Nicotinic acetylcholine receptor variation and response to smoking cessation therapies

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Objective **To evaluate the association of nicotinic acetylcholine receptor (nAChR) single nucleotide polymorphism (SNP) with 7-day point prevalence abstinence (abstinence) in randomized clinical trials of smoking cessation therapies in individuals grouped by pharmacotherapy randomization to inform the development of personalized smoking cessation therapy.**

Materials and methods **We quantified association of four SNPs at three nAChRs with abstinence in eight randomized clinical trials. Participants were 2633 outpatient treatment-seeking, self-identified European ancestry individuals smoking at least 10 cigarettes/day, recruited through advertisement, prescribed pharmacotherapy, and provided with behavioral therapy. Interventions included nicotine replacement therapy (NRT), bupropion, varenicline, placebo (PLA), or combined NRT and bupropion, and five modes of group and individual behavioral therapy. Outcome measures tested in multivariate logistic regression were end of treatment and 6 month (6MO) abstinence, with demographic, behavioral, and genetic covariates.**

Results **'Risk' alleles previously associated with smoking heaviness were significantly ($P<0.05$) associated with reduced abstinence in the PLA pharmacotherapy group (PG) at 6MO [for rs588765, odds ratio (95% confidence interval) 0.41 (0.17–0.99)], and at end of treatment and at 6MO [for rs1051730, 0.42 (0.19–0.93) and 0.31 (0.12–0.80)], and with increased abstinence in the NRT PG at 6MO [for rs588765, 2.07 (1.11–3.87) and for rs1051730, 2.54 (1.29–4.99)]. We observed significant heterogeneity in rs1051730 effects ($F=2.48$, $P=0.021$) between PGs.**

Conclusion **chr15q25.1 nAChR SNP risk alleles for smoking heaviness significantly increase relapse with PLA treatment and significantly increase abstinence with NRT. These SNP–PG associations require replication in independent samples for validation, and testing in larger sample sizes to evaluate whether similar effects occur in other PGs.** ***Pharmacogenetics and Genomics*** **23:94–103** ©

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Introduction

Tobacco use is the largest preventable cause of death in the USA [1] and worldwide [2]. Most smokers wish to stop, and both behavioral counseling and pharmacotherapies increase abstinence rates two-to-three fold compared with placebo (PLA) abstinence rates in randomized clinical trials (RCTs), though there are differences in the effectiveness of the therapy [3]. Yet, the majority of smokers are not able to quit long-term with either behavioral therapy and/or pharmacotherapy. Thus, there is a critical need to enhance the effectiveness of smoking cessation treatments. One approach to improve cessation rates would be to identify factors that indicate which individuals will be benefited the most from which treatment and to develop algorithms to incorporate these factors into clinical practice. These factors could include sex, nicotine dependence, comorbidity, the rate of nicotine metabolism, pharmacogenetic variation, or combinations of factors [4–11].

Evidence that reveals interactions between smoker characteristics, medications, and cessation success suggests that effective algorithms to assign medication may be possible. For example, there is evidence that the rate of nicotine metabolism predicts which smokers will be more successful at quitting with bupropion (BUP) [12] and with transdermal nicotine replacement therapy (NRT) [8,13], and that more highly dependent smokers

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benefit more from combination pharmacotherapies than do less dependent smokers [14]. Despite such findings, at present, no algorithm for the assignment of smoking cessation medication has been demonstrated to be useful in clinical practice, and none is widely used. More research is needed on this topic. Nicotinic acetylcholine receptor (nAChR) locus single nucleotide polymorphisms (SNPs) have been found to be related with measures of nicotine dependence [15–42], response to tobacco [43–45], and smoking cessation [23,46–52] and, therefore, may prove useful in optimizing assignment of smoking cessation pharmacotherapies. We choose four nAChR SNPs among many possible nAChR SNPs with a-priori evidence for an association with nicotine dependence, with response to nicotine or with smoking cessation. We choose these four based on substantial and repeated a-priori evidence of association with nicotine dependence and with abstinence (below). The a-priori associations represented by these four nAChR SNPs are the only association signals investigated across the eight RCTs to date.

rs2072661, in the 3′ untranslated region of *CHRNB2* at chr1q21.3, has been associated with abstinence in RCT randomizing participants to BUP or PLA; initial response to tobacco in adolescent samples; short-term abstinence in a crossover smoking cessation trial of NRT and PLA; baseline Fagerström test for nicotine dependence (FTND) score among treatment-seeking smokers; and nausea among treatment-seeking smokers randomized to behavioral therapies and prescribed varenicline (VAR) [35,43,46,53,54]. Candidate gene, genome-wide association studies, and meta-analytic studies with a nicotine dependence phenotype have identified three different loci represented by SNPs rs1051730, rs578776, and rs588765 at chr15q25.1 in *CHRNA5* and *CHRNA3* [30]. rs1051730 and correlated SNPs have been associated with nicotine dependence and lung cancer [18–20,22,55], abstinence [23,50], and smoking likelihood during pregnancy [48]. rs578776 and correlated SNPs have been associated with nicotine dependence [18,22,27,30] and abstinence [49]. rs588765 and correlated SNPs have been associated with nicotine dependence [27,30] and with abstinence [51]. Recent research using a single RCT has demonstrated that individuals with chr15q25.1 risk haplotypes [22,23] exhibit statistically significantly reduced abstinence when randomized to PLA versus no effect on abstinence when randomized to active pharmacotherapy [52], encouraging further exploration of chr15q25.1 associations with response to multiple pharmacotherapies and cessation outcomes in treatment-seeking smokers.

