

Impact of the interactions of *CYP2B6**6 and *CYP2A6* Polymorphisms on the treatment of nicotine dependence

Abstract

Introduction: *CYP2B6* and *CYP2A6* metabolize nicotine and are involved in nicotine dependence treatment. Although evidence suggests they may interact to influence nicotine dependence treatment outcome, in terms of nicotine dependence and withdrawal syndromes and therapy types (placebo, bupropion, and NRT), the significance of their interaction in nicotine cessation has not been fully substantiated and clarified.

Methods: A total of 1862 individuals, consisting of Caucasians and African Americans were studied. Nicotine dependence and withdrawal syndrome scores were evaluated using the Fagerström Test for Nicotine Dependence (FTND) and Wisconsin Inventory of Smoking Dependence Motives (WISDM) scales, respectively. Participants were required to smoke at least ten cigarettes per day and underwent specific therapy types (placebo, NRT, or bupropion) over a 2-week period, and subsequently reported their quitting status at 6 months post-treatment. Participants were also screened for SNPs *CYP2A6**1A (*rs1137115*), *1H (*rs616636070*), *4A (*rs28399434*), *9A (*rs28399443*), *12A (*rs28399442*), and *CYP2B6**6 (*rs3745274*) for nicotine genotype analysis.

Results: Gene variants were uniformly distributed in the population with p-values > 0.05 based on the chi-squared test. Logistics regression analysis showed *CYP2A6**4A was most significantly associated to the odds ratio (OR) of quitting smoking in each treatment group with nicotine dependence syndrome (OR=1.61, 95% CI 1.31-1.96), and *4A in individuals with nicotine withdrawal syndrome (OR=1.70, 95% CI 1.15-1.95). ANOVA test also showed a strong main interaction effect between *CYP2B6**6, *1A, *4A, and *12A gene variants in the bupropion group.

Conclusion: *CYP2A6* and *CYP2B6**6 may interact to increase the likelihood of a successful outcome of nicotine dependence treatment.

Keywords: nicotine dependence, nicotine withdrawal syndromes, smoking cessation, nicotine metabolism, nicotine genes metabolizer, nicotine dependence treatment, personalized medicine, nicotine genes candidates, *CYP2A6* genetics variants, therapy, gene-gene interactions, slow and faster nicotine metabolizers

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Abbreviations: BGT, behavioral genetic theory; CYP P-450, cytochrome P450 enzyme genes; CO, carbon monoxide; FTND, fagerström test for nicotine dependence; GED, general education degree; NRT, nicotine replacement therapy; SNPs, single nucleotides polymorphisms; UW-TTURC, university of wisconsin transdisciplinary tobacco use research center; 3HC/COT, 3'hydroxycotinine/cotinine; OR, odd ratio

Introduction

Emerging evidence supports a possible interaction between *CYP2A6* and *CYP2B6* influencing the outcome of nicotine dependence treatment.^{1,2} Smokers who carried the homozygous form of the genetic variant *CYP2B6**6 and *CYP2A6* slow nicotine metabolizers receiving bupropion/nicotine replacement therapy (NRT) experienced a longer abstinence period than smokers carrying a for mentioned gene variants who received placebo treatment.^{2,3} Ring and Valdez also found that *CYP2B6**6 haplotypes variants Q172H, and K262R were linked to nicotine and cotinine clearance in individuals with *CYP2A6* faster and slow nicotine metabolizers, and with a greater

association in individuals with slow nicotine metabolizer *CYP2A6* genotype; and observed that these gene variants may statistically interact significantly to mediate nicotine clearance ($p < 0.002$), and cotinine clearance ($p < 0.003$).³

CYP2A6 slow nicotine metabolizers may protect individuals from nicotine addiction, since their defective structure and function inhibit the synthesis of sufficient *CYP2A6* enzyme required for nicotine metabolism, as it occurs in normal nicotine metabolizers. Therefore, these genes information may help in the development of individualized nicotine dependence treatment.⁴ Individual carrying the *CYP2A6* high-activity alleles (*CYP2A6**1/*1, *1/*9, *1/*4, *9/*9) may have the urge for cigarette smoking a day earlier compare to low-activity carriers (*CYP2A6**4/*9, *4/*4), thus indicating a noticeable association between *CYP2A6* polymorphism activities and nicotine dependence.^{5,6} Additionally, *CYP2A6* high-activity carriers experience frequent and serious nicotine withdrawal symptoms and adverse events, compared to other groups, during smoking dependence treatment, showing that *CYP2A6* is heavily implicated in nicotine dependence and treatment.

