genotyping relevant nAChR variants before the onset of taking varenicline to personalize therapy. Those with CHRN22 minor alleles could receive the standard course of treatment with the usual rate of titration to the full dose (1 mg b.i.d.). Those with CHRN22 major alleles could (1) be encouraged to consistently take varenicline with food and water; (2) receive a more extended course of titration from the lower to the higher sustained dose (perhaps up to two weeks); (3) remain at the lower dose for the entire course of treatment; or (4) in cases of extreme sensitivity, be prescribed a concomitant therapeutic agent to reduce nausea such as a 5-hydroxytryptamine receptor 3 (HTR3) or neuropeptide 1 (NK1R) antagonist. \(^{9}\) At this stage of knowledge, however, randomized, prospective pharmacogenetic trials are needed to determine the effectiveness of such approaches to the preemptive management of nausea and
whether doing so results in desired clinical outcomes (decreased stopping of the medication, improved adherence and higher overall quit rates).

**Study limitations**
Potential limitations of the study include its reliance on self-report for medication adherence and smoking outcomes. Because this open-label study was conducted in a real-world setting and used telephone and mailed data collection methods, more intensive monitoring was not feasible. The direct inquiry of the experience of nausea at each follow-up is different than the method used to assess side effects in a standard clinical trial, and could result in a higher frequency than previously reported. Finally, DNA samples were not obtained from all members of the COMPASS study. Although there were no differences in reported nausea severity at 21 days between those who did and did not provide a biospecimen for genotyping, those who did so were significantly older than those who did not. As nausea severity at 21 days was associated negatively with age (younger participants reported higher nausea), it is likely that the strength of the observed associations between nausea and correlates (genetic and otherwise) was attenuated.

**Future directions**
The possibility that nausea is directly produced by agonism of CHRN2 receptors by varenicline will need to be confirmed through analysis of gene–nausea associations in another clinical trial setting. Moreover, other plausible explanations of the association observed here exist will also need to be examined. It is possible, for example, that variation in CHRN2 enhances the nausea associated with smoking abstinence even in the absence of varenicline, although, at present, there is insufficient evidence to view nausea as a specific abstinence effect. This could be examined in a clinical trial arm that involves behavioral counseling paired with placebo medication. Although the occurrence of nausea is much lower for other smoking cessation medications, such as nicotine replacement therapy and bupropion (~10% of users), the specificity of the association could also be determined by examination of the gene–nausea association in the presence of these medications. A second possibility that will require further research is that CHRN2 variation contributes to nausea in individuals who smoke while also taking varenicline. Laboratory studies of the effects of varenicline in the presence and absence of concurrent smoking could be conducted under controlled conditions to examine this hypothesis. A number of side effects, in addition to nausea, have been reported following use of varenicline. Any one or combination of these could result in lower levels of patient adherence to the recommended regimen, thereby reducing varenicline’s overall effectiveness in clinical settings. Because varenicline is one of the most effective medications currently available for smoking cessation when taken as prescribed, further investigation of the relation between the complete side effect profile and its subsequent impact on adherence is warranted.

**Conflict of interest**
Varenicline and the nominal support for recruiting participants was provided by Pfizer. Neither entity had any role in the study design, collection, analysis and interpretation of data, in the writing of the report or in the decision to submit the report for publication. Swan, Javitz, Jack, Wessel, Michel, Hinds, Stokowski, McClure, Catz, Richards, Zbikowski, Deprey, McAfee, Conti and Bergen declare that all financial and material support for this work was provided by their primary employer. Javitz, Jack, Wessel, Michel, Hinds, Stokowski, McClure, Catz, Richards, Zbikowski, Deprey, McAfee and Bergen declare that, except for income provided from their primary employer, no other financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional services nor are there personal financial holdings that could be perceived as constituting a potential conflict of interest. Dr Swan received financial support from Pfizer to attend a 1-day advisory meeting in 2008. Dr Conti was a paid consultant for Pfizer in 2008. Dr Wessel is currently employed by Indiana University. Dr Hinds is currently employed by 23 and Me. Dr Stokowski is currently employed by Tandem Diagnostics and Dr McAfee is currently employed by the Centers for Disease Control and Prevention.

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Varenicline, nausea and nicotinic receptors

GE Swan et al

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