The Pharmacogenetics of Nicotine Addiction Treatment (PNAT) consortium was formed in 2005 to identify the role of pharmacokinetic and pharmacodynamic gene variation on nicotine dependence and metabolism phenotypes, with a focus on smoking cessation and medication response, and to generate the evidence base to optimize the use of pharmacotherapies for smoking cessation. In this analysis, we carried out analyses of the association of nAChR candidate gene variation with abstinence at the end of treatment (EOT) and at 6 month (6MO) after the quit date in 2633 treatment-seeking smokers enrolled in eight RCTs of smoking cessation. We carried out analyses by pharmacotherapy group (PG), including predictor SNP regression, sensitivity, mediation, and receiver–operator curve analyses. We carried out these analyses to address the following questions: (a) are any of the four nAChR SNPs of a-priori interest significantly associated with abstinence in smokers grouped by pharmacotherapy, and (b) how do the results help our understanding of the pharmacogenetic mechanisms that operate in smoking cessation?

This research uses the largest combined sample and the most comprehensive group of smoking cessation pharmacotherapies to be submitted to pharmacogenetic analyses. In our analyses, we have adjusted for trial randomization arm, participant demographics, nicotine dependence measures, and genetic covariates. This study refines previous pharmacogenetic smoking cessation associations at four nAChR SNPs of current interest, identifies novel associations of two nAChR loci on smoking cessation outcomes in individuals randomized to NRT, and identifies at least two mechanisms by which a nAChR SNP may influence abstinence. The significant SNP–PG association results require testing in independent RCT arms to validate the specific PG associated effects. Additional testing in larger numbers of RCTs arms, and using multiple treatment meta-analysis techniques, may establish whether there are specific SNP associations with PGs not identified in this analysis.

Materials and methods

Participants

Informed written consent was obtained by the investigators of each RCT, and approval was obtained from the appropriate institutional review boards [56–62].

Data sources, study selection, and phenotype data extraction

We utilized data from eight RCTs with participant clinical outcome and genetic data [56–62] [Table 1 and Supplemental digital contents 1–4 (*http://links.lww.com/FPC/A554*): RCT design characteristics; behavioral and demographic variables selected for analysis; inclusion and exclusion criteria for eight RCTs; pharmacotherapy and behavioral therapy to EOT and 6MO of eight RCTs by randomization arm]. The individuals included in the analysis represented 44% of individuals randomized to treatment in the eight RCTs, and 81% of individuals for whom we had received RCT data and biospecimens

Table 1 RCT participant characteristics, pharmacotherapy, EOT, and 6MO abstinence

RCT	3A	3B	5	6A	6B	9A	9B	9C
Investigator	Lerman	Lerman	Swan	Hall	Hall	Baker	Baker	Baker
N[a]	378	416	487	150	174	173	171	684
Age (years) [mean (SD)]	46.7 (11.4)	44.4 (11.5)	49.1 (11.5)	41.8 (9.6)	57.3 (5.9)	37.7 (11.2)	41.3 (10.8)	44.4 (11.8)
BMI [mean (SD)]	27.5 (5.5)	26.5 (4.7)	27.8 (5.8)	26.5 (4.7)	26.5 (5.9)	26.6 (5.7)	26.7 (5.5)	28.8 (6.7)
College (%)	51.9	46.1	25.5	51.7	58.6	19.7	18.2	22.8
Female (%)	46.8	54.6	68.8	38.0	41.4	53.8	58.5	60.0
Married (%)	49.2	47.3	69.0	24.7	28.7	44.8	48.0	47.2
FTND [mean (SD)]	5.55 (2.2)	5.17 (2.1)	5.15 (2.1)	4.82 (2.1)	4.87 (2.1)	5.13 (2.4)	5.76 (2.1)	5.21 (2.2)
CPD [mean (SD)]	23.7 (9.2)	21.8 (9.4)	20.2 (8.3)	19.1 (7.4)	20.8 (8.8)	21.5 (8.3)	24.1 (9.7)	21.5 (8.8)
Pharmacotherapy[b]	NRT	BUP, PLA	VAR	NRT + BUP	NRT + BUP	BUP, PLA	BUP, PLA	NRT, BUP, PLA
Randomization arms	2	2	3	5	4	4	2	4
EOT ABS	0.325	0.272	0.554	0.642	0.672	0.272	0.216	0.418
6MO ABS	0.198	0.219	0.431	0.460	0.626	0.145	0.205	0.317

