

# Pharmacogenetic Association of the Galanin Receptor (*GALR1*) SNP rs2717162 with Smoking Cessation

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Galanin modulates dopaminergic neurotransmission in the mesolimbic dopamine system, thereby influencing the rewarding effects of nicotine. Variants in the galanin receptor 1 (*GALR1*) gene have been associated with retrospective craving severity and heaviness of smoking in prior research. We investigated pharmacogenetic associations of the previously studied *GALR1* polymorphism, rs2717162, in 1217 smokers of European ancestry who participated in one of three pharmacogenetic smoking cessation clinical trials and were treated with nicotine patch ( $n=623$ ), nicotine nasal spray ( $n=189$ ), bupropion ( $n=213$ ), or placebo ( $n=192$ ). The primary endpoint was abstinence (7-day point prevalence, biochemically confirmed) at the end of treatment. Cravings to smoke were assessed on the target quit day (TQD). The longitudinal regression model revealed a significant genotype by treatment interaction ( $P=0.03$ ). There was a reduced odds of quitting success with the presence of at least one minor (C) allele in the bupropion-treated group (OR = 0.43; 95% CI = 0.22–0.77;  $P=0.005$ ) but equivalent quit rates by genotype in the nicotine-replacement therapy groups. This genotype by treatment interaction was reproduced in a Cox regression model of time to relapse ( $P=0.04$ ). In the bupropion trial, smokers carrying the C allele also reported more severe TQD cravings. Further research to identify functional variants in *GALR1* and to replicate pharmacogenetic associations is warranted.

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## INTRODUCTION

The neuropeptide galanin is distributed throughout the brain (Melander *et al*, 1988; Takatsu *et al*, 2001) and has been implicated in learning and memory (Crawley, 2008; Rustay *et al*, 2005), feeding behavior (Crawley, 1999), and drug dependence (Picciotto *et al*, 2010). Galanin modulates mesolimbic dopaminergic neurotransmission (Robinson and Brewer, 2008; Tsuda *et al*, 1998), and there is evidence that the inhibition of midbrain DA activity by galanin may be mediated, in part, via the galanin receptor 1 (*GALR1*) receptor subtype (Counts *et al*, 2002). Galanin, acting through its receptor subtypes, has been shown in rodent models to modulate reward and withdrawal responses from drugs of abuse (Hawes *et al*, 2008; Zachariou *et al*, 1999). Of relevance to nicotine dependence, mice lacking the galanin peptide display decreased sensitivity to nicotine reward (Neugebauer *et al*, 2011). Further, galanin, mediated via

*GALR1*, blocks nicotine reward and reverses physical nicotine withdrawal signs (Jackson *et al*, 2011).

Translating these preclinical observations, two recent studies have explored the role of variation in the *GALR1* gene in nicotine-dependent phenotypes. In European- and African-American smokers, multiple single-nucleotide polymorphism (SNP) markers in the *GALR1* gene were associated with self-reported heavy smoking (Jackson *et al*, 2011). In addition, an analysis of treatment-seeking smokers identified a highly significant association of a single *GALR1* SNP, rs2717162, with retrospective reports of tobacco craving during a previous quit attempt (Lori *et al*, 2011).

Extending these observations, we examined the association of rs2717162 in *GALR1* to prospectively assessed smoking cessation and tobacco craving in 1217 smokers of European ancestry who participated in one of three pharmacogenetic smoking cessation clinical trials: (1) a bupropion placebo-controlled randomized trial ( $n=405$ ) (Lerman *et al*, 2006); (2) an open-label trial of nicotine patch ( $n=441$ ) (Schnoll *et al*, 2010); and (3) a randomized open-label trial of nicotine patch vs nicotine nasal spray ( $n=371$ ) (Lerman *et al*, 2006; Lerman *et al*, 2004). Data were pooled across trials to test for the presence of a genotype by treatment interaction on quitting success,

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time to relapse, and subjective craving on the first day of abstinence.

