

Title

A genome-wide association study of nausea incidence in varenicline-treated cigarette smokers

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Abstract (250/250 words)

Introduction: Varenicline is the most efficacious smoking cessation treatment, however long-term cessation rates tend to be <25%. Nausea, the most common side effect of varenicline, observed in ~28% of individuals treated, peaks early following treatment initiation and reduces cessation success. Genetic variation influences treatment response, however genetic contributors to individual differences in side effects are less understood. **Methods:** We conducted a genome-wide association study of nausea incidence at one week following the initiation of varenicline treatment (corresponding to the target quit date) in 189 cigarette smokers of European ancestry (NCT01314001). Additive genetic models examining the likelihood of experiencing any versus no nausea controlled for population substructure, age, and sex. Variants with minor allele frequencies (MAF)≥10% were considered. **Results:** Fifty-seven (30.2%) out of 189 participants reported nausea. The top variant associated with nausea was rs1568209 (OR=2.61 for A vs. G allele; 95% CI=1.65,4.15; P=2.1e-7; MAF=48.7%), mapping to the *SLCO3A1* drug transporter gene on chromosome 15. In the same trial, rs1568209 was not associated with nausea in either the nicotine patch (P=0.56; n=181) or placebo (P=0.59; n=174) arms. In varenicline-treated smokers, the incidence of nausea was higher in females (44.6%; n=74) versus males (20.9%; n=115) (P=0.001), however there was no evidence of a difference in the influence of rs1568209 on nausea between the sexes (P for sex*genotype interaction=0.36). Future studies in larger samples are required to test the robustness of this finding. **Conclusions:** Variation in *SLCO3A1* may influence the risk for developing nausea in varenicline-treated smokers, which may alter adherence and cessation.

Implications: Varenicline-associated nausea reduces adherence and limits cessation success. Previous candidate gene association studies showed genetic factors influence nausea on varenicline. This pilot genome-wide investigation of nausea, the most common side effect associated with varenicline treatment and an importance cause of treatment discontinuation, suggests the potential involvement of common variation in the *SLCO3A1* drug transporter gene.

Introduction

Cigarette smoking remains a leading preventable cause of morbidity and mortality (1). Nicotine is the principal psychoactive compound in cigarette smoke, mediating its reinforcing effects through neuronal $\alpha_4\beta_2$ nicotinic receptors (2). Three FDA-approved medications are available to treat nicotine dependence: nicotine replacement therapy, bupropion, and varenicline (1). Varenicline, which acts as a partial agonist at $\alpha_4\beta_2$ nicotinic receptors, is the newest and most efficacious smoking cessation aid (1, 3). In head-to-head clinical trials, long-term quit rates at 6-months are ~24% with varenicline treatment, compared to ~17% and ~19% for bupropion and nicotine replacement therapy, respectively (1, 3).

Nausea is the most common side effect associated with varenicline treatment (4). Varenicline-associated nausea is dose-related and peaks early following the initiation of treatment (3). To help reduce the incidence and severity of side effects, varenicline dose is titrated upwards during the first week of treatment, from 0.5 mg once/day to 0.5 mg twice/day to 1 mg twice/day (3, 4). Even with dose titration, ~28% of smokers receiving varenicline treatment in clinical trial settings report nausea (3), and nausea is a commonly cited reason for discontinuation of treatment (5). We have shown that in varenicline-treated smokers, nausea is indirectly associated with lower quit rates, in a relationship that is mediated by reduced varenicline adherence (6). A greater understanding of the factors that increase the risk for varenicline-associated nausea will enable improved treatment approaches that increase medication adherence and promote cessation.