Furthermore, studies have suggested that *CYP2B6*6* alleles occur at a much higher frequency, with considerable inter variability between ethnic groups, and are associated with an increase of nicotine metabolism,^{4,5} indicating that these alleles may influence the development of nicotine dependence. *CYP2B6* represents 6% of the total liver cytochrome P450 enzyme genes (*CYP* P-450) content and metabolizes drugs, such as bupropion in nicotine therapy, which underlines their importance in nicotine dependence treatment.^{3,5,6} Analysis of both genes, with a view to understanding their involvement in metabolism of smoking cessation therapies, using larger numbers of participants required to detect the statistical effect of gene-gene interactions, may help to shape individualized treatment of nicotine dependence.^{3,4}

This study examined the impact of the effect of interactions between *CYP2A6* (slow and normal alleles) and *CYP2A6*6* in relation to nicotine dependence risk factors, such as nicotine dependence and withdrawal syndromes, and therapy type (bupropion or NRT). To date, the influence of *CYP2A6* and *CYP2B6* interactions on nicotine treatment or abstinence still remains a subject of debate, since there are conflicting reports of whether these genetic interactions may influence nicotine abstinence. Ring and Valdez described a significant interaction between *CYP2A6* and *CYP2B6* gene variants.³ In contrast, in a statistical study of the association between *CYP2A6* and *CYP2B6* genes in nicotine dependence treatment, Lee et al.,⁶ did not find any detectable gene-gene interactions causing a significant effect on abstinence in the treatment arm at 6-month post-treatment. Candidate nicotine genes (*CYP2A6* and *CYP2B6*), nicotine dependence and withdrawal syndromes, and types of treatment, such as bupropion and NRT, constitute major interacting factors which play a determining role in nicotine dependence treatment.⁵⁻⁷ Also interactions between *CYP2A6* and *CYP2B6* gene variants may play a critical role in the treatment and recovery of patients, because they may not only catalyze medications used, but they also metabolize nicotine.¹⁻³ Understanding how these major nicotine gene interactions affect the outcome of nicotine treatment in relation to therapy types and the pattern of nicotine dependence and withdrawal syndromes, based on their frequency according to race/ethnic groups,⁵ may help in the development of individualized nicotine dependence treatment.⁶⁻⁸ Statistical analysis of the association of clinical factors, as well as demographic characteristics, interacting together and treatment may help to understand the pattern of smoking behaviors in a population in order to improve nicotine cessation outcomes.^{3,6,7}

Novel individualized treatment of nicotine dependence depends on an in-depth knowledge of the interactions of primary nicotine metabolizer genes, which might include some biological factors, that interfere with a successful outcome of nicotine dependence treatment.⁸⁻¹⁰ Currently, only 5-20% of patients maintain their abstinence after 6 months to 1 year of nicotine dependence therapy,¹¹⁻¹³ showing that nicotine dependence remains difficult to treat. Clinically, nicotine withdrawal syndromes consists of an urge to smoke at a certain time during treatment or an urge to smoke earlier in the morning or after a long period without nicotine, while nicotine dependence syndrome is excessive smoking due to an addiction to nicotine.¹¹⁻¹³ These smoking syndromes or diseases have been linked to *CYP2A6* and *CYP2B6*6* metabolism enzyme encoding and regulatory activities.³⁻⁵

The aim of this study was to determine whether interactions of *CYP2A6* nicotine gene variants with *CYP2B6*6* impact the outcome

of nicotine dependence treatment, as both genes are known to influence nicotine dependence by increasing or decreasing nicotine uptake, distribution, and clearance through their ability to metabolize nicotine.^{5,9,14} As study framework, we used the behavioral genetic theory (BGT) which proposes that genes play a role in human behavior, with the primary goal of establishing co relational relationships between genes and behavioral development or disorder etiology.^{13,14}

Using logistic regression in a cross-sectional methodology, we assessed the statistical significance of the impact of the associations of *CYP2A6* gene variants (slow and fast nicotine metabolizer) and *CYP2B6*6* with therapy types (bupropion and NRT) and with nicotine dependence and withdrawal syndromes, respectively, on nicotine dependence treatment outcome. We used ANOVA (3×2) to evaluate statistically the main effect of gene interactions between *CYP2A6* with *CYP2B6*6* gene variants in the bupropion and NRT groups. These statistical tests would describe the roles of the interaction of these gene variants in nicotine dependence treatment and provide useful information that can be used as significant predictors and indicators in the diagnosis of nicotine dependence and the prognosis of nicotine dependence treatment.