## SUBJECTS AND METHODS

### Participants and Procedures by Clinical Trial

#### *Bupropion trial*

**Sample.** Treatment-seeking smokers responding to media advertisements were screened for eligibility from April 1999 to October 2001 at Georgetown University (Washington, DC) and SUNY Buffalo (New York). Inclusion criteria were: ages 18–65 and a smoking rate of  $\geq 10$  cigarettes a day for the prior 12 months. Exclusion criteria included a history of DSM-IV axis I psychiatric disorder, seizure disorder, current use of psychotropic medications, and pregnancy or lactation. In the full cohort of smokers, there were 555 trial participants, including 436 of self-reported European ancestry. Of these smokers, DNA was available for 428, and *GALR1* genotyping was completed for 405 subjects (failed SNP assays and DNA samples with low call rates were removed from the data set after confirming replicate concordance). Prior analyses of ancestry informative markers in this sample revealed no evidence for population stratification (Lerman *et al*, 2006). Of the 405 eligible participants, 55% were female, 46% were college graduates, and the average age was 44.4 (SD = 11.6). On average, participants smoked 22 cigarettes per day (SD = 9.4). The mean score on the Fagerström Test for Nicotine Dependence (FTND) was 5.1 (SD = 2.1).

**Procedures.** The study was approved by the University of Pennsylvania and SUNY Buffalo institutional review boards. Participants at both sites received identical assessments of demographics, smoking rate, and nicotine dependence assessed with the FTND (Heatherton *et al*, 1991) and provided a blood sample for genotyping. All participants received 10 weeks of either bupropion or matched placebo, initiated 2 weeks prior to the target quit date following a pre-quit counseling session. Bupropion was administered according to the guidelines (150 mg/day  $\times$  3 days, followed by 300 mg/day). Participants received seven sessions of standardized behavioral group counseling during the medication phase. Self-reported smoking was assessed using the time-line follow-back procedure (Brown *et al*, 1998). Consistent with prior pharmacogenetic trials (Lerman *et al*, 2006; Ray *et al*, 2010), the primary outcome was biochemically verified (saliva cotinine  $\leq 15$  ng/ml) 7-day point-prevalence abstinence at the end of treatment (EOT) and 6-months post target quit date. As per convention (SRNT, 2002), participants who reported smoking within 7 days before the assessment, failed to provide a cotinine sample, or provided a cotinine sample  $> 15$  ng/ml were considered non-abstinent.

#### *Nicotine patch trial*

**Sample.** Treatment-seeking smokers were screened at the University of Pennsylvania (Philadelphia, PA) from 2004 to 2008, using methods and inclusion criteria similar to those described above. In the full cohort of 568 trial participants, there were 478 smokers of self-reported European ancestry. Of these, DNA was available for 451 and *GALR1* genotyping was completed for 441 subjects (failed SNP assays and DNA

samples with low call rates were removed from the data set after confirming replicate concordance). Prior analyses of ancestry informative markers in this sample revealed no evidence for population stratification (Ray *et al*, 2010). Of the final 441 participants, 42% were female, 35% were college graduates, and the average age was 44.9 (SD = 10.4). On average, participants smoked 22 cigarettes per day (SD = 9.0). The mean score on the FTND was 5.3 (SD = 2.2). Thus, this sample was highly comparable to the bupropion trial sample.

**Procedures.** The study protocol was approved by the University of Pennsylvania institutional review board. Participants completed pretreatment assessments identical to those described above for the bupropion trial. After a pre-quit counseling session, transdermal nicotine-patch therapy was initiated on the target quit date and continued for 8 weeks; all participants received three sessions of behavioral counseling during the treatment period (Schnoll *et al*, 2010). The primary outcome was 7-day point-prevalence abstinence at the end of 8-week treatment. The only difference in outcome assessment is that self-reported smoking for the 7 days prior to the EOT survey was biochemically verified using exhaled breath carbon monoxide (CO) (cotinine verification was not used because the treatment contained nicotine). The primary outcome was biochemically confirmed 7-day point-prevalence abstinence at the EOT. As per convention (SRNT, 2002), participants who reported smoking within 7 days before the assessment, failed to provide a CO, or provided a CO  $> 10$  p.p.m were considered non-abstinent.

