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Varenicline for Smoking Cessation: Nausea Severity and Variation in Nicotinic Receptor Genes

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Abstract

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, FDA-approved varenicline regimen for smoking cessation. Included in the analysis were 397 participants from a randomized clinical effectiveness trial with complete clinical and DNA resequencing data (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_{ACT}<.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

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nausea. As nausea is the most common reason for discontinuance of varenicline, further pharmacogenetic investigations are warranted.

Keywords

varenicline; nausea; smoking cessation; adherence

Introduction

Varenicline tartrate (Chantix®, Pfizer) was developed as a partial agonist at the α4β2 nicotinic acetylcholine receptor (nAChR)[1, 2] and was approved by the FDA for smoking cessation in May 2006 following a series of Phase 3 randomized clinical trials (RCTs). 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 act as a partial and full agonist at α3β4 and α7 nAChRs, respectively, and as a partial agonist at α3β2 and α6-containing receptors although with lower efficacy[4].

The most common adverse drug reaction (ADR) reported by people taking varenicline is nausea and its occurrence is dose-related. In an analysis of RCTs, 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, 5]. In an ongoing analysis of adverse events within a cohort of more than 2500 patients prescribed varenicline in a nonclinical trial setting in the UK[6], nausea/vomiting was the most frequent suspected ADR among the 51% of patients reporting an ADR 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 ADR by varenicline users, a significantly higher rate than for placebo (29.5% vs. 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% vs. 4.3%). nAChRs play 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)[8, 9]. While we are unaware of any published studies of genetic variation in relation to varenicline-related nausea, recent evidence suggests a role for variation in the β_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 FDA-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, nonadherence, and point-prevalent smoking at 12 weeks); and, d) association analyses of common and rare variants of the *CHRNA2-7* and *CHRNB1-4* nAChR subunit genes with nausea severity at 21 days subsequent to the use of varenicline.

Methods

Population

Current smokers (≥10 garettes per day over the past year, N=1,202) were recruited from members of Group Health (GH), 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 Inc.).

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 one week prior to the quit date. Telephone follow-up interviews were conducted by non-intervention study staff at 21 days, 12 weeks, and six months after the target quit date (TQD). All recruitment, consent, screening and data collection methods were reviewed and approved by the Institutional Review Boards of SRI International (SRI), Group Health (GH), and Free & Clear (F&C).

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 (FTND)[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 due to 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 seven days. Quit outcomes did not differ based on modality (phone, Web, 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, while 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 (PNAT) consortium (http://www.pharmgkb.org/contributors/pgrn/pnat_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[13]. In that study, a recently developed (454) and a traditional (Sanger) method of resequencing[20, 21] were utilized 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] ≥ 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 Yi be the nausea severity for the i-th individual, agei be the person's age, age_sqi be the square of age, genderi be an indicator for male gender, SNPi be either an indicator for a dominant genotype or a variable taking values of 0, 1, or 2 for an additive model, and ei be an independent normally distributed error term. The following model was fit:

$$(1)\ Y_i = b_0 + b_1 \times age_i + b_2 \times age\_sqi + b_3 \times gender_i + b_4 \times SNP_i + e_i$$

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

$$(2)\ Y_i = b_0 + b_1 \times age_i + b_2 \times age\_sqi + b_3 \times gender_i + e_i$$

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

$$(3)\ Z_i = Y_i - b^*_0 - b^*_1 \times age_i - b^*_2 \times age\_sq_i - b^*_3 \times gender_i$$

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 FTND 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_{ACT})[22] and via permutation testing.

For rare variants, gene-based association tests were performed by the cohort allelic sum test (CAST) and by the weighted sum statistic (WSS)[23]. CAST 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 WSS 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 (MDMR) was also employed 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 MDMR 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 (LD) values D′ and r^2 were calculated for three common *CHRNB2* SNPs from the COMPASS sequence data using Haploview[28]. For the nAChR subunit genes that are clustered in the genome (*CHRNB3* and *CHRNA6* at chr8p11, and *CHRNA5*, *CHRNA3* and *CHRNB4* at chr15q25.1), CAST, WSS and MDMR 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 to 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 nonsmoking 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 (±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 (OR=1.24, 95% CI: 1.08-1.42; P=0.002), with increased likelihood of stopping due to side effects at 12 weeks (OR=1.58, 95% CI: 1.34-1.86; P<0.001), and of having smoked (7-day point prevalence smoking) at the 12-week follow-up (OR=1.20, 95% CI: 1.05-1.37; P=0.008).