Methods

Study population and data collections

Participants were selected from 9000 nicotine-dependent individuals in Madison and Milwaukee, Wisconsin, USA, seeking nicotine cessation treatment. Among them, 2725 smokers aged between 18 and 80 years were previously sampled⁹ to participate in randomized clinical trials¹¹⁻¹³ organized by The University of Wisconsin Transdisciplinary Tobacco Use Research Center (UW-TTURC) from 2001 to 2009. We obtained approval from National Institutes of Health (reference OMB control number 0925-0670) for genotype and phenotype data requested for our study. An appropriate sampling technique was used to establish a nicotine dependence sample population of 1972 individuals, with the correct power of 80% and a level of significance of 0.05, thus indicating that there was a 5% chance of making a type 1 error or incorrectly rejecting the null hypothesis and to detect an effect size $f=0.25$ of genes variants. After rigorous data processing, 1862 phenotype and genotype samples were used for statistical analysis (Table 1).

Data and statistical analysis

Data collected provided the following information: nicotine dependence scores (1-10) on the Fagerström Test for Nicotine Dependence (FTND) scale, withdrawal syndrome scores (1-10) on the WSDN scale, each participant's therapy type (bupropion, NRT, or placebo), and their quitting status after 6 months post-treatment. In addition to their genotype data, SNPs and their corresponding markers were matched as described in a previous study which reviewed and validated nicotine dependence and withdrawal syndrome scores and other variables (age, educational background, ethnicity, and gender) using the Statistical Package for Social Sciences (SPSS/PASW) utilities to eliminate data redundancy¹⁴ (Table 1).

We compare the means and standard deviations of nicotine phenotypes, according to age, gender, education levels, and race/ethnicity (Table 1). The level of statistical significance for these measurements was set at $\alpha < .05$. Descriptive statistics (maximum, means, standard deviations, percentages, frequencies, and 95% confidence intervals [CIs] of study variables) were used to describe

the characteristics of the sample population. Logistic regression in power interaction analysis (therapy type ×dependence score ×genes variants=quitting status) was used to determine the association between independent and dependent variables, adjusting for covariates, including age, educational background, gender, and race/ethnicity, since these factors are known to be possible etiological contributors

to nicotine dependence.^{10,12} Nicotine dependence and withdrawal syndromes, genetic slow nicotine metabolizers (CYP2A6*1H,*4A,*9A, and *12A) and normal nicotine metabolizers *1A and CYP2B6*6 and therapy types (NRT and burpropion) were used as dependent variables run against the quitting status as the independent variable.

Table 1 Baselines demographic characteristics of study sample

Characteristic	N	Percentage (%)
Age Bracket		
18-30	284	15.3
31-50	1099	59
51-70	466	25
71-80	13	0.7
Ethnicity		
African American	262	14.1
Caucasian	1600	85.9
Educational Background		
Less Than High School/GED	182	9.8
High School Degree/GED	486	26.1
Some Colleges Education	802	43.1
College Graduate	392	21.1
Gender		
Male	772	41.4
Female	1090	58.6
Other baseline measurements		
Overall NRT Bupropion	Placebo	
Sample N(%)	1862 (100%)	840(45.1%) 652 (35%) 370(19.1%)
Age Mean (SD)	42.2 (11.8)	45(10.5) 42.6 (11) 41.3(11.4)
FTND Score Mean (SD)	4.93 (1.31)	4.65 (1.2) 4.34(1.1) 4.28 (1.0)
WISDN Mean(SD)	4.41 (2.15)	4.14 (2.12) 4.01(2.4) 4.28 (2.1)
Cigarettes smoked/day	21.4 (8.9)	21.0 (8.3) 21.4 (8.2) 21.6 (9.1)
Baseline CO level Mean(SD)	25.8 (12.5)	24.5 (13.3) 25.0 (10.7) 24.6 (12.0)
End-6-month quitting rate %	29%	34.3% 22.13% 16.2%

Other Baseline Measurements

Note: N, sample; GED, general equivalence diploma; SD, standard mean; FTND, fagerstroöm test; CO, carbon monoxyde;WISDM, wisconsin inventory of smoking dependence motives (WISDM); NRT, nicotine replacement therapy