#### *Nicotine patch vs nicotine spray trial*

**Sample.** Treatment-seeking smokers were recruited at Georgetown University and the University of Pennsylvania (Philadelphia, PA), from February 2000 to August 2003, using methods identical to those described above. In the full cohort of 600 trial participants, there were 397 smokers of self-reported European ancestry. Of these, DNA was available and *GALR1* genotyping was completed for 371 participants (nicotine patch,  $n = 182$ ; nicotine nasal spray,  $n = 189$ ) (failed SNP assays and DNA samples with low call rates were removed from the data set after confirming replicate concordance). Prior analyses of ancestry informative markers in this sample revealed no evidence for population stratification (Lerman *et al*, 2006). Of the 371 participants, 47% were female, 52% were college graduates, and the average age was 46.3 (SD = 11.5). On average, participants smoked 23 cigarettes per day (SD = 9.3). The mean score on the FTND was 5.5 (SD = 2.2).

**Procedures.** The institutional review boards from both universities approved the research protocols. The procedures and outcome assessments were identical to those described above for the nicotine-patch trial, except that participants were randomized to nicotine patch or nicotine nasal spray, and 7 sessions of behavioral group counseling were delivered during the 8-week treatment period. As per convention (SRNT, 2002), participants who reported smoking within 7 days before the assessment, failed to provide a CO, or provided a CO  $> 10$  p.p.m were considered non-abstinent.

### Genotyping

Genotyping for the *GALR1* SNP rs2717162 was completed using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA). PCR was performed with 2.25 ng of DNA, 2.5  $\mu$ l of ABI Taqman Universal Mastermix, 0.125  $\mu$ l of water, and 0.125  $\mu$ l of 40X Assay by Design SNP Assay for the *GALR1* variant (ABI, Foster City, CA). The 5- $\mu$ l reactions were performed in a 384-well plate (ABI). The plates were thermal cycled using the same conditions described previously (Lerman *et al*, 2006). The plates were scanned utilizing the Allelic Discrimination End-Point Analysis on the ABI Prism 7900HT Sequence Detection System. The allelic discrimination data were analyzed by the AutoCall algorithm of the SDS v2.1 Software (ABI).

### Craving assessment

In all the three trials, craving was assessed as the sum of two items from a withdrawal symptom checklist, validated in previous research (Killen and Fortmann, 1997; Wang *et al*, 2007). On a Likert scale (0 = not present, 1 = mild, 2 = moderate, and 3 = severe), participants rated cravings for cigarettes and urges to smoke. For this analysis, we focused on cravings reported on the target quit date in biochemically verified abstainers at the target quit day (TQD) visit.

### Statistical analysis

Our outcomes of interest were point-prevalence abstinence (binary) at 8 weeks and 6 months, time to first smoking lapse (continuous), and craving (continuous) at TQD. Preliminary analyses of bivariate associations of outcomes with genotype were conducted using contingency tables ( $\chi^2$ ), *t*-tests, or one-way ANOVA as appropriate. To estimate pharmacogenetic associations, data were pooled across trials; there were no significant differences in genotype, demographic, or smoking history variables by treatment arm. The primary endpoint, point-prevalence abstinence, was analyzed by longitudinal logistic regression, estimated using generalized estimating equation methods. The model included the *GALR1* rs2717162 genotype (dichotomized, TT = 0, C\* = 1), treatment group, time point, and time  $\times$  treatment interactions. We also included sex and nicotine dependence level as controlling variables and tested treatment  $\times$  genotype interactions for inclusion. We analyzed time to first cigarette (censored at week 8) using Cox regression. Finally, we analyzed the effect of genotype on quit-day craving (urge to smoke) using multiple linear regression, adjusted for sex, nicotine dependence score, genotype, and treatment. All analyses were conducted using STATA (Stata Corporation, College Station, TX). With respect to statistical power, the 410 subjects in the bupropion trial yielded 80% power to detect a 13% difference in quit rates by genotype. The detectable difference was 18% for nicotine spray and 10% for the combined patch trials.

## RESULTS

### Descriptive Data

Descriptive data below are presented by treatment arm consistent with the pooled analysis estimating genotype effects across treatments.