Pretreatment correlates of nausea at 21 days

Age, gender, years of formal schooling, FTND 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 *vs.* 1.1, $t_{(302)}$=−5.16, P<0.0001), while 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) while 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 FTND score (r=0.00, P=0.991), and years of formal schooling (r=0.09, P=0.062) 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

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 *CHRNB2* (rs2072660, β=−0.428; rs2072661, β=−0.443; rs4292956, β=−0.542) and *CHRNB1* (rs2302764, β=0.337). Permutation analysis resulted in nearly identical significance values. The three *CHRNB2* variants are found within the *CHRNB2* 3′ untranslated region within 224 basepairs of each other. D' 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 *CHRNB2* variants and the 21 day nausea severity rating: rs2072660 ($P_{ACT,\ Additive}$=0.013, $P_{ACT,\ Dominant}$=0.019); rs2072661 ($P_{ACT,\ Additive}$=0.021; $P_{ACT,\ Dominant}$=0.016); and, rs4292956 ($P_{ACT,\ Additive}$=0.120; $P_{ACT,\ Dominant}$=0.045).

Individuals with one or two copies of the minor alleles of these *CHRNB2* SNPs exhibited the following unit decreases in 21-day mean nausea severity relative to those without the minor allele: rs2072661, 0.44 (mean [SD] = 1.81 [1.53] *vs*. 1.37 [1.51]; P=0.004); rs2072660, 0.43 (1.80 [1.54] *vs*.1.37 [1.50]; P=0.006); and, rs4292956, 0.54 (1.69 [1.55] *vs*.1.15 [1.33]; P=0.021).

No significant associations between rare variation in *CHRNB2* and the 21 day nausea severity rating score were observed from either the CAST (P>0.07) or WSS (P>0.06) tests (Table 3). Significant associations between common and rare variants combined and 21 day nausea severity were identified at *CHRNB2* by both the allele sharing (P=0.02) and

weighted allele sharing (*P*=0.01) MDMR tests (Table 4). Subsequent *post hoc* testing revealed that this association was due to the effects of common variants only (both tests, *P*=0.02).

Discussion

The present analysis identified common variants in *CHRNB2* 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 *CHRNB2* minor alleles ranges from 6.6% to 24.5% in this 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[29] samples JPT, CEU, CHB and YRI, respectively, suggesting that approximately 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 and colleagues reported a relation between one of the *CHRNB2* SNPs examined here (rs2072660) and feelings of dizziness or nausea (tobacco sensitivity) shortly after smoking initiation in 1068 young adults aged 17-21 years[10]. The direction of the association noted by Ehringer *et al* 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 *CHRNB2* promoter and 3′UTR variants, Ehringer *et al*[30] assessed 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. While Ehringer *et al* did not observe association of *CHRNB2* SNPs with dizziness in the COGEND sample, Hoft *et al* report association of a *CHRNB2* promoter variant (rs2072659) 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* 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[11]. Another investigation showed the major allele of rs2072660 to be associated with an increased number of days of abstinence following treatment with nicotine patch[32]. 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 *CHRNB2* 3′UTR variants and nicotine dependence[34-37] or smoking behaviors[38, 39]. The rare variant analyses at *CHRNB2* identified *P* values ranging from 0.06 to 0.44. Thus, the possible contribution from rare variants at *CHRNB2* to 21 day nausea severity requires further study, e.g., resequencing of additional samples and/or *in silico* assessment of rare variant function.

Possible mechanisms

While 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 (CTA) paradigms may be one potential model to study the aversive effects of drugs at high doses. Studies involving wild type and *CHRNB2* knockout mice revealed that while nicotine produced CTA in both genotypes, the magnitude of the effect was less in the mutant mice, thereby implicating the *CHRNB2* 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[8] which comprise the nAChRs upon which varenicline exerts action.[40]