Twin studies have shown that cigarette smoking behaviours including initiation, quantity, nicotine dependence, and cessation are highly heritable (7). Smoking cessation outcomes on pharmacotherapy are influenced by a variety of genetic factors (reviewed in (8)). Genetic variation also influences treatment side effects; for example, prior candidate gene studies revealed significant associations between variants in nicotinic receptor subunit genes (e.g. *CHRNA4* and *CHRNA5*) and nausea severity on varenicline (9, 10). Variation in additional targets, including *SLC22A2*, which codes

for the major drug transporter (i.e. OCT2) for varenicline, was also associated with nausea (10). To expand beyond these candidate gene investigations, we performed a pilot genome-wide association study (GWAS) of nausea incidence at one week following the initiation of varenicline treatment (corresponding to the target quit date) in European ancestry smokers. We focused on nausea at one week following treatment initiation as nausea incidence peaks early and then tapers over time (3).

Methods

Study Participants

Participants were 1246 adult smokers (≥ 10 cigarettes/day) from the PNAT2 clinical trial (full details are available elsewhere (11)) which comprised three treatment arms: varenicline ($n=420$), nicotine patch ($n=418$), and placebo ($n=408$). Participants were randomized to treatment based on their nicotine metabolite ratio (NMR), a biomarker of CYP2A6 activity and nicotine clearance (12), which was measured from blood or saliva samples collected at intake (**Figure S1**). In the varenicline arm, the dose of varenicline was titrated upwards from 0.5 mg once/day (days 1-3) to 0.5 mg twice/day (days 4-7) to 1 mg twice/day (days 8-84) (11). Participants were recruited from four clinical sites: the Centre for Addiction and Mental Health (CAMH), University of Pennsylvania, MD Anderson, and the State University of New York (SUNY) Buffalo. Trial procedures were approved by IRBs at all clinical sites and at the University of Toronto (11, 13).

Genome-Wide Association Study

Genome-wide genotyping was conducted at the Centre for Applied Genomics (SickKids Hospital, Toronto, Canada) using the Illumina HumanOmniExpressExome-8 v1.2 array and a custom add-on as previously described (14). Standard GWAS quality control procedures were performed on the whole PNAT2 dataset as described elsewhere (14). Individuals of European ancestry were identified genetically using HapMap 3 data and multidimensional scaling analysis (14). Following GWAS quality control procedures, imputation was performed using the Michigan Imputation Server (15) using ShapeIT v2.r790 and the HRC version r1.1 reference panel. The observed and expected P-values for variants with info (i.e. quality) scores ≥ 0.50 and minor allele frequencies (MAF) $\geq 1\%$ are shown in a quantile-quantile

plot in **Figure S2**. Due to the relatively small sample size, we focused on the results for common variants with $MAF \geq 10\%$.

Nausea was measured using a side effect questionnaire. Participants were directed to “rate the following symptoms according to how you have been feeling for the last week. Please select one response: none, mild, moderate, or severe”. Frequentist additive logistic regression models assessed the likelihood of experiencing any nausea (mild, moderate, or severe; coded as 1) versus no nausea (coded as 0) after the first 7 days of varenicline treatment (week one, **Figure S1**). The autosomes were analyzed using SNPTEST version 2.5.2 and the “method –expected” option was specified to control for genotype uncertainty (14). In addition to the first two principal components (to adjust for population substructure), age and sex were included as covariates due to their association with smoking cessation (16). Of the 204 European ancestry individuals in the varenicline arm of the PNAT2 trial, $n=189$ (92.6%) completed the nausea assessment at week one (i.e. target quit date) and were included in the GWAS.

Sensitivity Analyses

Sensitivity analyses examining the influence of rs1568209 on week one nausea were completed in varenicline-treated smokers, using frequentist additive logistic regression models (**Figure S1**). Because the NMR was used to randomize participants to treatment (11), sensitivity analysis #1 controlled for the NMR assessed at intake (in addition to principal components 1 and 2, sex, and age). Because body size can influence varenicline clearance (17), sensitivity analysis #2 controlled for body mass index (BMI) assessed at intake (in addition to principal components 1 and 2, sex, and age). Sensitivity analysis #3 controlled for nausea at week zero (in addition to principal components 1 and 2, sex, and age). Week zero nausea level was coded as 1 = any nausea and 0 = no nausea, and was assessed prior to the initiation of varenicline treatment. Sensitivity analysis #4 analyzed treatment adherent participants and controlled

for principal components 1 and 2, sex, and age. Participants were considered adherent if they had a detectable salivary varenicline concentration (i.e. varenicline ≥ 1 ng/ml (18)) two weeks following the initiation of varenicline treatment (i.e. a week after the target quit date). Varenicline levels were measured in saliva samples using LC-MS/MS (details available elsewhere (6, 18)). The sensitivity analyses were conducted in SNPTEST version 2.5.2 and the “method –expected” option was specified to control for genotype uncertainty.