**Bupropion.** For the rs2717162 SNP, 135 (63.4%) participants were homozygous for the T allele (TT), 68 (31.9%) were heterozygous (CT), and 10 (4.7%) were homozygous for the C allele (CC). The rs2717162 genotypes were in Hardy–Weinberg equilibrium ( $P = 0.71$ ). There were no significant differences in demographics, FTND, cigarettes per day, or treatment group assignment between genotype groups ( $P > 0.10$ ).

**Placebo.** For the rs2717162 SNP, 104 (54.2%) participants were homozygous for the T allele (TT), 76 (39.6%) were heterozygous (CT), and 12 (6.2%) were homozygous for the C allele (CC). The rs2717162 genotypes were in Hardy–Weinberg equilibrium ( $P = 0.70$ ). There were no significant differences in demographics, FTND, cigarettes per day, or treatment group assignment between genotype groups ( $P > 0.10$ ).

**Nicotine-patch treatment.** For the rs2717162 SNP, 366 (58.7%) participants were homozygous for the T allele (TT), 226 (36.3%) were heterozygous (CT), and 31 (5.0%) were homozygous for the C allele (CC). The rs2717162 genotypes were in Hardy–Weinberg equilibrium ( $P = 0.61$ ). There were no significant differences in demographics, FTND, or cigarettes per day between genotype groups ( $P > 0.10$ ).

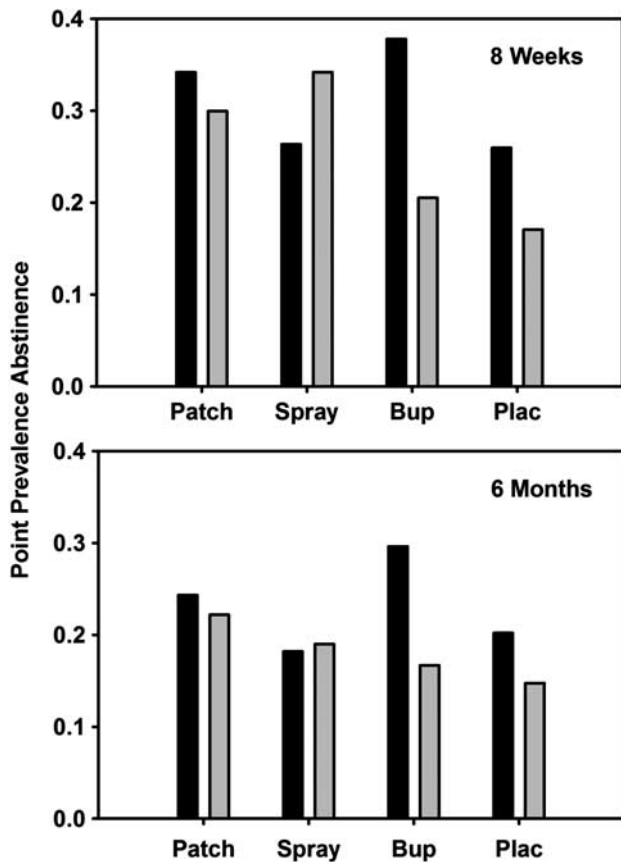
**Nicotine-spray treatment.** For the rs2717162 SNP, 110 (58.2%) participants were homozygous for the T allele (TT), 66 (34.9%) were heterozygous (CT), and 13 (6.9%) were homozygous for the C allele (CC). The rs2717162 genotypes were in Hardy–Weinberg equilibrium ( $P = 0.48$ ). There were no significant differences in demographics, FTND, or cigarettes per day between genotype groups ( $P > 0.10$ ).