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.[40] It is possible that varenicline results in nausea as a consequence of its agonist effects on presynaptic α4 and α6-containing receptors involved in the regulation of dopamine release in the striatum[42]. Although nausea and emesis have been observed in Parkinson's patients taking dopaminergic agonists[43], 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 α4β2 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 α4β2 agonists as a drug class and that the *CHRNB2* subunit, in particular, could partner with other alpha subunits to form a functional receptor that influences autonomic ganglia. Because nicotine has a high affinity for α4β2 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 prior to the onset of taking varenicline to personalize therapy. Those with *CHRNB2* minor alleles could receive the standard course of treatment with the usual rate of titration to the full dose (1mg bid). Those with *CHRNB2* 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 neurokinin receptor 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 utilized 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. While 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. Since 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 *CHRNB2* 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 *CHRNB2* 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[49]. This could be examined in a clinical trial arm that involves behavioral counseling paired with placebo medication. While the occurrence of nausea is much lower for other smoking cessation medications such as nicotine replacement therapy and bupropion (approximately 10% of users[50, 51]), 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 *CHRNB2* 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.

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References

1. Coe JW, Brooks PR, Vetelino MG, Wirtz MC, Arnold EP, Huang J, et al. Varenicline: an alpha4beta2 nicotinic receptor partial agonist for smoking cessation. J Med Chem. 2005; 48(10): 3474–3477. [PubMed: 15887955]
2. Rollema H, Chambers LK, Coe JW, Glowa J, Hurst RS, Lebel LA, et al. Pharmacological profile of the alpha4beta2 nicotinic acetylcholine receptor partial agonist varenicline, an effective smoking cessation aid. Neuropharmacology. 2007; 52(3):985–994. [PubMed: 17157884]
3. Cahill K, Stead LF, Lancaster T. Nicotine receptor partial agonists for smoking cessation. Cochrane Database Syst Rev. 2008; (3):CD006103. [PubMed: 18646137]
4. Mihalak KB, Carroll FI, Luetje CW. Varenicline is a partial agonist at alpha4beta2 and a full agonist at alpha7 neuronal nicotinic receptors. Mol Pharmacol. 2006; 70(3):801–805. [PubMed: 16766716]
5. Cahill K, Stead L, Lancaster T. A preliminary benefit-risk assessment of varenicline in smoking cessation. Drug Saf. 2009; 32(2):119–135. [PubMed: 19236119]
6. Kasliwal R, Wilton LV, Shakir SA. Safety and drug utilization profile of varenicline as used in general practice in England: interim results from a prescription-event monitoring study. Drug Saf. 2009; 32(6):499–507. [PubMed: 19459717]
7. Rigotti NA, Pipe AL, Benowitz NL, Arteaga C, Garza D, Tonstad S. Efficacy and safety of varenicline for smoking cessation in patients with cardiovascular disease: a randomized trial. Circulation. 2010; 121(2):221–229. [PubMed: 20048210]
8. Mandl P, Kiss JP. Role of presynaptic nicotinic acetylcholine receptors in the regulation of gastrointestinal motility. Brain Res Bull. 2007; 72(4-6):194–200. [PubMed: 17452281]
9. Sanger GJ, Andrews PL. Treatment of nausea and vomiting: gaps in our knowledge. Auton Neurosci. 2006; 129(1-2):3–16. [PubMed: 16934536]
10. Ehringer MA, Clegg HV, Collins AC, Corley RP, Crowley T, Hewitt JK, et al. Association of the neuronal nicotinic receptor beta2 subunit gene (CHRNB2) with subjective responses to alcohol and nicotine. Am J Med Genet B Neuropsychiatr Genet. 2007; 144B(5):596–604. [PubMed: 17226798]