Assessment of nicotine dependence and withdrawal syndromes

Participants' smoking status (required to have smoked at least ten cigarettes per day) was assessed by an alveolar carbon monoxide (CO) level of >9.⁹ The quitting status was self-reported by each participant using 'yes/no' response questionnaires, and confirmed using alveolar CO levels.^{11,14} Nicotine dependence syndrome was evaluated using the FTND scale, with low nicotine dependence given a score of 0-4 and high nicotine dependence a score of 5-10.^{11,14} Nicotine withdrawal syndrome was assessed using Wisconsin Inventory of Smoking Dependence Motives (WISDM)^{12,14} where a score of 0-4 was associated with low withdrawal syndrome and a score of 5-10 with high withdrawal syndrome⁹ (Table 1).

Genotyping

Genotyping was performed, as described in a previous study.¹⁴ In brief, genetic samples were genotyped in 96-well plates using the IlluminaHumanOmni2.5-4v1 D array. Gene samples were clustered and called using Genome Studio version 2010.2 with Genotyping Module version 1.7.4 and GenTrain version 1.0. Genotyping call rates for all SNPs were >>98.71% (mean=0.99, dev=0.0071, max=1.00, min=0.85).¹⁴ In this study, candidate gene variants of interest included *CYP2A6*1H* (*rs61663607*), *CYP2A6*4A* (*rs28399434*), *CYP2A6*9A* (*rs28399433*), and *CYP2A6*12A* (*rs28399442*) *CYP2B6*6* (*rs3745274*), and they were located in the 500-kb region on chromosome 19q13.2.⁹ These gene variants were identified previously as primary nicotine metabolizers that encode enzymes involved in nicotine metabolism (at different levels) and increased the likelihood of nicotine dependence by 30-40%, as well as smoking-related diseases, in individuals who carried these common risk alleles.^{3,7} Less than 5% of these gene variants consist of SNPs missing alleles. All SNPs used in this study were extracted from data previously analyzed using BEAGLE version 3.3.1 and Plink-1.07⁹ and converted to the most likely to genotypes using the BEAGLE utility "gprobs2linkage", based on the measurement of SNPs markers expression in association tests of unrelated individual imputed data.¹⁴

Results

Sample demographic characteristics

The study population consisted of 1600 (85.9%) Caucasians and 262 (14.1%) black Americans. Of these, 772 (41.4%) were males, and 1090 (58.6%) were females; 182 (9.8%) had an education level below high school/GED, 486 (26.1%) were high school graduates or have GED, 802 (43.1%) had some college education, and 392 (21.1%) were college graduates (Bachelors, Masters, and doctorate levels). Participants were adults and current smokers, seeking nicotine dependence treatment in three rigorous clinical trials. In addition, participants were between 18 and 80years old, with 59.0% aged between 31 to 50years old. A total of 32.1% of participants reported a low level of nicotine dependence, whereas 67.9% reported a high level of nicotine dependence. Moreover, 40.8% of all participants reported low scores of nicotine withdrawal syndrome during treatment, while 59.2% reported high scores of nicotine withdrawal syndrome (Table 2).

Results showed that 69.1% of all participants were identified as carriers of the normal nicotine metabolizer gene variant *CYP2A6*1A*. (43.6% of Whites (W) and 25.5% of African Americans (AA)) and for or the slow nicotine metabolizer gene variants, 46.9% (34.6% of W, and 12.3% AA) of participants were carriers of *CYP2A6*1H*, 56.5%

(39.2% of W and 17.3% AA) were carriers of *CYP2A6*4A*, 38.2% (26.9% of W, and 11.3% of A A) were carriers of *CYP2A*9A*, 58.6% (35.4% of W, and 23.2% of AA) were carriers of *CYP2*12*, and 48.1% (34.6 of W and 13.5% of AA) were carriers of *CYP2B6*6*. These genotype results were matched subsequently with other studies (Figure 1) (Figure 2). In the treatment groups, 45.1% of participants received NRT, 35% received bupropion, and 10.5% received placebo. Of all participants, 29.4% reported quitting smoking, and 70.6% did not quit after 6months of nondrug follow-up treatment.