### Abstinence

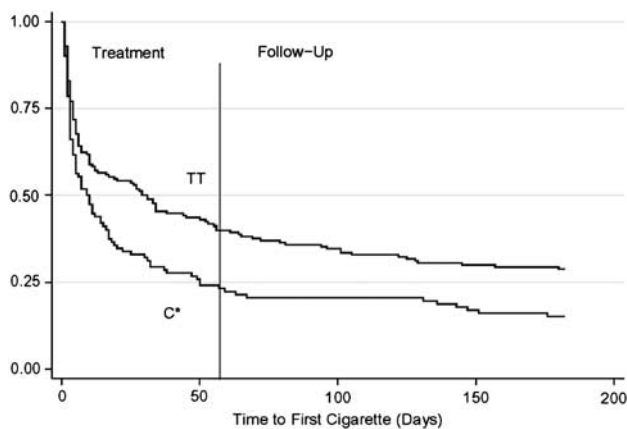
At the end of 8 weeks, 367 (30.1%) participants were abstinent. At the 6-month follow-up, 268 (22.0%) participants were abstinent. Abstinence rates by genotype and treatment are shown in Figure 1. The results of our longitudinal regression model of abstinence at EOT and 6-month follow-up revealed a significant genotype by treatment interaction ( $P = 0.03$ ); there was a reduced odds of quitting success with the presence of at least one minor (C) allele in the bupropion-treated group only (OR = 0.43; 95% CI = 0.22–0.77;  $P = 0.005$ ). There was no interaction of genotype by time point (EOT vs 6 M). Likewise, time to self-reported continuous abstinence failure using Cox regression revealed a significant genotype by treatment interaction ( $P = 0.04$ ) and an association of the C allele with faster time to smoking relapse in the bupropion-treated group (HZ = 2.33; 95% CI = 1.30–4.55;  $P = 0.005$ ) (Figure 2).

### Craving

In the bupropion trial, where significant associations of the C allele with relapse were observed, craving scores of abstainers on TQD ( $n = 264$ , saliva cotinine  $< 15$  ng/ml) were higher in the C-allele group (M = 5.78; SD = 1.40) compared with the TT group (M = 5.32; SD = 1.43;  $F(1, 262) = 6.85$ ;  $P = 0.009$ ). Results of the linear regression model of TQD craving, controlling for effect of treatment, revealed a significant genotype association for each copy of the risk allele ( $t(267) = 3.35$ ;  $P = 0.001$ ). Craving predicted reduced likelihood of abstinence (OR = 0.78; 95% CI = 0.67–0.92;  $P = 0.002$ ). However, inclusion of craving in the model did not have a meaningful effect on the genotype association with



**Figure 1** Biochemically verified 7-day point-prevalence abstinence rates at 8 weeks and 6 months by genotype and treatment arm. There was a significant genotype by treatment interaction ( $P=0.03$ ). Among participants in the bupropion trial, those carrying a minor (C) allele (gray bars) had significantly lower quit rates at the EOT compared with TT homozygotes (black bars), independent of treatment arm assignment (OR = 0.43; 95% CI = 0.22–0.77;  $P=0.005$ ). Among C allele carriers, quit rates differed in *post-hoc* testing ( $\chi^2=9.32$ ;  $P=0.025$ ).



**Figure 2** (Bupropion treatment) Relapse hazard for time to first cigarette. Subjects carrying at least one C allele relapsed significantly more quickly from TQD to EOT ( $P=0.005$ ), reported as time to first cigarette.

abstinence (OR changed from 0.52 to 0.56), suggesting that genotype associations are not mediated by craving. There were no significant genotype associations with craving in the nicotine-replacement therapy trials (all  $P$ -values  $>0.10$ ).

## DISCUSSION

This study examined the rs2717162 SNP in the *GALR1* gene for associations with smoking cessation and treatment response in a pooled analysis of 1217 smokers of European ancestry participating in one of three pharmacogenetic clinical trial cohorts. The presence of a genotype by treatment interaction indicated significant heterogeneity in genotype associations with abstinence in the different treatment arms. There was a reduced odds of quitting success and a faster rate of relapse with the presence of at least one minor (C) allele in the bupropion-treated group but not in the placebo, nicotine patch, or nicotine nasal spray groups.

Consistent with the observed pharmacogenetic association, among smokers treated with bupropion, the presence of at least one minor (C) allele was also associated with more severe cravings on the target quit date. In a recent study (Lori *et al*, 2011), the rs2717162 SNP was associated with self-reported cravings to smoke as experienced during a prior quit attempt. Although study participants were treatment-seeking smokers, the craving assessment was performed at baseline and was retrospective. Individuals carrying the T allele had higher craving scores than those carrying the minor C allele, which is the opposite of what we observed for target quit-date cravings in the bupropion trial. A second study (Jackson *et al*, 2011) used a recombinant inbred strain mouse model and human genetic approach to investigate the role of *GALR1* in nicotine dependence. In the mouse model, somatic signs of withdrawal were correlated with *GALR1* expression in the nucleus accumbens. In the human smoker cohorts, two *GALR1* variants were associated with heavy smoking, based on the reported number of cigarettes smoked per day. Data available from HAPMAP (hapmap.org) show that one of the significant markers, rs2717164, is in linkage disequilibrium (LD) with rs2717162. However, we did not find an association of this SNP with heavy smoking in our cohorts (results not shown).