11. Conti DV, Lee W, Li D, Liu J, Van Den Berg D, Thomas PD, et al. Nicotinic acetylcholine receptor beta2 subunit gene implicated in a systems-based candidate gene study of smoking cessation. Hum Mol Genet. 2008; 17(18):2834–2848. [PubMed: 18593715]
12. Halperin AC, McAfee TA, Jack LM, Catz SL, McClure JB, Deprey TM, et al. Impact of symptoms experienced by varenicline users on tobacco treatment in a real world setting. J Subst Abuse Treat. 2009; 36(4):428–434. [PubMed: 19004600]
13. Wessel J, McDonald SM, Hinds DA, Stokowski RP, Javitz HS, Kennemer M, et al. Resequencing of Nicotinic Acetylcholine Receptor Genes and Association of Common and Rare Variants with the Fagerstrom Test for Nicotine Dependence. Neuropsychopharmacology. 2010
14. McClure JB, Swan GE, Catz SL, Jack L, Javitz H, McAfee T, et al. Smoking outcome by psychiatric history after behavioral and varenicline treatment. J Subst Abuse Treat. 2010; 38(4): 394–402. [PubMed: 20363092]
15. McClure JB, Swan GE, Jack L, Catz SL, Zbikowski SM, McAfee TA, et al. Mood, side-effects and smoking outcomes among persons with and without probable lifetime depression taking varenicline. J Gen Intern Med. 2009; 24(5):563–569. [PubMed: 19238488]
16. Swan GE, McClure JB, Jack LM, Zbikowski SM, Javitz HS, Catz SL, et al. Behavioral counseling and varenicline treatment for smoking cessation. Am J Prev Med. 2010; 38(5):482–490. [PubMed: 20409497]
17. Fiore, MC.; Jaén, CR.; Baker, TB., et al. Clinical Practice Guideline. U.S. Department of Health and Human Services. Public Health Service: Clincial Practice Guideline; Rockville, MD: 2008. Treating Tobacco Use and Dependence: 2008 Update.
18. Heatherton TF, Kozlowski LT, Frecker RC, Fagerstrom KO. The Fagerstrom Test for Nicotine Dependence: a revision of the Fagerstrom Tolerance Questionnaire. Br J Addict. 1991; 86(9): 1119–1127. [PubMed: 1932883]
19. Nishita DM, Jack LM, McElroy M, McClure JB, Richards J, Swan GE, et al. Clinical trial participant characteristics and saliva and DNA metrics. BMC Med Res Methodol. 2009; 9:71. [PubMed: 19874586]
20. Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, et al. Genome sequencing in microfabricated high-density picolitre reactors. Nature. 2005; 437(7057):376–380. [PubMed: 16056220]
21. Sanger F, Nicklen S, Coulson AR. DNA sequencing with chain-terminating inhibitors. Proc Natl Acad Sci U S A. 1977; 74(12):5463–5467. [PubMed: 271968]
22. Conneely KN, Boehnke M. So Many Correlated Tests, So Little Time! Rapid Adjustment of P Values for Multiple Correlated Tests. American journal of human genetics. 2007; 81(6)
23. Madsen BE, Browning SR. A groupwise association test for rare mutations using a weighted sum statistic. PLoS Genet. 2009; 5(2):e1000384. [PubMed: 19214210]
24. Kryukov GV, Shpunt A, Stamatoyannopoulos JA, Sunyaev SR. Power of deep, all-exon resequencing for discovery of human trait genes. Proc Natl Acad Sci U S A. 2009; 106(10):3871–3876. [PubMed: 19202052]
25. Nievergelt CM, Libiger O, Schork NJ. Generalized analysis of molecular variance. PLoS Genet. 2007; 3(4):e51. [PubMed: 17411342]
26. Schork NJ, Wessel J, Malo N. DNA sequence-based phenotypic association analysis. Advances in genetics. 2008; 60:195–217. [PubMed: 18358322]
27. Wessel J, Schork NJ. Generalized genomic distance-based regression methodology for multilocus association analysis. American journal of human genetics. 2006; 79(5):792–806. [PubMed: 17033957]
28. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics. 2005; 21(2):263–265. [PubMed: 15297300]
29. Frazer KA, Ballinger DG, Cox DR, Hinds DA, Stuve LL, Gibbs RA, et al. A second generation human haplotype map of over 3.1 million SNPs. Nature. 2007; 449(7164):851–861. [PubMed: 17943122]
30. Ehringer MA, McQueen MB, Hoft NR, Saccone NL, Stitzel JA, Wang JC, et al. Association of CHRN genes with “dizziness” to tobacco. Am J Med Genet B Neuropsychiatr Genet. 2010; 153B(2):600–609. [PubMed: 19760673]