Table 2 Data processing results

Variables Description	N	Missing Variables	Mismatched and Missing Genotype
FTND	1972	6	-
WS	1972	28	-
Unknown	55	-	-
Other Races	4	-	-
Genotype ID	1972	-	51
Study Sample	1862	-	-
SNPs	Chi-Square (χ ²)	p-values	95%CI
<i>CYP2A6*1A</i>	1.12	0.501	0.227-0.684
<i>CYP2A6*1H</i>	0.711	0.61	0.304-0.955
<i>CYP2A6*4A</i>	0.223	0.697	0.330-0.874
<i>CYP2A6*9A</i>	0.34	0.708	0.210-0.925
<i>CYP2A6*12A</i>	0.278	0.841	0.430-0.970
<i>CYP2B6*6</i>	0.175	0.956	0.241-0.990

Genotype Chi-square (χ²)Test for Hardy Weinberg Equilibrium Note :Monte Carlos Sig: 0.01, 95% CI, confidence interval; FTND, fagerström test for nicotine dependence; ND, nicotine dependence syndrome score; SNPs, single nucleotide polymerase;WS, withdrawal syndrome score

Nicotine dependence and withdrawal syndromes in association with smoking quitting status at 6months post-treatment

Nicotine dependence syndrome: There was a significant association of slow nicotine metabolizers and *CYP2B6*6* in the overall group, as well as the bupropion and NRT groups, with a positive odds ratio (OR) for successful nicotine dependence treatment. In the overall group (N=1862), each slow nicotine metabolizer was associated significantly with an OR with *p*<.001 (*CYP2A6*1H* [OR=1.56, 95% CI 1.20-1.90]; **4A* [OR=1.61, 95% CI 1.31-1.96]; **9A* [OR=1.47, 95%CI 1.15-1.88]; and **12A* [OR=1.35, 95%CI 1.11-1.68]), indicating that the group of patients carriers of slow nicotine metabolizer gene variants and *CYP2B6*6*gene variant had 1.47, 1.50, 1.56, and 1.61 chance, to maintain abstinence for 6months post-treatment.

Results in the bupropion group (N=652) showed increased OR, (*CYP2A6*1H* [OR=1.60, 95% CI 1.20-2.06]; **4A* [OR=1.78, 95% CI 1.17-2.08]; **9A* [OR=1.57, CI 1.18-2.03]; and **12A* [OR=1.50, 95%CI 1.48-1.68]) indicating that, in nicotine cessation, bupropion may interact more effectively with nicotine genes involved in nicotine cessation and drug metabolism, thus increasing the odds for successful nicotine dependence treatment. The results in the NRT group (N=840)

showed decreased OR (*CYP2A6**1H [OR=1.50, 95% CI 1.18-1.67]; *4A [OR=1.55, 95% CI 1.31-1.76]; *9A [OR=1.49, 95% CI 1.16-1.60]; and *12A [OR=1.37, 95%CI 1.11-1.48]), indicating that NRT may interact less effectively with slow nicotine gene variants (Table 3). In addition, results of an association test of *CYP2A6**1A with *CYP2B6**6 in this model yielded ORs of (OR=1.25, 95% CI 1.30-1.45), (OR=1.40, 95% CI 1.85-1.99) and (OR=1.34, 95% CI 1.13-1.69) in the NRT, overall, and bupropion groups, respectively (Table 4).

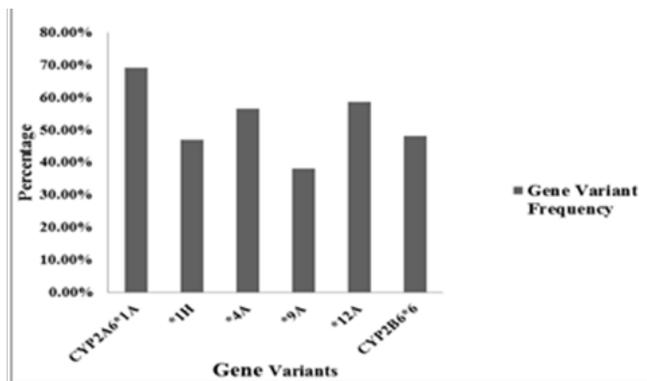


Figure 1 Genotype Frequency in General.