Sex-Based Analysis of the Top Variant in the GWAS (rs1568209)

In varenicline-treated smokers, a Chi-square test compared the incidence of nausea at week one in males and females. A logistic regression analysis that included main effects of sex and rs1568209 genotype (coded additively) and a sex*rs1568209 interaction term was used to examine potential sex differences in the influence of rs1568209 on nausea. The model controlled for principal components 1 and 2 and age. Analyses were conducted in SPSS version 27 (IBM, Armonk, New York, USA).

Analysis of the Top Variant in the GWAS (rs1568209) in the Nicotine Patch and Placebo Arms

Logistic regression modeling was used to examine the influence of rs1568209 (coded additively: GG=0, GA=1, AA=2) on nausea incidence (coded as 1 = any nausea, 0 = no nausea) in European ancestry smokers from the nicotine patch and placebo arms. In the nicotine patch arm, the nausea data used were measured at week 2, after one week of patch treatment (**Figure S1**). In the placebo arm, the nausea data used were measured at week 1, after one week of placebo treatment (**Figure S1**). Data were available on n=181 and n=174 individuals in the nicotine patch and placebo arms, respectively. Models controlled for principal components 1 and 2, sex, and age. Analyses were conducted in SPSS version 27 (IBM, Armonk, New York, USA).

Results

Participants (n=189; 39% female) were aged 46 years (SD=13) on average and smoked a mean of 19 (SD=6) cigarettes per day at baseline. Fifty-seven (30.2%) reported nausea (n=51 mild, n=6 moderate, n=0 severe). GWAS results for the top 20 variants with MAF \geq 10% are found in **Table S1**. The top variant was rs1568209 (OR=2.61 for A vs. G allele, 95% CI 1.65, 4.15; P=2.1e-7) (**Figure 1**), located in intron 2 of the *SLCO3A1* gene encoding the organic anion transporting polypeptide 3A1 (OATP3A1). The rs1568209 variant was directly genotyped by the array. The frequency of the rs1568209 A risk allele was 51.3%, consistent with its frequency in the European sub-population of 1000 Genomes. In contrast to varenicline-treated smokers, rs1568209 was not associated with nausea in either the nicotine patch (P=0.56) or placebo (P=0.59) arms (**Figure 2**); further controlling for the NMR did not change these results (the P-values were 0.56 and 0.77, respectively).

In sensitivity analyses, controlling for baseline NMR, nausea level at week zero, or BMI did not alter the relationship between rs1568209 and nausea on varenicline: the OR (95% CI) remained 2.61 (1.65, 4.15), and the P-values were 2.1e-7 (original analysis), 2.1e-7 (controlling for the NMR), 3.2e-7 (controlling for BMI), and 2.8e-7 (controlling for nausea at week zero). In comparing the total group (n=189) to the subset of varenicline adherent participants (n=151; 79.9% of participants), the effect size did not change substantially: the OR (95% CI; P-value) went from 2.61 (1.65, 4.15; P=2.1e-7) to 2.73 (1.61, 4.63; P=2.9e-6).

In the varenicline arm, more females (44.6%) than males (20.9%) reported nausea at week one (P=0.001) (**Figure S3**). There was no significant interaction between sex and rs1568209 on nausea (P=0.36), suggesting the association between rs1568209 and nausea is similar in males and females (**Figure S3**).

