

**Personalized smoking cessation: interactions between nicotine dose, dependence and quit success genotype score**

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**Abstract**

Improving and targeting nicotine replacement therapy (NRT) are cost-effective strategies for reducing adverse health consequences for smokers. Treatment studies document the efficacy of pre-cessation NRT and support important roles for level of nicotine dependence and pre-cessation smoking reduction in successful quitting. However, prior work has not identified the optimal pre-cessation dose or means for personalizing NRT. Genome wide association has identified groups of genomic markers that are associated with successful quitting, allowing us to develop a v1.0 “quit success” genotype score. We now report influences of v1.0 quit success genotype score, level of dependence and pre-cessation smoking reduction in a smoking cessation trial that examined effects of 21 vs 42 mg/24 h pre-cessation NRT. 479 smokers were randomized to 21 or 42 mg NRT, initiated 2 weeks prior to target quit dates. We monitored self-reported abstinence and end-expired air carbon monoxide (CO). Genotyping used Affymetrix arrays. The primary outcome was 10-week continuous smoking abstinence. NRT dose, level of nicotine dependence and genotype scores displayed significant interactive effects on successful quitting. Successful abstinence was also predicted by CO reductions during pre-cessation NRT. These results document ways in which smoking cessation strategies can be personalized based on levels of nicotine dependence, genotype scores and CO monitoring. These assessments, taken together, can help to match most smokers with optimal NRT doses and to rapidly identify some who may be better treated using other methods.

Cigarette smoking is a significant cause of premature death and disease <sup>1</sup>. Successfully quitting and maintaining abstinence reduce risks to smokers <sup>2-4</sup>. However, success rates following attempts to quit smoking remain modest. One year after unaided attempts to quit smoking, abstinence rates are less than 5% <sup>5, 6</sup>. Even with pharmacologic aids that include nicotine replacement therapy (NRT), bupropion and varenicline, long-term abstinence rates are less than 25% <sup>7, 8</sup>.

Pre-cessation NRT initiates nicotine patch treatment two weeks prior to a target quit date <sup>9, 10</sup>. The 21 mg/24 hour dose pre-cessation dose used to date is well tolerated by most smokers <sup>9, 10</sup> and consistently enhances quit rates: recent meta-analyses show a 1.8-2.2 fold greater likelihood of success than conventional NRT <sup>11, 12</sup>. However, neither dose/response data nor clear-cut recommendations for personalizing pre-cessation NRT have been reported.

More effective smoking cessation might result from personalizing existing treatments based on characteristics of individual smokers. Previous research has identified phenotypic and genotypic variables that might aid in predicting likelihood of success in smoking cessation and/or differential responses to different treatment regimens. In smoking cessation trials, smokers who **score** higher on the Fagerström Test for Nicotine Dependence (FTND), and thus display more prominent physiological dependence on nicotine, experience lower rates of success from conventional NRT regimens and may derive greater benefits from higher NRT doses <sup>13-15</sup>. In a trial of pre-cessation NRT with conventional, 21 mg/24h dosing, smokers with higher FTND scores displayed less benefit than those with lower scores <sup>10</sup>. We thus hypothesized, a priori, that more highly dependent smokers and smokers who find it more difficult to quit with conventional NRT doses might require higher NRT doses, both before and after their target quit dates.

Aside from level of nicotine dependence, other features could help to personalize NRT. After initiation of pre-cessation NRT, individual differences in abilities to decrease end-expired air carbon monoxide (CO) concentrations provide measures of reduced *ad lib* smoking that strongly predict the ability to achieve abstinence<sup>10</sup>. Inherited predispositions are also likely to play large roles. Classical genetic studies strongly support the idea that individual differences in abilities to achieve abstinence have significant genetic determinants<sup>16</sup>. Genome-wide association and candidate gene molecular genetic studies of quit success have identified a number of candidate “quit success” alleles<sup>17, 18</sup>. No individual variant provides a large effect, raising several of the issues discussed in recent reviews<sup>19-22</sup>. Nevertheless, GWA datasets from multiple independent smoking cessation samples identify many of the same small chromosomal regions to extents that, overall, are virtually never found by chance<sup>18</sup>. Thus, as noted in a recent white paper, when “each individual has a constellation of many variants (we need to).. integrate the sum of these variants and use them.. in the context of.. clinical information, some of which (may also be influenced by genetics)” (<http://www.genome.gov/27529204>). Based on this logic, we have both a) formulated and used a version 1.0 genetic score as an initial, testable index of genetic risk for quit success and b) anticipated (*and sought in primary analyses*), interactions between this score and level of nicotine dependence.