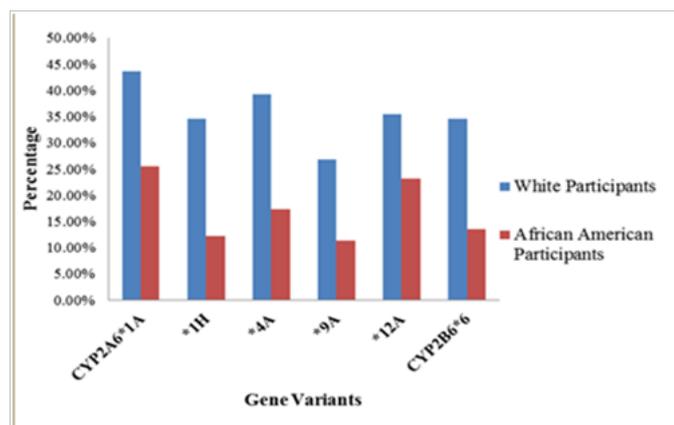


Figure 2 Genotype Frequency within Races.

Nicotine withdrawal syndrome

Nicotine withdrawal syndrome may interfere with nicotine cessation by causing individuals in the *CYP2A6* high-activity group to relapse,^{5,15} which may negatively influence outcomes of nicotine cessation treatment. Logistics regression analysis in participants carrying *CYP2A6* slow nicotine metabolizer variants and *CYP2B6**6 in the overall, NRT, and bupropion groups gave statistically significant results ($p < .001$) for each slow nicotine metabolizer. In the overall group ($N=1862$), results for each variant were as follows: (*CYP2A6**1H [OR=1.47, 95% CI 1.13-1.89]; *4A [OR=1.70, 95% CI 1.15-1.95]; *9A [OR=1.54, 95% CI 1.17-1.80]; and *12A [OR=1.32, 95% CI 1.09-1.64]), showing that these individuals experiencing withdrawal syndrome have a 1.32, 1.47, 1.54, and 1.70 times chance, respectively, to maintain abstinence for 6months post-treatment. In the bupropion group ($N=652$), the ORs were (*CYP2A6**1H [OR=1.55, 95% CI 1.05-1.67]; *4A [OR=1.79, 95% CI 1.15-1.92]; *9A [OR=1.60, 95% CI 1.03-1.68]; and *12A [OR=1.45, 95%CI 1.01-1.57]), for the group of people having slow nicotine metabolizers and *CYP2A6*.

In the NRT group ($N=840$), the ORs were (*CYP2A6**1H [OR=1.36, 95% CI .72-1.42]; *4A [OR=1.51, 95% CI .94-1.68]; *9A [OR=1.47, 95% CI 1.08-1.54]; and *12A [OR=1.35, 95%CI .64-1.48]) for maintaining abstinence for 6months post-treatment (Table 4). Testing in participants carrying the normal nicotine metabolizer *CYP2A6**1A in this model also gave statistically significant results, ($p < .01$) but with decreased ORs of (OR=1.19, 95% CI 1.15-1.35), (OR=1.45, 95%CI 1.08-1.69), and (OR=1.27, 95% CI 1.04-1.39), respectively, in the NRT, overall, and bupropion groups for maintaining abstinence for 6months post-treatment (Table 5).

Table 3 Test results of quitting status after 6 months posttreatment in slow

Genes SNPs	Pharmacotherapy	OR	95% CI
NRT-p-Value			
CYP2A6*1H rs61663607	0.0002	1.5	1.18-1.67
CYP2A6*4A rs28399434	0.00011	1.55	1.31-1.76
CYP2A6*9A rs28399433	0.0003	1.49	1.16-1.6
CYP2A6*12A rs28399442	0.0006	1.37	1.11-1.48
Bupropion-p-Value			
CYP2A6*1H rs61663607	0.0006	1.6	1.20-2.06
CYP2A6*4A rs28399434	0.00007	1.78	1.17-2.08
CYP2A6*9A rs28399433	0.000029	1.57	1.18-2.03
CYP2A6*12A rs28399442	0.0000045	1.50	1.48-1.68
Overall -p-Value			
CYP2A6*1H rs61663607	0.00004	1.56	1.20-1.9
CYP2A6*4A rs28399434	0.00031	1.61	1.31-1.96
CYP2A6*9A rs28399433	0.000071	1.47	1.18-1.88
CYP2A6*12A rs28399442	0.000014	1.35	1.11-1.68

nicotine metabolizers, having *CYP2B6**6 for nicotine dependence syndromes
SNPs, single nucleotides polymeras; OR, odd ratio; CI, confidence interval; NRT, nicotine replacement therapy; p, p-value

Table 4 Test results of quitting status after 6 months posttreatment in slownicotine metabolizers for nicotine having CYP2A6 for withdrawal syndromes.