Discussion

Nausea is the most common side effect associated with varenicline treatment, peaks early following treatment initiation, and is a commonly cited reason for treatment discontinuation (3, 5). This pilot GWAS of nausea at one week following the initiation of varenicline treatment revealed a top variant (i.e. rs1568209) mapping to intron 2 of *SLCO3A1* on chromosome 15. Although more females experienced nausea overall, consistent with previous reports (5, 9), the influence of rs1568209 on varenicline-associated nausea was similar in males and females. Post-hoc power analyses showed >80% power to detect main effects of sex and rs1568209 variation on nausea on varenicline. In contrast to the varenicline arm, rs1568209 was not associated with nausea in the nicotine patch or placebo arms.

The *SLCO3A1* gene encodes OATP3A1, a sodium-independent transporter of organic anions including prostaglandins, thyroxine, and vasopressin (19). Varenicline, an organic cation and basic compound, is thought to be transported by OCT2 (SLC22A2) (10). The rs1569209 variant is in high linkage disequilibrium (LD) with other intronic variants in *SLCO3A1* but is not associated with altered gene expression in GTEx (<https://gtexportal.org> (20)). There is one missense variant in the region, rs1517618 (MAF=17%), and it is in low-moderate LD with rs1568209 ($D'=0.52$, $r^2=0.06$ in European populations). The biological mechanism linking *SLCO3A1* variation to varenicline-associated nausea remains to be determined. However, some members of the OATP family may be able to transport basic drugs (21); if varenicline can be transported by OATP3A1, it may lead to altered varenicline concentration and risk for nausea.

In the GWAS Catalog (<https://www.ebi.ac.uk/gwas/>; accessed 24 September 2020), other variants in *SLCO3A1* have been associated with several traits including BMI, lung function, cognitive ability, and Alzheimer's disease. Variation in *SLCO3A1* has also been associated with nicotine dependence: rs7163369 was the top SNP ($P=3.3e-6$) found in a meta-GWAS of nicotine dependence (22).

Previous candidate gene association studies evaluated nausea on varenicline in European ancestry smokers (9, 10). In a sequencing study of 10 nicotinic acetylcholine receptor subunit genes, three variants found in the 3'-untranslated region (UTR) of *CHRNA2* (located on chromosome 1) were significantly associated with nausea severity (9). A separate study highlighted variants in several genes including *SLC22A2* (coding for the major varenicline transporter, OCT2; located on chromosome 6) and *CHRNA5* (located on chromosome 15) (10). The top variant (rs1568209) from our GWAS in Europeans (n=189) is also on chromosome 15, approximately 13.7 MB away from *CHRNA5*. In contrast to Europeans, rs1568209 (risk A allele frequency=14% in Africans from 1000 Genomes) was not associated with nausea in varenicline-treated PNAT2 participants of genetically African ancestry (n=135) (P=0.72), who experienced a similarly high overall incidence of nausea (29.6% versus 30.2% in Europeans). However, there was support for the *SLCO3A1* (chr 15) and *MIR552* (chr 1) regions (highlighted in **Table S1** for Europeans) being associated with nausea in African Americans: the minimum P-values in the regions were 0.00021 (~99 kb 3' of *SLCO3A1*) and 0.0013 (~131kb 3' of *MIR552*). Future studies examining genetic influences on varenicline-associated nausea in individuals of non-European ancestry may confirm these regions and reveal novel loci.

Strengths of our study include the genome-wide analysis, assessment of nausea at one week following treatment initiation, and the sensitivity analysis in participants with biologically-confirmed varenicline adherence. A limitation of our analysis is the relatively small sample size. Future studies are required to test the robustness of the association between rs1568209 and nausea on varenicline and the persistence of genetic effects throughout the treatment period.

In summary, this pilot GWAS of nausea incidence in varenicline-treated smokers yielded a top variant in the *SLCO3A1* drug transporter gene. The influence of rs1568209 on nausea incidence was not apparent in the nicotine patch or placebo treatment arms. Although varenicline is the most efficacious smoking cessation pharmacotherapy, early nausea is a common side effect which reduces varenicline

adherence and subsequent quit rates (6). If the association between rs1568209 and nausea proves robust, smokers with the rs1568209 A risk allele may benefit from a longer titration schedule, lower dose, adjuvant antiemetic treatment, or an alternative smoking cessation aid as part of a tailored smoking cessation approach to increase medication adherence and optimize quit success.