BUP, bupropion; CPD, cigarettes per day; EOT ABS, end of treatment abstinence; FTND, Fagerström test for nicotine dependence; 6MO ABS, 6 month abstinence; NRT, nicotine replacement therapy; NRT + BUP, combined NRT and BUP; PLA, placebo; RCT, randomized clinical trials; VAR, varenicline.
[a]*N* of self-identified White participants with DNA.
[b]NRT, BUP, PLA, VAR, NRT + BUP.

or DNA samples. Reasons for exclusion include: (a) a biospecimen was not collected [1595 (27.0%)]; (b) did not self-identify as White [1168 (19.7%)]; (c) were randomized to pharmacotherapy arms not selected for this analysis [490 (8.3%)]; (d) did not enter treatment after randomization [188 (3.2%)]; (e) DNA sample genotype completion rate was below a predetermined threshold [70 (1.2%)]; and/or (f) chromosomal sex did not match clinical sex [22 (0.4%)].

Genotyping and genotype data extraction

Genomic DNA was extracted from saliva [63], whole blood, or buffy coat, quantified and normalized to 50 ng/μl, and genotyped at the University of Southern California Epigenome Center and at the University of California San Francisco Institute for Human Genetics Genomics Core Facility. We extracted SNP genotype data from custom 1536 SNP Illumina GoldenGate panels (Illumina, San Diego, California, USA) interrogating candidate genes of interest to PNAT [46,64] and imputed genotype data where necessary. All genotyping included HapMap and replicated DNA samples. We reviewed and filtered GoldenGate genotyping data as described [46] for RCTs 3A and 3B and in a similar manner for the remaining RCTs by manual review of genotype cluster metrics, review of HapMap sample concordance, by successively filtering samples and SNPs with call rates below a defined threshold, and comparison of X chromosome heterozygosity and clinical sex. We estimated principal components of population genetic variation [65] among self-identified White participants using 45 ancestry informative markers genotyped across all individuals. Genotypes were imputed with IMPUTE v2.1.2 [66] using 1000 Genomes CEU (Utah residents with ancestry from northern and western Europe) August 2010 haplotype data at *CHRNB2* and chr15q25.1 [chr1: 154476304–154616304 and chr15: 78747906–79045112 (NCBI build 37)]. Imputed dosage was converted to genotypes with a 0.90 dosage probability cutoff using GTOOL v0.6.5. (*http://www.well.ox.ac.uk/~cfreeman/software/gwas/gtool.html*). rs2072661 and rs1051730 genotype data were extracted from GoldenGate genotyping data, and rs588765 genotype data were imputed for all RCTs. rs578776 genotype data were extracted from GoldenGate genotyping data for RCTs 3A and 3B, and imputed for the remaining RCTs. Among the expected 10 532 genotypes from four SNPs at 2633 individuals tested for association, the overall missing genotype rate was 1.3%, where 57.0% and 41.6% were extracted from GoldenGate genotyping data or imputed, respectively. A total of 97.7% of rs588765 and 98.8% of rs578776 imputed genotype dosage probabilities were within 10% of modal values. nAChR SNP minor allele frequencies did not differ significantly across the 26 arms. We evaluated rs2072661 and rs1051730 genotype distributions by randomization arm and observed two arm-by-SNP strata with Hardy–Weinberg equilibrium *P*-values of less than 0.05 versus 2.5 expected by chance [see table, Supplemental digital content 5 (*http://links.lww.com/FPC/A555*): nAChR SNPs counts and Hardy–Weinberg equilibrium *P*-value, by arm].

Logistic modeling of the effect of SNPs on EOT and 6MO abstinence

Multiple imputation by chained equations [67] was used to impute missing values 20 times for age (two individuals), education (10), marital status (seven), cigarettes per day (CPD) (seven), and FTND [68] score (42). Regression analyses were carried out on each imputed data set and the results were combined with adjustment to the variance of regression parameters to reflect the additional variance attributable to the imputations [69]. Regression analyses were run for all SNPs using an additive model (and for rs2072661, with the dominant model [35,46,53,54]), and with adjustment for the other chr15q25.1 SNPs [30], when appropriate. Regression analysis was carried out with data from all 26 arms (except for rs2072661, where we excluded the two arms from the RCT that discovered the SNP association)

and included variables for the SNPs, demographics [age (age and age^2), education (presence or absence of college degree), sex, marital status (married or other)], dependence measures [FTND and CPD (coded as in the FTND)], interactions with demographic variables (CPD × age, CPD × sex, and FTND × sex), the first 10 principal components of population genetic variation, and indicator variables for the 26 RCT arms and the PGs. These analyses were carried out as regression analyses including all 2633 individuals simultaneously, thus the number of variables is a small fraction (∼*2%*) of the number of individuals. Regression analyses assessed the homogeneity of SNP effects between PGs, and quantified effects of SNP across all PGs.

Post-hoc analyses performed and general considerations

Regression analysis of chr15q25.1 SNPs evaluated SNP effects excluding dependence covariates. Multiple mediation analyses tested whether nicotine dependence measures mediated the association of rs1051730 with 6MO abstinence, controlling for other chr15q25.1 SNPs, demographics, population genetic variation, and relevant RCT arms [70]. Receiver-operating characteristic (ROC) analyses of abstinence compared the contribution of nicotine dependence and genetic variables with a base model with demographic variables. Statistical analyses were carried out using STATA 12.0 (StataCorp, College Station, Texas, USA). Power analyses were carried out using Quanto [71]. α-value for all tests was 0.05.