We thus now report a comparison of 21 and 42 mg/day pre-cessation NRT in smokers categorized based on levels of nicotine dependence, v1.0 quit success genotype scores and changes in expired air CO during the two week pre-cessation treatment period. We document the previously-reported predictive value of CO reductions during pre-cessation treatment. We also identify novel interactive effects of genotype score, dependence level and nicotine dose in influencing smoking abstinence outcomes in both European-American and African-American smokers. We describe suggestions for personalized pre-cessation NRT that arise from this work, especially as it is replicated in additional samples.

## MATERIALS AND METHODS

### Study Design

The study had a randomized, double-blind, parallel-arm, placebo-controlled factorial design with two levels of nicotine dependence and two nicotine doses. Cigarette smokers interested in quitting were subdivided *a priori* into low- and high- dependence subgroups (FTND scores  $\leq 6$  or  $> 6$ , respectively <sup>23</sup>) and were randomly assigned to 21 mg/24 h or 42 mg/24h nicotine patch doses.

### Recruitment, eligibility and compensation

Adult smokers expressing a desire to quit smoking were recruited through newspaper, radio and television advertisements, flyers, and word-of-mouth and were screened by telephone and physical examination at one of four North Carolina centers. Participants provided written informed consent, reported smoking an average of  $\geq 10$  cigarettes that each yielded  $\geq 0.5$  mg nicotine per day, displayed end-expired air CO  $\geq 10$  ppm and failed to display any exclusionary feature on history, physical exam or laboratory evaluation (Supplement 1). Participants were compensated up to \$140 for seven sessions.

### Study Procedures

After screening and enrollment in the study, participants returned to the research center for seven sessions (Fig. 1), during which brief ( $< 15$  min) supportive counseling was provided, clinical trial materials were dispensed and dependent measures assessed. Dependent measures included self-reported smoking, end-expired air CO, withdrawal symptoms and other adverse effects.

Each participant wore two skin patches daily for eight weeks, beginning two weeks prior to the target quit date (Fig. 1). In the 21 mg nicotine dose condition, one active patch (GlaxoSmithKline) was applied

in the morning and one placebo patch (Rejuvenation Labs, Inc.) applied at noon. In the 42 mg nicotine dose condition, two active patches were worn. NRT doses were gradually reduced beginning 4 or 6 weeks after the quit date for the 42- and 21 mg/24 h groups, respectively. Participants with sleep disturbances removed patches at bedtime and applied new ones upon awakening. Subjects experiencing other symptoms of nicotine toxicity reduced doses, until symptoms abated, according to the following sequence: reduce morning patch from 21- to 14- to 7- to 0 mg/day then discontinue the afternoon patch. All participants were provided with denicotinized cigarettes (< 0.05 mg nicotine yield; Vector Tobacco Co., Mebane, NC) to smoke during the 2-week pre-cessation period, to minimize the likelihood of potential adverse effects of high dose NRT.

#### **Genotyping and assignment of genetic background groups**

DNA was extracted from blood, quantitated and genotyped using Affymetrix 6.0 microarrays as described (Supplement S2). Genotypes for each individual passed Affymetrix quality control metrics and provided calls for  $\geq 97\%$  of SNP genotypes. Imputation using PLINK<sup>24</sup> with confidence threshold > 0.95 determined most missing genotype calls.

Genetic background was assigned for each individual based on principal component analyses of data from all SNPs. Two principal components separated a cluster of individuals who almost all reported European ancestry from a cluster whose members reported predominantly African-American ancestry (Supplementary Fig. S1). Three individuals who fell between these clusters and identified themselves as of mixed ethnic/racial backgrounds were classed as indeterminant and excluded from further statistical analyses.

**Assignment of v 1.0 quit success genotype scores**

Genotypes and v1.0 scores were assigned for each participant by investigators blinded to clinical phenotype. We assessed alleles at the 12,058 SNPs (listed in Appendix A of <sup>25</sup>, see <http://www.sciencedirect.com/science/MiamiMultiMediaURL/B6T63-4W8KHMN-2/B6T63-4W8KHMN-2-1/5019/html/S0376871609000921/85fe35dc441ab557783c8a1ce3038044/f.doc?MMCV=widget>), for which at least one of three previously-reported smoking cessation success clinical trial samples had identified nominally-highly-significant ( $P < 0.01$ ) differences between successful vs unsuccessful quitters, weighting data from these SNPs based on strength and replicability of the associations (Supplement 3)

<sup>18</sup>.

**Analyses**

*Primary outcome:* Continuous abstinence from the target quit date through the end of treatment (10 weeks) was assessed based on self reports of continuous abstinence (i.e., no lapses) that were confirmed by end-expired CO levels  $\leq 10$  ppm. Participants who withdrew from the study or were lost to follow-up were classified as nonabstinent. Secondary abstinence outcomes included a) 4-week continuous abstinence during weeks 7-10 after the target quit date, and b) 7 day, point abstinence at 6 months.