31. Hoft NR, Stitzel JA, Hutchison KE, Ehringer MA. CHRNB2 Promoter Region: Association with subjective effects to nicotine and gene expression differences. Genes Brain Behav. 2010
32. Perkins KA, Lerman C, Mercincavage M, Fonte CA, Briski JL. Nicotinic acetylcholine receptor beta2 subunit (CHRNB2) gene and short-term ability to quit smoking in response to nicotine patch. Cancer Epidemiol Biomarkers Prev. 2009; 18(10):2608–2612. [PubMed: 19755656]
33. Etter JF, Hoda JC, Perroud N, Munafo M, Buresi C, Duret C, et al. Association of genes coding for the alpha-4, alpha-5, beta-2 and beta-3 subunits of nicotinic receptors with cigarette smoking and nicotine dependence. Addict Behav. 2009; 34(9):772–775. [PubMed: 19482438]
34. Feng Y, Niu T, Xing H, Xu X, Chen C, Peng S, et al. A common haplotype of the nicotine acetylcholine receptor alpha 4 subunit gene is associated with vulnerability to nicotine addiction in men. Am J Hum Genet. 2004; 75(1):112–121. [PubMed: 15154117]
35. Li MD, Beuten J, Ma JZ, Payne TJ, Lou XY, Garcia V, et al. Ethnic- and gender-specific association of the nicotinic acetylcholine receptor alpha4 subunit gene (CHRNA4) with nicotine dependence. Hum Mol Genet. 2005; 14(9):1211–1219. [PubMed: 15790597]
36. Weiss RB, Baker TB, Cannon DS, von Niederhausern A, Dunn DM, Matsunami N, et al. A candidate gene approach identifies the CHRNA5-A3-B4 region as a risk factor for age-dependent nicotine addiction. PLoS Genet. 2008; 4(7):e1000125. [PubMed: 18618000]
37. Bergen AW, Conti DV, Van Den Berg D, Lee W, Liu J, Li D, et al. Dopamine genes and nicotine dependence in treatment-seeking and community smokers. Neuropsychopharmacology. 2009; 34(10):2252–2264. [PubMed: 19494806]
38. Lueders KK, Hu S, McHugh L, Myakishev MV, Sirota LA, Hamer DH. Genetic and functional analysis of single nucleotide polymorphisms in the beta2-neuronal nicotinic acetylcholine receptor gene (CHRNB2). Nicotine Tob Res. 2002; 4(1):115–125. [PubMed: 11906688]
39. Silverman MA, Neale MC, Sullivan PF, Harris-Kerr C, Wormley B, Sadek H, et al. Haplotypes of four novel single nucleotide polymorphisms in the nicotinic acetylcholine receptor beta2-subunit (CHRNB2) gene show no association with smoking initiation or nicotine dependence. Am J Med Genet. 2000; 96(5):646–653. [PubMed: 11054772]
40. Andrews PL, Horn CC. Signals for nausea and emesis: Implications for models of upper gastrointestinal diseases. Auton Neurosci. 2006; 125(1-2):100–115. [PubMed: 16556512]
41. Shoaib M, Gommans J, Morley A, Stolerman IP, Grailhe R, Changeux JP. The role of nicotinic receptor beta-2 subunits in nicotine discrimination and conditioned taste aversion. Neuropharmacology. 2002; 42(4):530–539. [PubMed: 11955523]
42. Salminen O, Murphy KL, McIntosh JM, Drago J, Marks MJ, Collins AC, et al. Subunit composition and pharmacology of two classes of striatal presynaptic nicotinic acetylcholine receptors mediating dopamine release in mice. Mol Pharmacol. 2004; 65(6):1526–1535. [PubMed: 15155845]
43. Stowe RL, Ives NJ, Clarke C, van Hilten J, Ferreira J, Hawker RJ, et al. Dopamine agonist therapy in early Parkinson's disease. Cochrane Database Syst Rev. 2008(2):CD006564. [PubMed: 18425954]
44. Rowbotham MC, Duan WR, Thomas J, Nothaft W, Backonja MM. A randomized, double-blind, placebo-controlled trial evaluating the efficacy and safety of ABT-594 in patients with diabetic peripheral neuropathic pain. Pain. 2009; 146(3):245–252. [PubMed: 19632048]
45. DiFranza JR, Savageau JA, Fletcher K, Ockene JK, Rigotti NA, McNeill AD, et al. Recollections and repercussions of the first inhaled cigarette. Addict Behav. 2004; 29(2):261–272. [PubMed: 14732415]
46. Pomerleau CS, Pomerleau OF, Namenek RJ, Marks JL. Initial exposure to nicotine in college-age women smokers and never-smokers: a replication and extension. J Addict Dis. 1999; 18(3):13–19. [PubMed: 10507578]
47. Pomerleau OF, Pomerleau CS, Namenek RJ. Early experiences with tobacco among women smokers, ex-smokers, and never-smokers. Addiction. 1998; 93(4):595–599. [PubMed: 9684398]
48. Toll BA, McKee SA, Martin DJ, Jatlow P, O'Malley SS. Factor structure and validity of the Medication Adherence Questionnaire (MAQ) with cigarette smokers trying to quit. Nicotine Tob Res. 2007; 9(5):597–605. [PubMed: 17454716]