Results and discussion

Variation between RCTs

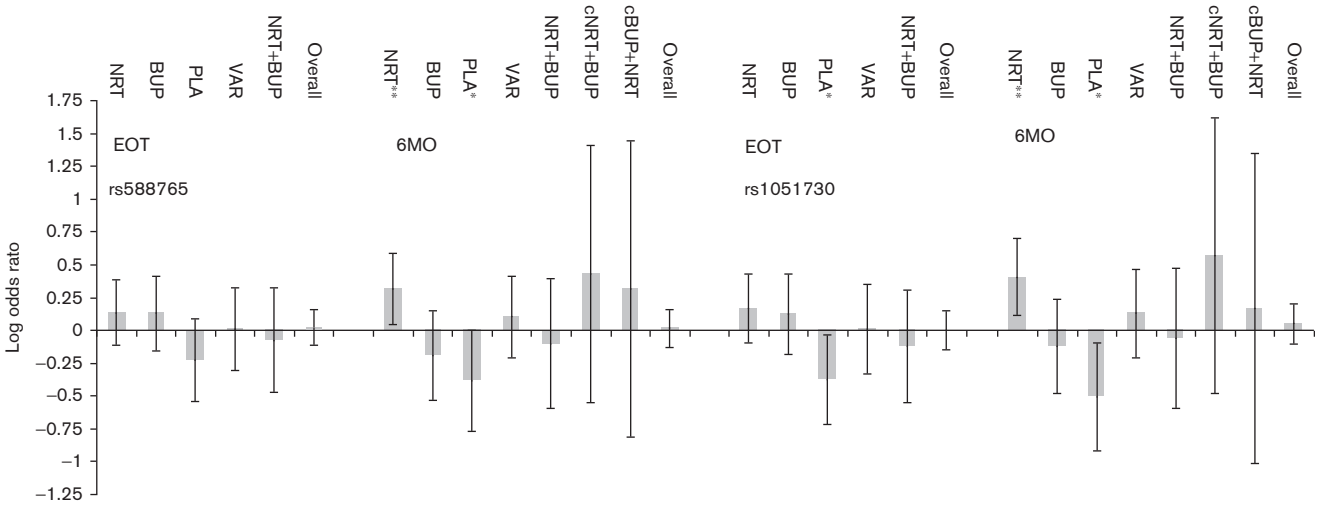
The eight RCTs exhibit similar design features and ascertainment criteria, but differ in prevalence of baseline variables and EOT and 6MO abstinence (Table 1 and see tables, Supplemental digital contents 1–4, *http://links.lww.com/FPC/A554*). RCT 5 was conducted in a healthcare setting [57], and the other RCTs were conducted at Universities. All RCTs were conducted in the USA metropolitan regions. Two RCTs were designed as pharmacogenetic efficacy trials [56], one as a comparative effectiveness trial [57], and the remaining as comparative treatment efficacy trials [58–62]. All RCTs required at least 10 CPD and age more than 18 years, although one RCT was focused on older smokers [59]. All RCTs had similar exclusion criteria that included reproductive/lactation criteria for women, severe current cardiovascular, neurological, or psychiatric disorders, medical contraindications for pharmacotherapy treatment, and current use of psychiatric drugs. All RCTs provided multiple sessions of group or individual counseling, where one RCT randomized participants to web-based counseling, proactive telephone-based counseling, or both modalities [57]. Therapy randomization from baseline to EOT was to five different pharmacotherapies [NRT, BUP, PLA, VAR, or combined NRT and BUP (NRT + BUP)], which could be combined with different behavioral therapies [group counseling (five or seven sessions), individual counseling (six, seven, or eight sessions), and web-based counseling, proactive telephone-based counseling, or both]. Combined PG sizes at EOT were 748, 595, 479, 487, and 324, respectively. Most RCT arms received no further therapy from EOT to 6MO; individuals in the two arms that received NRT + BUP from baseline to EOT were randomized to several pharmacologic and behavioral treatments from EOT to 6MO (see table, Supplemental digital content 4, *http://links.lww.com/FPC/A562*), resulting in a total of seven different PGs at 6MO, the five original PGs, chronic NRT and BUP (cNRT + BUP), and chronic BUP and NRT (cBUP + NRT). Combined PG sizes at 6MO were the same for the first four PGs and 161, 98, and 65, respectively, for the three NRT + BUP PGs. Seven RCTs performed biochemical verification of abstinence [56–62]. All RCTs evaluated 7-day point prevalence abstinence at EOT (8–12 weeks postquit), and at 6MO.