Logistic regression analyses were conducted to seek statistically-significant effects on quit success based on: nicotine patch dose (21 mg vs 42 mg), level of dependence ( $\leq 6$  vs  $>6$  FTND score), and v1.0 quit-success genotype scores ( $\leq$  median vs  $>$  median), with emphasis on interactions among these factors. In preplanned analyses, we compared individuals with upper- to lower half v1.0 quit success scores, seeking: a) to avoid any assumptions regarding the linearity of influences across the range of genotype scores; b) to ensure that equal numbers of subjects were classified “low” or “high” in each racial/ethnic subgroup; and c) to parallel the analyses used for FTND scores and changes in CO levels (*see below*).

Median v1.0 scores and proportions of smokers with FTND scores  $\leq 6$  were similar in European- (384; 54%) and African-American (392; 49%) participants.

To verify that participants assigned to each of the four treatment groups did not exhibit disparate v1.0 quit success genotype scores due to stochastic influences<sup>26</sup>, we conducted one preplanned interim analyses of data from the first 203 participants. There was no evidence for significant stratification in individuals assigned to each of the four treatment arms (*data not shown*). In addition, quit-success v1.0 genotype scores appeared to interact with dependence level and nicotine dose. Highly-dependent smokers with low quit-success genotype scores benefited to greater extents from the 42 mg NRT dose (*data not shown*).

Based on results of interim analyses and our *a priori* hypotheses positing interactions between dose and level of dependence, the final analysis of the complete dataset (n =457) included the following terms: nicotine dose (21 mg vs 42 mg); FTND dependence score ( $\leq 6$  vs  $> 6$ ); quit-success genotype score ( $\leq$  median vs  $>$  median); nicotine dose x dependence; and nicotine dose x dependence x genotype score. Separate follow-up analyses for the European- and African- American samples explored the robustness of the nicotine dose x dependence x v1.0 genotype score interaction.

Frequencies with which adverse events occurred in the two nicotine dose conditions were compared using  $\chi^2$  tests.

## RESULTS

Most smokers who contacted the research center met eligibility criteria and were enrolled. Most enrollees participated successfully (Fig. 2).

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Logistic regression of data from the primary outcome, 10-week continuous abstinence, yielded a significant three-way interaction between nicotine dose, dependence and quit-success genotype score ( $P = 0.015$ ), exponentiated coefficient = 0.56 (95%CI = 0.35-0.91). The interaction noted in interim analysis of the initial set of subjects was also noted in separate analysis of data from the second half of the subject group (*data not shown*). In the entire group of subjects, the two-way interaction of nicotine dose and FTND score was also significant ( $P = 0.02$ ), exponentiated coefficient = 1.78 (95% CI = 1.10-2.87). Fig. 3 displays the rate of 10-week continuous abstinence as a function of nicotine dose, dependence level and genotype score. The 42 mg nicotine dose condition tended to enhance treatment outcome for highly dependent smokers with low v1.0 scores ( $P = 0.06$ ). By contrast, this high dose patch impeded abstinence for less dependent smokers with low v1.0 scores ( $P = 0.009$ ). The nicotine dose x dependence x genotype score interaction was also significant for 4-week continuous abstinence at the end of treatment ( $P = 0.02$ , exponentiated coefficient = 0.62, 95% CI = 0.42-0.93), a secondary outcome (Supplementary Fig. S2). Moreover, the 6-month point abstinence results showed the same pattern of three-way interaction ( $P = 0.03$ , exponentiated coefficient = 0.62, 95% CI = 0.40-0.96), see Supplementary Fig. S2.

There were concordant results from analyses that separated European- and African-American smokers. For smokers with European ancestry ( $n=369$ ), the 10-week continuous abstinence outcome showed a nicotine dose x dependence x v1.0 genotype score interaction ( $P = 0.05$ , exponentiated coefficient = 0.60, 95% CI = 0.35-1.00). With respect to 4-week continuous abstinence and 6-month point abstinence, the subsample showed a similar, though nonsignificant, trend ( $P = 0.12$ , exponentiated coefficient = 0.71, 95% CI = 0.46-1.09;  $P = 0.19$ , exponentiated coefficient = 0.72, 95% CI = 0.45-1.17). For smokers with African ancestry ( $n=88$ ), this three-way interaction displayed a trend similar to that noted for the entire sample for both 10-week ( $P = 0.15$ , exponentiated coefficient = 0.33, 95% CI = 0.07-1.48)

and 4-week continuous abstinence ( $P = 0.08$ , exponentiated coefficient = 0.38, 95% CI = 0.13-1.12).

The three-way interaction did reach significance in this subsample for 6-month point abstinence ( $P = 0.02$ , exponentiated coefficient = 0.20, 95% CI = 0.05-0.78).