49. Hughes JR. Effects of abstinence from tobacco: valid symptoms and time course. Nicotine Tob Res. 2007; 9(3):315–327. [PubMed: 17365764]

50. Mills EJ, Wu P, Lockhart I, Wilson K, Ebbert JO. Adverse events associated with nicotine replacement therapy (NRT) for smoking cessation. A systematic review and meta-analysis of one hundred and twenty studies involving 177,390 individuals. Tob Induc Dis. 2010; 8:8. [PubMed: 20626883]

51. Hughes JR, Stead LF, Lancaster T. Antidepressants for smoking cessation. Cochrane Database Syst Rev. 2007; (1):CD000031. [PubMed: 17253443]

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[1] (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 due to side effects (% yes)	38.0	39.9	0.634

[1]FTND=Fagerström Test of Nicotine Dependence

Table 2

nAChR gene common variant association with nausea severity at 21 days

SNP ID	*A1*[1]	*A2*[2]	*Gene*	*Type*	*MAF*	P_{Add}[3]	P_{Dom}[4]
rs2280781	C	T	*CHRNB2*	5′ UTR	0.101	0.359	0.278
rs4845378	G	T	*CHRNB2*	intron	0.097	0.259	0.259
rs2072660	**T**	**C**	***CHRNB2***	**3′ UTR**	**0.244**	**0.004**	**0.006**
rs2072661	**G**	**A**	***CHRNB2***	**3′ UTR**	**0.245**	**0.006**	**0.005**
rs4292956	**C**	**T**	***CHRNB2***	**3′ UTR**	**0.066**	0.056	**0.021**
rs2472553	G	A	*CHRNA2*	non-syn	0.139	0.465	0.791
rs13277254	G	A	*CHRNB3*	up	0.234	0.889	0.837
rs13280301	A	G	*CHRNB3*	up	0.178	0.948	0.786
rs13277524	G	T	*CHRNB3*	up	0.234	0.889	0.837
rs6474413	C	T	*CHRNB3*	up	0.232	0.986	0.896
rs4950	G	A	*CHRNB3*	5′ UTR	0.236	0.901	0.855
rs2304297	G	C	*CHRNA6*	3′ UTR	0.244	0.288	0.281
rs71653603	C	T	*CHRNA7*	syn	0.060	0.878	0.878
rs569207	C	T	*CHRNA5*	intron	0.196	0.466	0.542
rs16969968	G	A	*CHRNA5*	non-syn	0.367	0.790	0.698
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*	syn	0.367	0.802	0.675
rs3743075	T	C	*CHRNA3*	syn	0.378	0.981	0.533
rs3743074	G	A	*CHRNA3*	intron	0.378	0.969	0.587
rs8040868	T	C	*CHRNA3*	syn	0.429	0.376	0.491
rs8192475	C	T	*CHRNA3*	non-syn	0.050	0.362	0.362
rs12914008	G	A	*CHRNB4*	non-syn	0.051	0.208	0.208

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SNP ID	*A1*[1]	*A2*[2]	*Gene*	*Type*	*MAF*	P_{Add}[3]	P_{Dom}[4]
rs3813567	G	A	*CHRNB4*	up	0.157	0.781	0.824
rs2302765	T	C	*CHRNB1*	intron	0.159	0.182	0.142
rs12452047	A	G	*CHRNB1*	intron	0.166	0.235	0.172
rs7210231	C	A	*CHRNB1*	intron	0.199	0.268	0.242
rs2302761	C	T	*CHRNB1*	intron	0.202	0.192	0.183
rs2302763	T	C	*CHRNB1*	intron	0.164	0.462	0.394
rs2302764	**T**	**C**	***CHRNB1***	**3′ UTR**	**0.160**	0.062	**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*	syn	0.460	0.331	0.369
rs1044396	G	A	*CHRNA4*	syn	0.458	0.180	0.177
rs2229960	A	G	*CHRNA4*	syn	0.059	0.627	0.681
rs2229959	C	A	*CHRNA4*	syn	0.122	0.702	0.858
rs1044394	A	G	*CHRNA4*	syn	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*	syn	0.065	0.358	0.444

[1] A1=allele 1;

[2] A2=allele 2;

[3] P_{Add}= *P* of additive model;

[4] P_{Dom}= *P* of dominant model.