Association of nAChR SNPs with abstinence by pharmacotherapy randomization

rs2072661 is not significantly associated with reduced abstinence in any PG with either transmission model [see table, Supplemental digital content 6 (*http://links.lww.com/FPC/A562*): effects of rs2072661 on EOT and 6MO abstinence, 24 arms]. There are two PG groups that exhibit *P*-values of less than 0.10, however, these differ in transmission model, abstinence time point, and PG.

rs588765 and rs1051730 are significantly associated with abstinence [Fig. 1 and see table, Supplemental digital content 7 (*http://links.lww.com/FPC/A557*): effects of chr15q25.1 nAChR SNPs on abstinence, 26 arms]. The minor allele of rs588765 is significantly associated with reduced abstinence in the PLA PG at 6MO [odds ratio = 0.414 (95% confidence interval) (0.171–0.999) $P = 0.049$], and with increased abstinence in the NRT PG at 6MO [2.074 (1.111–3.871) 0.022]. The minor allele of rs1051730 is significantly associated with reduced abstinence in the PLA PG at EOT [0.422 (0.191–0.934) 0.033] and at 6MO [0.312 (0.122–0.802) 0.016], and with increased abstinence in the NRT PG at 6MO [2.540 (1.293–4.987) 0.007]. The effect of rs1051730 on abstinence differs significantly between PGs at 6MO ($F_{6,28\,652} = 2.48$; $P = 0.021$). The degrees of freedom of the *F* statistic reflect imputation of multiple datasets. The significant test of homogeneity is likely due to the significant and opposite effects of rs1051730 on abstinence in individuals randomized to PLA versus NRT. In sensitivity analyses not adjusting for nicotine dependence measures [see table, Supplemental digital content 8 (*http://links.lww.com/FPC/A558*): effects of chr15q25.1 nAChR SNPs on abstinence, excluding dependence

Fig. 1



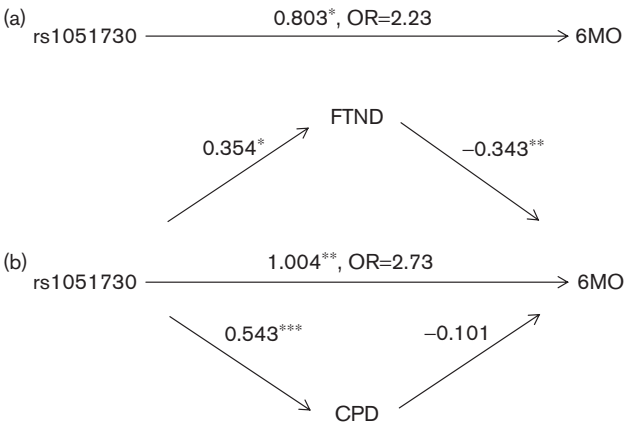
Effects of rs588765 and rs1051730 on abstinence at end of treatment (EOT) and 6 months (6MO) by pharmacotherapy group [nicotine replacement therapy (NRT), bupropion (BUP), placebo (PLA), varenicline (VAR), NRT and BUP (NRT + BUP), chronic NRT and BUP (cNRT + BUP), and chronic BUP and NRT (cBUP + NRT)]. *$P<0.05$, **$P<0.01$.

measures, 26 arms], rs1051730 associations with abstinence remain statistically significant with modestly reduced effect sizes.

Mediation analysis

We carried out post-hoc multivariate mediation analyses to evaluate the association of rs1051730 with nicotine dependence measures, nicotine dependence measure associations with 6MO abstinence, and rs1051730 direct effects on 6MO abstinence in the PLA and in the NRT PGs. We restricted these analyses to rs1051730 because of the significant effect sizes observed with this locus on 6MO abstinence with and without adjustment for multiple nicotine dependence measures. We observe a significant mediational path through the FTND score in the association of rs1051730 with 6MO abstinence in the NRT PG, but not in the PLA PG, perhaps because of sample size limitations [Fig. 2 and see table, Supplemental digital content 9 (*http://links.lww.com/FPC/A559*): mediation of rs1051730 association with 6MO abstinence by nicotine dependence measures in individuals randomized to NRT and PLA]. The direct effect of rs1051730 on abstinence with both FTND and CPD included in the mediation model is significant [2.73 (1.34–5.53) 0.005], and the pseudo-r^2 is 0.083. rs1051730 is significantly and positively associated with CPD and with FTND score ($P < 0.001$ and $P = 0.016$, respectively). FTND score is significantly negatively associated with 6MO abstinence [0.71 (0.57–0.89) 0.003], whereas CPD is nonsignificantly negatively associated with 6MO abstinence. The effect of rs1051730 on 6MO abstinence excluding both nicotine dependence measures from the model is 2.23 (1.12–4.44) 0.022, with pseudo-r^2 of 0.058. Thus, rs1051730 has a stronger relation with 6MO abstinence when the dependence measures are included in the model than when they are not, that is, the dependence measures are acting as suppressors in this mediation model [72].