Abstinence outcomes were also predicted by individuals' abilities to reduce smoking during the 2-week pre-cessation period, as measured by decreases in end expired air CO levels. Smokers whose CO dropped by more than the median (55.6% decrease) showed a substantially higher rate of 10-week continuous abstinence than smokers who did not show this decrease: 37.6 % vs 11.2% ( $P < 0.001$ , odds ratio = 4.77, 95% CI = 2.81-8.11; *for results from each treatment group, see Fig. 4*). A similar result was obtained for 4-week continuous abstinence (52.1% vs 26.5%;  $P < 0.001$ , odds ratio = 3.01, 95% CI = 1.97-4.60) and 6-month point abstinence (40.2% vs 18.9%;  $P < 0.001$ , odds ratio = 2.89, 95% CI = 1.83-4.57).

However, the pre-cessation decrease in CO did not explain all of the influences on quit success provided by other measures of individual differences. The three-way interaction of nicotine dose, FTND score and quit-success genotype score remained statistically significant even after inclusion of a term in the logistic regression model that reflected CO decreases ( $P = 0.04$  for 10-week abstinence).

### **Adverse effects**

Treatment was generally well tolerated. Three percent of participants withdrew from the study due to adverse effects. The most frequently reported patch-related adverse effects were: vivid dreams (83.5%), itching or burning (55.3%), insomnia (47.2%), rash (20.7%), nausea (15.9%), headache (26.1%), and vomiting (2.5%). There was a trend toward more frequent nausea in the 42 mg dose condition ( $P =$

0.06). Each of two women who received 42 mg doses reported syncope on one occasion, each in conjunction with nausea or vomiting; neither reported any sequelae.

## DISCUSSION

Nicotine replacement strategies, including pre-cessation NRT, aid smoking cessation with low cost and well-characterized safety profile. Personalizing NRT in ways that are informed by pre-cessation CO reductions and interactions between dependence and a v1.0 “quit success” genotype score appears to offer an attractive way of increasing cessation success for many smokers.

The plausibility of the results of this study is increased by several *a priori* considerations. The extent of nicotine dependence, assessed by FTND score, was likely, *a priori*, to influence success in quitting smoking, based on large amounts of evidence from many studies of smoking cessation, including some with pre-cessation NRT<sup>5, 9</sup>. Genetic influences on smoking cessation were also likely, *a priori*, to influence smoking cessation success, based on the substantial heritable influences documented in twin studies<sup>27</sup>. Retrospective molecular genetic studies identified SNP markers that provided nominally-significant associations with quit success in one or more of three initially-reported samples<sup>18</sup>. Many of the genomic regions identified by these SNPs have also been identified by studies of quit success in three additional samples<sup>28, 29</sup>. Since none of these associations is of large magnitude, we assembled a v1.0 molecular genetic measure relevant to quit success by combining data from groups of SNPs that provide nominally-significant associations with this phenotype in three prior studies. This “baseline” characteristic of each participant was joined by data from CO reductions during pre-cessation NRT, since prior data supported the predictive power of such CO reductions.

Remarkably, each of these participant characteristics exerted a significant direct or interactive influence on smoking cessation success in ways that depended on NRT dose. Our results support the hypothesis that different nicotine doses are more efficacious for different subgroups of smokers. The higher, 42 mg/24h NRT dose benefited the highly dependent smokers with low v1.0 genotype scores. By contrast, this high dose impeded quitting by less-dependent smokers with low v1.0 scores. The robust interactions between nicotine dose, dependence and genotype score are supported by: 1) the results of interim analysis having been borne out in the entire sample; and 2) replication within separate subsamples of smokers with European or African ancestry. These observations represent an apparent success in applying an “integrate(d).. sum of (genomic).. variants.. use(d)..in the context of.. (appropriate) clinical information” to prospectively help predict a clinical outcome. This use of data from sets of SNPs that individually provide modest association signals appears to document clinical utility for genome wide association data from this trait with polygenic genetic architecture <sup>19-22</sup>.

In addition to supporting a personalized treatment approach that takes into account baseline smoker characteristics, our results suggest that treatment algorithms could adapt therapeutic approaches based on smokers’ initial reduction in *ad lib* smoking in response to pre-cessation nicotine patch treatment. Smokers whose end-expired air CO level decreased >55.6% before the target quit date exhibited abstinence rates more than three times higher than those showing smaller decreases in CO. By rapidly identifying individuals who show insufficient CO reductions during pre-cessation NRT and hence are less likely to quit, we might avoid failed quit attempts through prompt application of alternative and/or more intensive treatments. Several such treatments, bupropion and varenicline, are more costly than NRT and appear to display greater risks <sup>30-32</sup>. A logical, stepped-care approach would thus entail initial use of pre-cessation NRT, assignment of nicotine dose based on dependence level and quit-success genotype scores, identification of individuals who do not reduce CO sufficiently and prompt

reassignment of such individuals to alternative therapies. Identification of the best alternatives for individuals who display insufficient CO reductions in response to NRT appears to provide a fruitful area for future study.