The divergence of the findings for cravings in the current trial and prior studies are likely due to differences in study design and phenotype assessment. The current study assessed craving and smoking cessation prospectively because retrospective reports of prior quit attempts can be subject to bias (Shiffman *et al*, 2008). Further, the current pharmacogenetic analysis indicates that genotype associations are modified by smoking-cessation treatment. In addition, the SNPs examined by the prior study and our study are not known to be functional, thus, associations may be due to a rare functional variant in LD with rs2717162 (Bodmer and Bonilla, 2008). A more complete annotation of SNPs in the *GALR1* gene could be generated through re-sequencing and functional studies to identify potential causative variants associated with smoking-related phenotypes.

Although the mechanisms underlying the observed associations of *GALR1* variation with craving and abstinence are largely unknown, there is a biological rationale to support a potential contribution of *GALR1* to these phenotypes. Galanin and *GALR1* are found in cortico-limbic regions (Holmes *et al*, 2003) and galanin receptors are highly expressed on neuronal terminals in striatum and nucleus accumbens (Hawes *et al*, 2008). These regions and

circuits are important in abstinence-induced cravings (Wang *et al*, 2007). In addition, galanin is co-expressed with acetylcholine in the paraventricular nucleus (PVN), and galanin injection into the PVN can cause release of dopamine and inhibit acetylcholine release in the nucleus accumbens (Rada *et al*, 1998). Acetylcholine acts on nAChRs located on glutamate and GABA neuronal terminals in the VTA, which project to the nucleus accumbens, amygdala, and prefrontal cortex (Kalivas and Volkow, 2005). Thus, galanin may exert its effects on dopamine release through the cholinergic system (Ericson and Ahlenius, 1999; Picciotto, 2010).

The observed pharmacogenetic effect is also biologically plausible, but alternate interpretations are speculative at this time. Although the results indicate that the genotype association is significant in the bupropion arm only, visual inspection of Figure 1 reveals a similar trend for the placebo arm; in both arms, C-allele carriers have higher relapse rates. Thus, it is less likely that the results reflect an enhanced response to bupropion in smokers homozygous for the T allele. Another possibility is that there is a liability to relapse in the C allele carriers that is not modified by bupropion (or placebo) but is reversed by nicotine-replacement therapy. Indeed, inspection of Figure 1 shows higher quit rates in the C allele carriers treated with nicotine patch or nicotine spray relative to placebo or bupropion. Although nicotine treatment (nicotine patch or spray) has agonist effects at the nAChRs, bupropion therapy has antagonist effects (Slemmer *et al*, 2000). An agonist at nAChRs may complement the effects of *GALR1* on dopamine-mediated neurotransmission but an antagonist blocks the effects of nicotine and may not offset the involvement of *GALR1*.

There are limitations of this study that should be considered. Although the study sample of 1217 participants is larger than most prior pharmacogenomic analyses and was amply powered to detect genotype by treatment effects, the number of individuals homozygous for the minor allele was relatively small; thus, we dichotomized the genotype variable. Importantly, the functional consequences of rs2717162 are unknown and it is likely that the associations observed in this study and prior studies are due to other SNPs in the *GALR1* gene. We selected only one SNP based on the strong genetic association with retrospective craving observed previously (Lori *et al*, 2011).

Preclinical studies that alter the expression and activity of *GALR1* and analyze a broader array of dependence-associated behavioral phenotypes in rodents would be useful to increase our understanding of the role of galanin and the *GALR1* in smoking cessation. Re-sequencing of *GALR1* would be valuable to identify the functional variants that may be contributing to craving and abstinence. If further investigations confirm the association of *GALR1* with nicotine dependence and smoking cessation, this could lead to the development of novel therapeutics to aid in smoking cessation.

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## DISCLOSURE

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