Genes SNPs	Pharmacotherapy	OR	95% CI
NRT-p-Value			
CYP2A6*1H rs61663607	0.00062	1.36	0.72-1.42
CYP2A6*4A rs28399434	0.00012	1.51	0.94-1.58
CYP2A6*9A rs28399433	0.00023	1.47	1.18-1.88
CYP2A6*12A rs28399442	0.00006	1.35	1.11-1.68
Bupropion-p-Value			
CYP2A6*1H rs61663607	0.000047	1.55	1.05-1.67
CYP2A6*4A rs28399433	0.000064	1.79	1.15-1.92
CYP2A6*9A rs28399434	0.000053	1.6	1.03-1.68
CYP2A6*12A rs28399442	0.0000203	1.45	1.01-1.57
Overall -p-Value			
CYP2A6*1H rs61663607	0.000431	1.47	1.13-1.89
CYP2A6*4A rs28399434	0.000052	1.7	1.15-1.95
CYP2A6*9A rs28399433	0.000095	1.54	1.17-1.80
CYP2A6*12A rs28399442	0.000017	1.32	1.09-1.64

SNPs, single nucleotides polymeras; OR, odd ratio; CI, confidence interval; NRT, nicotine replacement therapy

Table 5 Test results of quitting status after 6months posttreatment in normal nicotine metabolizers for nicotine dependence and withdrawal syndromes

Logistics regression test results for nicotine syndrome			
Genes SNPs	Pharmacotherapy	OR	95% CI
NRT-p-Value			
	0.00056	1.25	0.130-1.45
Bupropion-p-Value			
CYP2A6*1A rs1137115	0.0002	1.40	1.85-1.99
Overall -p-Value			
	0.0006	1.34	1.13-1.69

Table continued...

Predictor	df	F	η ²	p-value
Bupropion	1	2.106	0.003	0.221
<i>CYP2A6*12A</i> × <i>CYP2B6*6</i> × Bupropion	1	132.38	0.059	0.0001
Error	32.2			

Predictor	df	F	η ²	p-value
<i>CYP2A6*1A</i>	1	1.105	0.001	0.0001
<i>CYP2B6*6</i>	1	0.894	0.001	0.345
Bupropion	1	2.256	0.003	0.112
<i>CYP2A6*1A</i> × <i>CYP2B6*6</i> × Bupropion	1	127.72	0.065	0.0006
Error	38.2			

ANOVA Predicting Significant Main Interaction Effect of *CYP2A6*1A* by *CYP2B6*6*

Note: df, degree of freedom; η², partial eta square

Discussion: interpretation of statistical results and study limitation

Statistical results interpretations

This was an association study aiming to analyze statistically the interaction of factors that contribute to nicotine dependence and withdrawal syndromes in nicotine cessation trial. The statistical model involved the use of logistics regression analysis to determine whether measurable factors identified to have been implicated in nicotine dependence development (dependence scores, genetic variants, and demographic factors) were in a positive relationship with nicotine treatment outcome. The model focuses particularly on how the association of gene variants *CYP2A6* and *CYP2B6*6* with other factors may statistically increase the odds for successful nicotine dependence treatment. For example, the effect size of slow nicotine metabolizer *CYP2A6*4A* of 0.468 increased (Exp[0.468]=1.61 or OR=1.61) by 1.61 times the likelihood of a successful outcome of nicotine dependence treatment.

The results confirmed that individuals dependent on nicotine by carrying specific *CYP2A6* alleles with high activity of nicotine metabolism (*CYP2A6*1A*) would face difficulty in maintaining nicotine abstinence (low OR average of 1.20).^{13,14} Individuals carrying *CYP2A6* alleles with lower or zero nicotine metabolism activity have a high chance to maintain nicotine abstinence for 6months post-treatment (average OR=1.60). Moreover, the odds of maintaining nicotine abstinence for 6months post-treatment were higher in the bupropion group, compared to other group therapies. This is in agreement with evidence that *CYP2B6*6* have an affinity for metabolizing bupropion, thus increasing its pharmacological effect in treatment. *CYP2A6*4A* was associated with a higher OR in all groups,

supporting the assumption that carrying at least one loss-of-function allele (i.e., *CYP2A6*1H*, *CYP2A6*4A*) or carrying two decrease-of-function alleles (*CYP2A6*9* or **12*), or any combination of a loss-of-function allele with a decrease-of function allele,¹³⁻¹⁵ may help individuals to smoke less. In reality, whether an individual is a carrier of normal or slow metabolizers, it seems difficult for individuals undergoing treatment to stop smoking, as shown in previous studies where only 5-15% maintained abstinence was reported, due to multiple nicotine-related factors leading to nicotine dependence, including gene-gene interactions.^{10,11}