The current study, while offering support for the interactive effects of NRT dose, dependence level and quit-success genotype score, also has limitations. This randomized clinical trial might not replicate in other samples or generalize to clinical practice. The US Food and Drug Administration has not approved use of pre-cessation NRT, NRT doses > 21 mg/24 h or denicotinized cigarettes in conjunction with NRT. Initial studies do support efficacy of denicotinized cigarettes<sup>27</sup>. Pre-cessation treatment is approved in Australia and the UK, and is under consideration by other regulatory authorities. The v1.0 genotype score, while displaying substantial promise, was based on data from European-American participants in only three sets of clinical trials<sup>18</sup>. We anticipate that the power of this approach will be further enhanced as data from additional studies allows us to improve this score. Finally, even after future results replicate those in the present report, it will be necessary to surmount barriers to acceptance prior to implementation in smoking cessation clinical trials and/or in clinical practice. These barriers include costs, perceptions about genotyping by researchers, physicians and patients, and other practical issues such as turnaround time for testing<sup>33,34</sup>.

In summary, the present results provide support for a *personalized and adaptive* approach to smoking cessation treatment, which tailors the dose of NRT to the baseline phenotypic and genotypic characteristics of the individual smoker, and adapts the treatment based on early assessments of therapeutic response. In the foreseeable future, implementing such an algorithm in clinical practice should markedly enhance smokers' chances of ridding themselves of their health-damaging addiction to cigarettes.

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**Figure legends**

Fig. 1. Study timeline.

Fig. 2. Depiction of participant recruitment, eligibility assessment, allocation to treatment conditions and disposition.

Fig. 3. Smoking abstinence as a function of nicotine patch dose, dependence and quit success genotype score.

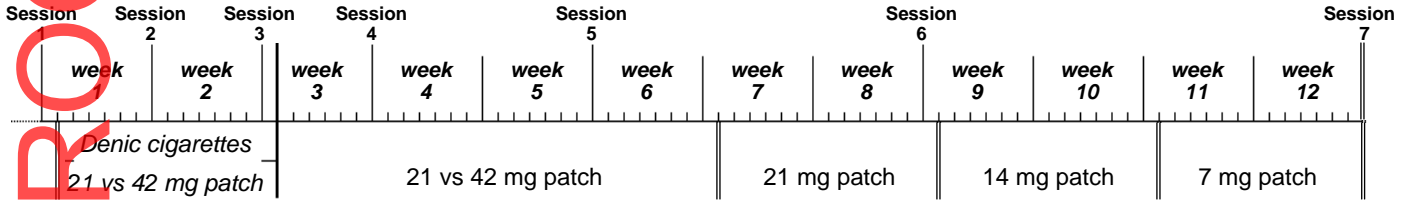
Fig. 4. Continuous 10-week smoking abstinence as a function of end-expired air CO reduction during the 2-week pre-cessation period.

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**Table 1.** Baseline Participant Characteristics

		<i>Low dependence smokers</i>		<i>High dependence smokers</i>	
		<b>21mg</b>	<b>42mg</b>	<b>21mg</b>	<b>42mg</b>
Age mean (SD)		43.2 (11.1)	42.2 (11.4)	45.0 (11.2)	43.8 (10.9)
Gender (male/female)		45/70	46/70	51/67	60/58
Race/ethnicity					
European-American	n (%)	92 (80.0)	80 (70.0)	99 (83.9)	87 (73.7)
African-American	n (%)	19 (16.5)	27 (23.3)	17 (14.4)	24 (20.3)
Other	n (%)	4 (3.5)	9 (7.8)	2 (1.7)	7 (5.9)
Nicotine yield	mean (SD)	0.87 (0.2)	0.90 (.90)	0.91 (.28)	0.96 (.27)
Cigarettes/day	mean (SD)	19.4 (8.0)	18.9 (6.6)	29.4 (9.9)	28.2 (11.2)
Years smoked	mean (SD)	23.8 (10.5)	22.7 (11.1)	26.8 (11.6)	25.6 (10.6)
FTND score	mean (SD)	4.6 (1.6)	4.7 (1.5)	8.0 (1.0)	8.0 (1.0)
End-expired air CO	mean (SD)	24.9 (9.5)	24.3 (9.7)	31.0 (13.1)	29.7 (10.8)

Target quit date



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933 Assessed for eligibility