Fig. 2



Mediation of rs1051730 association with 6MO abstinence by nicotine dependence measures Fagerström test for nicotine dependence (FTND) and cigarettes per day (CPD). (a) Association of rs1051730 with 6MO abstinence without adjustment for nicotine dependence measures. The total path from rs1051730 to 6MO abstinence (not including the nicotine dependence measures FTND and CPD) is statistically significant at *$P<0.05$. (b) Mediation analyses of rs1051730 with 6MO abstinence with nicotine dependence measures. The direct path has a larger effect size and is more significant (**$P<0.01$), than the total path in (a) above, due to the negative effects of FNTD and CPD on the total path. The path from rs1051730 through FTND to 6MO abstinence is statistically significant at *$P<0.05$. The path from rs1051730 through CPD to 6MO abstinence is not statistically significant, though the association of rs1051730 with CPD is statistically significant at ***$P<0.001$.

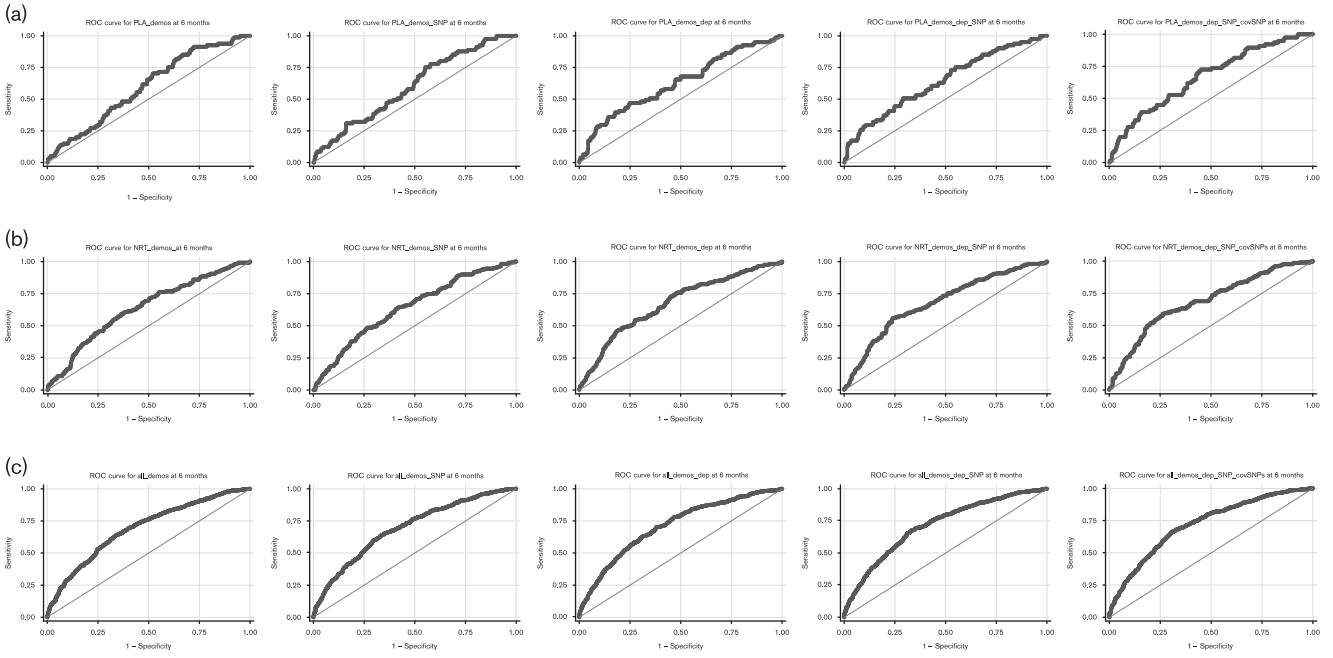
ROC analysis

We performed post-hoc ROC analyses to evaluate the contributions of demographic, dependence, and genetic variables to predict abstinence at 6MO. We evaluated ROC models for the association of rs1051730 with abstinence in the PLA PG at 6MO ($N \sim 467$), in the NRT PG at 6MO (~ 740), and in all PGs at 6MO (sample size ~ 2592) [Fig. 3 and see table, Supplemental digital content 10 (*http://links.lww.com/FPC/A560*): area under the curve (AUC) mean and 95% CI estimates from PLA, NRT, and all PG models]. The ROC AUC values increase when pharmacotherapy is added, for example, with the addition of NRT or all PGs, compared with PLA, and, within each set of ROC models, the ROC AUC increases when including additional variables in the model. For the PLA models, the AUC of the full model is significantly greater than PLA models with demographic variables or demographic variables and rs1051730. For NRT or all PG models, the inclusion of dependence variables, dependence variables and rs1051730, or dependence variables, rs1051730, and covariate SNPs (rs588765 and rs578776), results in ROC curves with significantly greater AUC than the models with only demographic variables, or with demographic variables and rs1051730. This suggests that with or without pharmacotherapy, information imparted by dependence measures and covariate SNPs increases the ability to predict abstinence outcomes. For example, for a specificity of 0.50, the sensitivity of the full model in the PLA, NRT, and all PGs setting is 0.73, 0.72, and 0.81 versus 0.68, 0.70, and 0.76 for the model with only demographic variables, respectively.