The structure of chromosome 19q13.2 shows that the position of *CYP2B6* is close to that of *CYP2A6*, suggesting a possible interaction with each other.² According to Russell,¹⁶ no gene was expressed alone as a phenotype, which, in turn, suggests that phenotypes are the result of highly complex and integrated patterns and molecular reactions under the control of multiple gene functions. In fact, genetic “epistasis” consists of the interaction of many genes for the expression of a specific phenotype or for a specific function (Table 7).¹⁶

As shown in this study, quantitative ANOVA evaluation of the main effect of the interaction of slow and normal nicotine metabolizer gene variants of *CYP2A6* with *CYP2B6*6* gene variant in nicotine dependence treatment using bupropion produced a greater main effect, thus increasing the likelihood for a successful nicotine dependence treatment outcome, as described by Ring and Valdez.³

Study limitations

Our study findings would help in the development of tailored nicotine addiction treatment strategies, but many challenges must be overcome before it is possible to fully incorporate these findings into successful individualized nicotine treatment plans and public health

practices. This study used an association methodology that did not analyze cause-effects and included only a few gene variants and ethnic groups (black and white Americans). In addition, the study did not use all SNPs but only a sufficient number of SNPs to harness the gene-gene and gene-environment effects needed and may be limited by admixture phenomena which need more investigation, although

the study has adjusted for race, age and gender. In addition, further studies should replicate the one reported here on other populations comprising more diverse ethnic groups and larger sample sizes, also by considering other sets of alleles in order to test other gene-gene and gene-environment interactions.⁵ Further analyses within each race/ethnic group would also help.

Table 7 Brief descriptive title of the tables

Table 1: demographic characteristics of study sample	Table 2: data processing results	Table 3	Table 4	Table 5	Table 6
Description: Provides Demographic characteristics of sample population use in the study, and other Baseline Characteristics measurements	Description: Data processing results by matching and excluding missing, redundant variables phenotypes and genotypes. The second part shows the result of Chi-square (χ^2) Test for Hardy Weinberg Equilibrium of each SNPs used in this study	Provides Test Results of Quitting Status After 6 Months Posttreatment in Slow Nicotine Metabolizers, having CYP2B6*6 for Nicotine Dependence Syndromes	Test Results of Quitting Status After 6 Months Posttreatment in Slow Nicotine Metabolizers, group having CYP2A6 for Withdrawal Syndromes	Test Results of Quitting Status After 6 Months Posttreatment in Normal Nicotine Metabolizers for Nicotine Dependence and Withdrawal Syndromes.	ANOVA Test to quantify the Main Interaction Effect between CYP2A6*4A by CYP2B6*6, and CYP2A6*12A by CYP2B6*6 in Bupropion Group

Conclusion

Nicotine candidate genes that are involved in nicotine dependence development interact, thus reinforcing the impact of their activities, as suggested by the “epistasis” theory.¹⁶ As found in this study, logistics regression analysis predicted a significant impact of the interaction of normal and slow nicotine metabolizers of CYP2A6 gene variants with CYP2B6*6 gene variant on nicotine dependence treatment outcome. ANOVA results showed a significant main interaction effect between nicotine metabolizer gene variants of CYP2A6 and CYP2B6*6 gene variant in relation to therapy type in the treatment of nicotine dependence syndrome. Findings in this study provide supportive evidence that gene-gene interactions increase the impact of their activities in the development and treatment of behavioral disease.³⁻⁵

In addition, this study provides supportive evidence that interactions of slow and normal nicotine metabolizer gene variants with CYP2A6 and CYP2B6*6 may increase the likelihood of a successful outcome of nicotine dependence treatment over normal nicotine metabolizers. Gene variants CYP2A6 and CYP2B6 are more likely to have additive effects for a positive outcome of nicotine dependence treatment, which would help in the design of personalized treatment of nicotine dependence.⁴

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Conflict of interest

The author declares no conflict of interest.

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