454 Excluded  
395 Did not meet inclusion criteria  
18 Did not provide consent  
41 Other reasons

479 Randomized

Enrollment

Allocation

Participation

Analysis

120 Assigned to  
21 mg patch,  
Low Dependence group

120 Assigned to  
42 mg patch,  
Low Dependence group

119 Assigned to  
21 mg patch  
High Dependence group

120 Assigned to  
42mg patch,  
High Dependence group

43 Withdrew (or were lost  
to follow-up) between  
Randomization and 10wks  
Post Quit Date

53 Withdrew (or were lost  
to follow-up) between  
Randomization and 10wks  
Post Quit Date

50 Withdrew (or were lost  
to follow-up) between  
Randomization and 10wks  
Post Quit Date

51 Withdrew (or were lost  
to follow-up) between  
Randomization and 10wks  
Post Quit Date

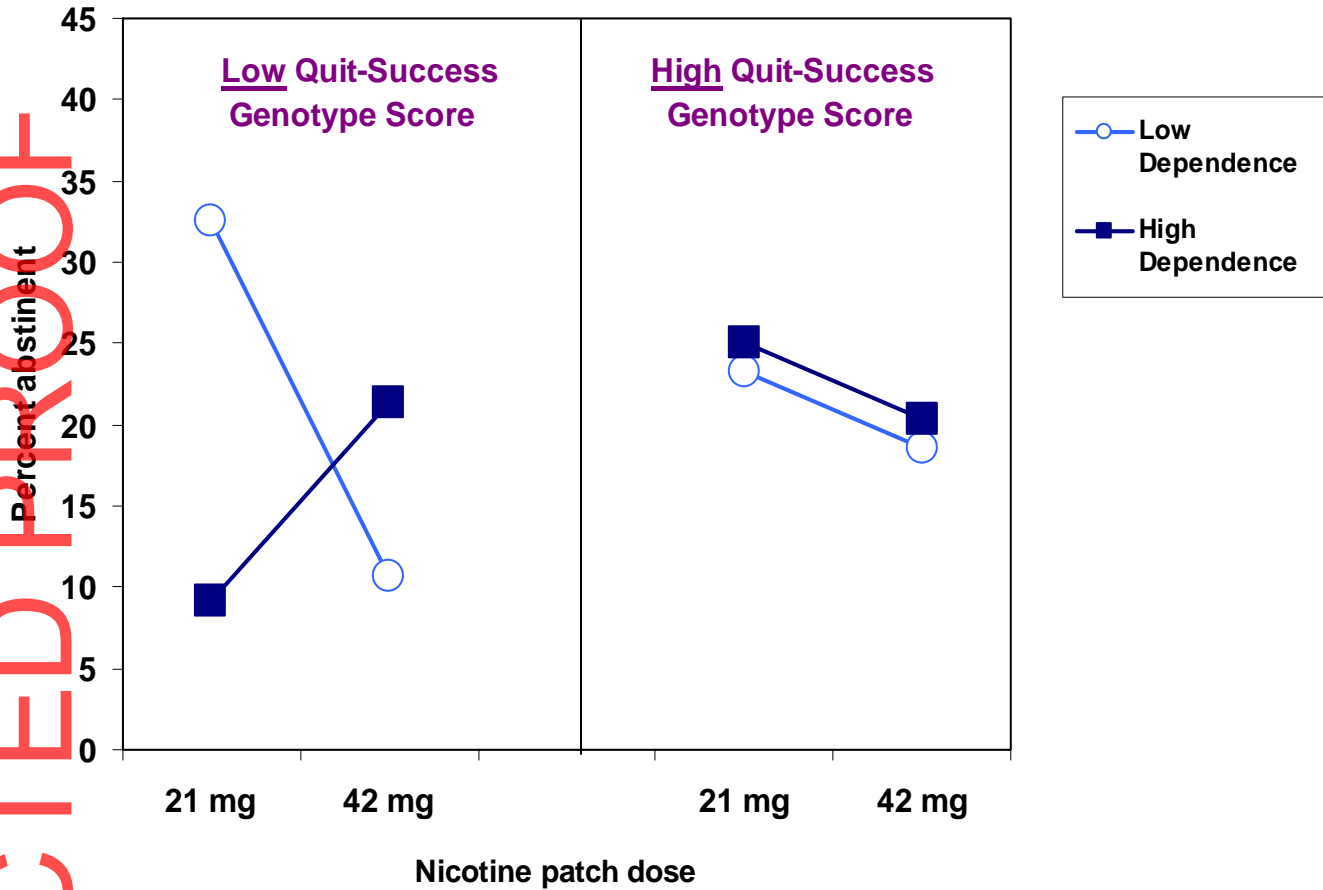
115 Analyzed  
5 Excluded from analysis  
due to use of excluded  
medications

116 Analyzed  
4 Excluded from analysis  
due to use of excluded  
medications

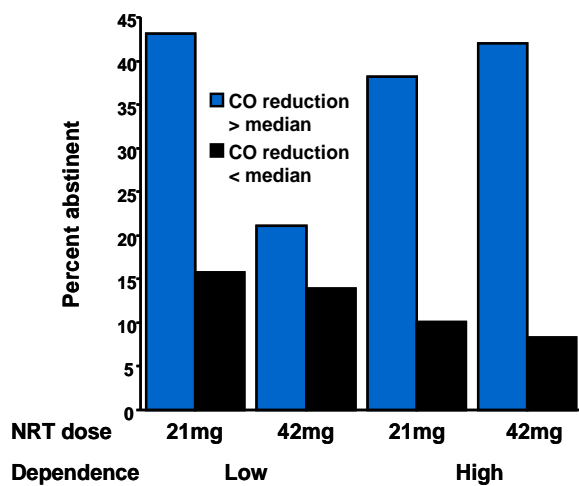
118 Analyzed  
1 Excluded from analysis  
due to use of excluded  
medications