Chr15q25.1 SNPs

Two chr15q25.1 SNPs (rs588765 and rs1051730) exhibit statistically significant associations with quitting success in individuals randomized to PLA and NRT, but not in individuals randomized to other pharmacotherapies. These results were obtained by analysis of a total of 2633 self-identified White participants from eight RCTs containing 26 therapy randomization arms, adjusted for PG, RCT arm, demographics, dependence measures, and population genetic variation (and chr15q25.1 SNPs, where appropriate). rs578776, another chr15q25.1 SNP, is not statistically significantly associated with abstinence at either time point or in any PG. This may be because of its more modest effect size or its inverse association with smoking heaviness [30]. rs588765 associations with abstinence appear to be somewhat smaller in magnitude than those observed with rs1051730, concordant with previously observed effects on smoking heaviness [30]. Focusing on the results of analysis of rs1051730, we observed that the minor allele is associated with reduced abstinence in the PLA PG at EOT and at 6MO, and with increased abstinence in the NRT PG at 6MO.

Fig. 3



Receiver-operating characteristic (ROC) curves for (a) placebo (PLA), (b) nicotine replacement therapy (NRT), and (c) all pharmacotherapy groups (all PG) models at 6MOs. ROC curves are shown for models including demographic variables (demos), demographic variables and rs1051730 (demos_SNP), demographic and dependence variables (demos_dep), demographics and dependence variables and rs1051730 (demos_dep_SNP), and all variables with other chr15q25.1 SNPs, rs588765 and rs578776 (demos_dep_SNP_covSNPs). SNP, single nucleotide polymorphism.

The directionality of the effect on abstinence in individuals prescribed PLA is expected, as previously shown for one trial included in this analysis [52], however, the directionality with the NRT PG is unexpected, given the prior associations of rs1051730 with nicotine dependence [23], with reduced abstinence at 4 weeks in multiple RCTs that randomize participants to NRT [50], and considering the inverse associations of nicotine dependence and abstinence [5,6]. We adjusted for the nicotine dependence measures CPD and FTND in our models because we previously observed significant inverse associations of these measures with abstinence in the eight RCTs (data not shown), concordant with a published meta-analysis [6]. Association analyses of chr15q25.1 SNPs and abstinence relations that exclude nicotine dependence measures modestly reduced rs1051730 SNP effects, suggesting that the influences of rs1051730 and nicotine dependence on abstinence are related. Mediation analysis examined the relations of rs1051730 with abstinence in the PLA and NRT PGs at 6MO, and the extent to which this relation was mediated by nicotine dependence measures; we observed significant mediation effects only in the NRT PG at 6MO. The mediation analysis suggests that rs1051730 significantly increases measures of nicotine dependence [18,19,25], that nicotine dependence significantly decreases abstinence likelihood [6], and that there is a mechanism other than nicotine dependence through which rs1051730, in the presence of NRT and at 6MO, increases abstinence.

Mechanisms

Mechanisms that underlie the two distinct association results involving rs1051730 can be postulated on the basis of recent studies in neurogenetics, neuroscience, and pharmacology. α5 knockout mice self-administer nicotine more vigorously than wild-type mice [73], show reduced seizure and hyperlocomotive sensitivity to nicotine [74], and exhibit conditioned place preference for nicotine at doses that are aversive in wild-type mice [75]. These properties are thought to result from the α5 subunit regulation of the medial habenulointerpeduncular nuclear tract [73]. Functional MRI in healthy human smokers has characterized functional connectivity (circuits) [76], including a dorsal anterior cingulate cortex to ventral striatal circuit inversely associated with FTND, and multiple distinct intracingulate cortex and cingulate cortex to frontal region circuits strengthened by nicotine patch administration. In additional studies in smokers and nonsmokers, and in individuals who do and who do not meet criteria for axis I disorders [26], rs16969968 (highly correlated with rs1051730 in European ancestry populations, and coding for CHRNA5 p.Asp398Asn, where Asn398 is associated with reduced nAChR function [77]) was observed to be associated with functional connectivity within the same FTND-associated cingulate circuit. In population samples, rs1051730 has been associated with reduced working memory performance [78]. In a laboratory study of abstinent smokers, transdermal nicotine has been associated with improvements in working memory [79]. Finally, reduced working memory performance in abstinent smokers has been associated with relapse over 7 days in individuals receiving PLA and exposed to a smoking lapse [80].

These findings suggest a hypothesis that can be tested in treatment-seeking smokers. Smokers with reduced α5 subunit function and associated increased nicotine dependence might be expected to have more difficulty quitting. The observation in this analysis that smokers with reduced α5 subunit function treated with NRT have increased overall abstinence rates, and the increased direct effect of rs1051730 on abstinence in mediation analysis, reflect a mechanism that is distinct from the effects of rs1051730 on nicotine dependence, and of nicotine dependence upon abstinence. Prescribed NRT may improve cognitive performance that assists abstinent smokers to maintain normal brain functioning after quitting smoking, and this effect may be stronger for individuals carrying the risk allele of rs1051730. Retrospective analyses of RCT arms randomizing individuals to PLA or NRT, and/or a prospective genotype and NRT-stratified trial, with the appropriate genetic, behavioral, and cognitive function data, could test this hypothesis.