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Declaration of Interests

R. F. Tyndale has consulted for Quinn Emanuel and Ethismos. The other authors declare no conflicts of interest. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Author Contributions

MJC, JK, and RFT were responsible for the study concept and the analysis plan. CL and RFT designed and oversaw the original study. MJC performed data analysis. All authors contributed to the interpretation of the findings. MJC and RFT wrote the paper and revised it after critical review by CL and JK. All authors reviewed and approved the manuscript.

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Figures Legends

Figure 1. The top variant in the GWAS of week one nausea on varenicline was rs1568209 which is located in intron 2 of *SLCO3A1* on chromosome 15. Linkage disequilibrium patterns (r^2 values) are based upon the hg19/1000 Genomes November 2014 release European reference population. The P-value is from the GWAS after adjusting for principal components 1 and 2, sex, and age. This Locus Zoom plot includes genotyped and imputed variants, with minor allele frequencies $\geq 1\%$ and imputation info scores ≥ 0.50 .

Figure 2. The top SNP (rs1568209) from the nausea GWAS in the varenicline arm was not associated with nausea in either the nicotine patch or placebo arms among European ancestry smokers. The P-value in varenicline-treated smokers, assessed at week 1 (one week after varenicline was initiated) is from the GWAS and controls for principal components 1 and 2, sex, and age. The P-values in smokers treated with nicotine patch (assessed at week 2, one week after nicotine patch was initiated) and placebo (assessed at week 1, one week after placebo was initiated) are from additive logistic regression models adjusting for principal components 1 and 2, sex, and age. Further controlling for the nicotine metabolite ratio did not substantially alter the findings in any of the three treatment arms. In the GWAS in the varenicline arm, the imputed genotypes of all 189 participants with nausea data were analyzed. When extracting specific rs1568209 genotype data from these 189 participants, one individual had an unclear genotype and was thus excluded from this figure.

Supplementary Information Titles

Figure S1. Timeline of PNAT2 clinical trial interventions and assessments. The nicotine metabolite ratio was ascertained from blood or saliva samples collected at intake (when participants were smoking as usual). Nausea was assessed at three time-points: week 0, week 1, and week 2. In the varenicline arm, the GWAS was performed on nausea assessed at week 1 (i.e. one week following the initiation of varenicline treatment). The top SNP in the GWAS of nausea on varenicline at week 1 was rs1568209. In the placebo arm, the top SNP-based analysis of nausea was performed at week 1 (one week after the initiation of placebo treatment). In the nicotine patch arm, the top SNP-based analysis of nausea was performed at week 2 (one week after the initiation of patch treatment). At week 2, varenicline concentrations were measured from saliva samples to determine adherence to varenicline treatment. Week 12 corresponded to the end-of-treatment phase for the trial.

Figure S2. Quantile-Quantile plot depicting the expected P-value against the observed P-value in the GWAS of nausea incidence at one week. This QQ plot includes genotyped and imputed variants, with minor allele frequencies $\geq 1\%$ and imputation info scores ≥ 0.50 .

Figure S3. Relationships between sex, rs1568209 genotype, and nausea on varenicline at week one. In varenicline-treated smokers from PNAT2, there was a higher incidence of nausea at one week in females compared to males **(A)**. There was no interaction between sex and genotype on the likelihood of experiencing nausea at one week (P value for sex*rs1568209 genotype interaction = 0.36) **(B)**. The logistic regression model in **(B)** included main effects of sex (OR=9.66, 95% CI=1.91, 48.8; P=0.006) and rs1568209 genotype (coded additively; OR=9.63, 95% CI=1.46, 63.6; P=0.019), a sex*rs1568209 interaction term (OR=0.58, 95% CI=0.18, 1.85; P=0.36), and controlled for principal components 1 and 2 and age.

Table S1. Results for the GWAS of nausea incidence at week one in varenicline-treated smokers.