118 Analyzed  
2 Excluded from analysis  
due to use of excluded  
medications

Molecular Medicine  
Continuous abstinence (through week 10)



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**Supplement 1: Exclusionary characteristics**

Subjects could be included if they were 18-65 years old, reported smoking an average of > 10 cigarettes that each yielded > 0.5 mg nicotine per day, and displayed end-expired air CO > 10 ppm. Exclusion criteria included: coronary heart disease; myocardial infarction; cardiac rhythm disorder; chest pains (unless history, exam, and EKG clearly indicated a non-cardiac source); cardiac disorder (including but not limited to valvular heart disease, heart murmur, heart failure); hypertension (systolic >140 mm Hg, diastolic >100 mm Hg for individuals with diagnosed hypertension, and > 160/100 for those with no previous diagnosis); hypotension (systolic <90 mm Hg, diastolic <60 mm Hg); history of skin allergy; active skin condition (psoriasis) within the last five years; skin disorder except minor skin conditions (including but not limited to facial acne, minor localized infections, and superficial minor wounds.); liver or kidney disorder (except kidney stones, gallstones); gastrointestinal problems or disease other than gastroesophageal reflux or heartburn; ulcers; lung disorder (including but not limited to COPD, emphysema, and asthma); brain abnormality (including but not limited to, stroke, brain tumor, seizure disorder); history of fainting; problems giving blood samples; difficulty passing urine; diabetes treated with insulin, non-insulin treated diabetes (unless glucose was < 180mg/dcl and HbA1c was < 7%); current cancer or treatment for cancer in the past 6 months (except basal or squamous cell skin cancer); other major medical condition; current psychiatric disease (with the exception of depression, anxiety disorders, OCD and ADHD); alcohol or drug abuse; use of an investigational drug within the last 30 days, psychiatric medications (including antidepressants, anti-psychotics or any other medications known to affect smoking cessation (e.g., clonidine); use of smokeless tobacco (chewing tobacco, snuff), cigars, pipes, nicotine replacement therapy, or other smoking cessation treatment within the last 2 weeks; pregnancy or nursing mothers.

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**Supplement 2: Genotyping**

DNA was extracted from blood samples using QIAamp Blood DNA Maxi Kits (Valencia, CA). DNA was quantitated using OD260 and picogreen (Invitrogen, Carlsbad, CA), and genotyped using Affymetrix 6.0 arrays. Briefly, 250 ng of individual DNA was digested using *Sty* I or *Nsp* I, ligated to corresponding oligonucleotide adaptors and amplified using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City CA). Conditions were 3 min at 94°C, 30 cycles of 30 sec at 94°C, 45 sec at 60°C, 15 sec at 68°C and a final 7 min 68°C extension. PCR products were purified using magnetic beads (Agencourt Ampure, Beckman Coulter, Beverly, MA) and a filtration system (MultiScreen Deep Well Solvinert Low Binding Hydrophilic PTFE; Millipore, Billerica, MA). 135 µg of purified PCR products were digested for 35 min at 37°C with 30 units of DNase I to produce 30-200 bp fragments, as verified by agarose gel electrophoresis. Fragments were end-labeled using terminal deoxynucleotidyl transferase and biotinylated dideoxynucleotides and hybridized to Affymetrix 6.0 arrays which were stained, washed and scanned as described<sup>35-38</sup>.

**Supplement 3: assignment of v 1.0 quit success genotype scores**

Genotypes and v1.0 scores were assigned for each participant by investigators blinded to clinical phenotype. We assessed alleles at the 12,058 SNPs for which at least one of three previously-reported smoking cessation success clinical trial samples had identified nominally-highly-significant ( $P < 0.01$ ) differences between successful vs unsuccessful quitters, based on strength and replicability of the associations<sup>18</sup>. We eliminated SNPs for which HapMap minor allele frequencies were 0 and SNPs for which opposite phase of association was demonstrated in two samples. Average p values for SNPs that displayed nominally-significant associations in multiple samples were determined, a factor [F1 = 0.01 –  $P(\text{average})$ ] was determined for each SNP. F1 factors for all such SNPs were scaled such that a subject homozygous for all of these abstinence-associated SNPs would thus score 900. SNPs that were