Conti *et al*. [46] identified the association of rs2072661 with abstinence in analyses of a double-blind randomized controlled trial of PLA or active BUP, for example, a SNP of 0.40 (0.25–0.67) at EOT and 0.31 (0.18–0.55) at 6MO. The associations of this SNP with a variety of smoking related phenotypes [35,43,54], including short-term cessation in a crossover trial of NRT and PLA patch [53], suggested that rs2072661 might exhibit effects on abstinence with other PGs and that we might more accurately quantify its association in larger samples. However, we did not observe statistically significant association of rs2072661 with any PG when analyzing 24 arms of seven RCTs, that is, excluding the two arms of the RCT in which the abstinence association was discovered [46]. If the main effect size of rs2072661 on abstinence in RCT participants is weaker than the effect observed by Conti and colleagues, which is expected [81], analysis of additional RCT arms will be necessary to validate the original [46] or subsequent associations [53], or discover novel associations.

Limitations

Limitations of our analyses include sample size limitations on statistical power, RCT participant heterogeneity, and assumptions about variable effects required by our pooled regression analyses. Sample size limitations on statistical power (see table, Supplemental digital content 11, *http://links.lww.com/FPC/A561*: odds ratio detectable

with 80% power) may underlie our inability to make statements about chr15q25.1 nAChR SNP pharmacogenetic effects in individuals randomized to BUP, VAR, or combined therapies. Increasing PG sample sizes in future analyses will increase power to detect pharmacogenetic effects, but will still require integrated data analysis choices to be made. Although there are differences in baseline, treatment, and outcome variables among the RCTs, ascertainment characteristics of the RCTs are similar and there are no significant differences in nAChR SNP allele or genotype frequencies among the RCTs. In the analyses reported here, we utilize one approach to performing integrated data analysis, namely, pooled regression analysis. Heuristically, all of the studies contributed to the estimation of the regression coefficients for each demographic and dependence variable, which were assumed to have the same value across arms; each individual arm contributed to estimation of an arm-specific level variable, allowing for different abstinence rates across arms, and each individual arm contributed to estimation of a pharmacotherapy-specific coefficient for the SNP variable, and, if present, pharmacotherapy-specific coefficients for covariate SNPs. This approach was implemented because many of the arms had insufficient observations for reliable estimation of SNP effects if all of the covariates had been included and regressions were performed separately by arm.

Summary

Treatment-seeking smokers with the minor alleles of chr15q25.1 SNPs rs588765 or rs1051730, versus those without these alleles, are less likely to achieve 6MO abstinence if prescribed PLA, and more likely to achieve 6MO abstinence if prescribed NRT. However, identification and characterization of biomarkers that support the personalization of smoking cessation therapy will be challenging. For example, differences in prediction of abstinence between ROC models with and without rs1051730 (Fig. 2) were a fraction (average of 10%) of the AUC change observed when nicotine dependence measures are added to the ROC models. The modest improvement in prediction attributable to genetic variables versus the larger impact of dependence measures on abstinence likelihood suggests that risk models will include multiple nongenetic and genetic variables [10]. The analysis of multiple RCTs in an integrated data analysis framework to validate the novel association of rs1051730 with abstinence in individuals randomized to NRT, and to discover, and then to validate, additional novel biomarker associations with abstinence, will be necessary to develop algorithms for smoking cessation treatment assignment, that is, personalized medicine [82]. The goal of developing predictive models of treatment response to be implemented into clinical practice will require collaborative efforts from each of the domains of research, policy, industry, and healthcare.

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Dr Bergen, Dr Javitz, Krasnow, and Dr Swan had full access to all of the data in the study and take responsibility for the integrity and the accuracy of the data analysis.

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Conflicts of interest

Dr Bergen has received research support from Medco Health Solutions and Affymetrix. Dr Javitz has conducted research sponsored by Pfizer, SmithKline Beecham, CV Therapeutics, Biogen, Berlex Laboratories, Johnson & Johnson, Ciba-Geigy, Angiotech, Merck & Co., Eli Lilly, and the Pharmaceutical Researchers and Manufacturers Association. He has also been a paid expert witness in litigation against tobacco companies relating to advertising to minors. Edlund is currently employed by BioRealm LLC and the University of Southern California. Dr Hall is the recipient of a materiel grant from Pfizer Pharmaceuticals. Dr Benowitz has served as a consultant to several pharmaceutical companies that market smoking cessation products, including Pfizer, GlaxoSmithKline, and Novartis. He has also been a paid expert witness in litigation against tobacco companies relating to nicotine

addiction. Dr Tyndale owns shares and participates in Nicogen Research Inc., a company focused on novel smoking cessation treatment approaches. No Nicogen funds were used in this work and no other Nicogen participants reviewed the manuscript. Dr Tyndale has received financial support from Novartis and McNeil to participate in one-day advisory meetings in 2008 and 2011, respectively. Dr Lerman has served as a consultant and/or has received research funding from AstraZeneca, GlaxoSmithKline, Targacept, Pfizer, and Novartis. Dr Swan received financial support from Pfizer to attend a one-day advisory meeting in 2008. For the remaining authors there are no conflicts of interest.

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