Table 3

nAChR gene rare variant association with nausea severity at 21 days

Gene	$CAST_\beta$[1]	P_1[2]	P_2[3]	$CAST_\beta$[4]	P_1	P_2	WSS_β[5]	P_1	P_2
CHRNB2	0.85	0.07	0.09	0.85	0.07	0.07	0.0040	0.07	0.06
CHRNA2	−0.73	0.12	0.11	−0.73	0.12	0.09	−0.0004	0.62	0.56
CHRNB3	−0.90	0.25	0.29	−0.90	0.25	0.21	−0.0040	0.25	0.27
CHRNA6	0.09	0.93	0.93	0.09	0.93	0.93	0.0004	0.93	0.95
chr8p11[6]	−0.57	0.37	0.37	−0.57	0.37	0.37	−0.0025	0.37	0.34
CHRNA7	−0.30	0.78	0.77	−0.30	0.78	0.80	−0.0013	0.79	0.79
CHRNA5	−1.02	0.19	0.20	−0.44	0.19	0.22	−0.0003	0.68	0.74
CHRNA3	0.26	0.81	0.88	0.26	0.81	0.80	0.0010	0.35	0.38
CHRNB4	−0.11	0.81	0.76	−0.27	0.36	0.37	0.0002	0.87	0.83
chr15q25.1[7]	−0.29	0.45	0.53	−0.33	0.13	0.15	−0.0003	0.67	0.69
CHRNB1	−0.99	0.20	0.23	−0.99	0.20	0.17	−0.0003	0.71	0.62
CHRNA4	−0.63	0.32	0.45	−0.63	0.32	0.34	−0.0002	0.84	0.95

[1] Cohort Allelic Sum Test, $CAST_\beta$, MAF < 1%;

[2] P_1 = P-value from standard F-test;

[3] P_2 = P-value from permutation testing;

[4] $CAST_\beta$, MAF < 5%;

[5] Weighted Sum Statistic, WSS_β

[6] Analysis of *CHRNB3* and *CHRNA6* variants together;

[7] Analysis of *CHRNA5*, *CHRNA3* and *CHRNB4* variants together.

Table 4

nAChR gene common and rare variant association with nausea severity at 21 days

		MDMR[1] Allele Sharing			MDMR Weighted Allele Sharing		
Gene	**N SNPs**	**pseudo-F**	**P**	**% variation**	**pseudo-F**	**P**	**% variation**
CHRNB2	24	4.70	**0.02**	0.012	5.58	**0.01**	0.014
CHRNB2[2]	5	5.30	**0.01**	0.013	5.55	**0.01**	0.014
CHRNB2[3]	19	1.21	0.42	0.003	0.56	0.44	0.001
CHRNA2	11	0.32	0.68	0.008	1.34	0.26	0.003
CHRNB3	12	0.16	0.77	0.004	−0.01	0.92	0.000
CHRNA6	3	1.00	0.32	0.003	−0.90	0.56	−0.002
chr8p11[4]	15	0.16	0.78	0.004	0.15	0.74	0.004
CHRNA7	4	−0.22	0.97	−0.006	−26.88	0.92	−0.073
CHRNA5	15	0.12	0.82	0.003	0.11	0.74	0.003
CHRNA3	33	−0.06	0.95	−0.001	0.22	0.71	0.006
CHRNB4	15	0.72	0.52	0.002	0.16	0.78	0.004
chr15q25.1[5]	63	0.08	0.90	0.002	0.07	0.83	0.002
CHRNB1	25	1.81	0.18	0.005	2.24	0.11	0.006
CHRNA4	31	0.69	0.53	0.002	20.67	0.18	0.050

[1] Multivariate distance-based matrix regression (MDMR);

[2] Post-hoc MDMR test performed with common variants only;

[3] Post-hoc MDMR test performed with rare variants only;

[4] Analysis of *CHRNB3* and *CHRNA6* variants together;

[5] Analysis of *CHRNA5*, *CHRNA3* and *CHRNB4* variants together.