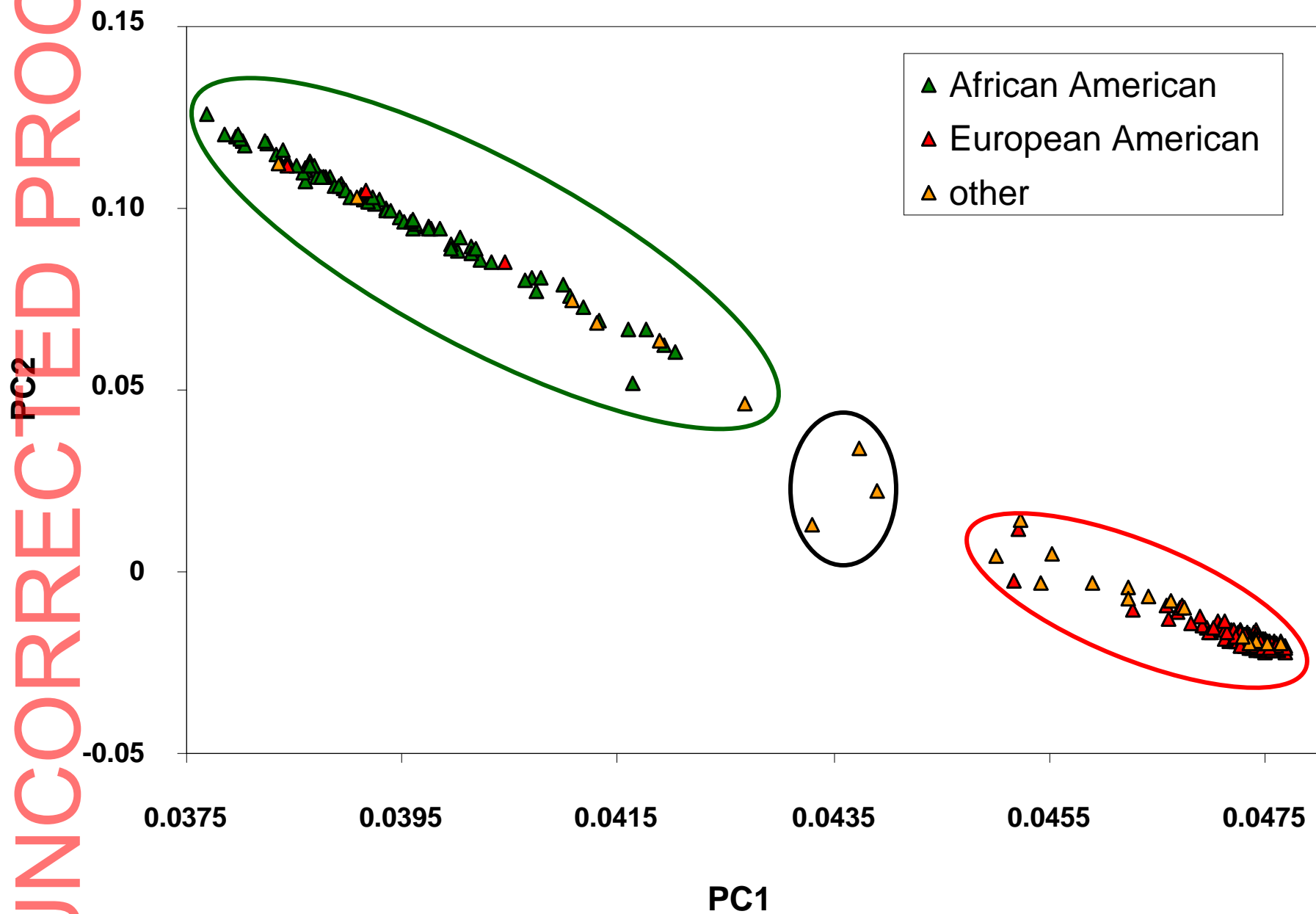
associated with abstinence in only one of the three prior samples were scaled to a total of 100. For each subject, an individual abstinence score was determined by 1) multiplying the factor for each SNP by the count of abstinence alleles for that SNP, and then 2) summing those values for the set of all SNPs. Each individual could thus achieve a quit-success genotype score of 1000. In preplanned analyses, we compared individuals with upper- to lower half quit success scores, seeking to a) minimize effects of unknown distributions and other properties of these scores and b) parallel the analyses used for FTND scores and changes in CO levels.

#### **Supplementary Figures:**

Fig. S1. Principal component analysis based on 1 million SNP genotypes separate the participants in this trial according to their ethnic background (PC1 and PC2 together account for over 95% of meaningful variance). Individuals were assigned to the groups based on predominant ethnicity in the PC1/PC2 cluster (African American cluster circled green, European American cluster circled red). 3 individuals with ambiguous cluster assignment were excluded from the analysis (circled black). The legend indicates “self reported” ethnicity.

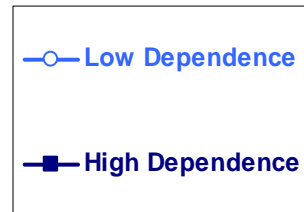
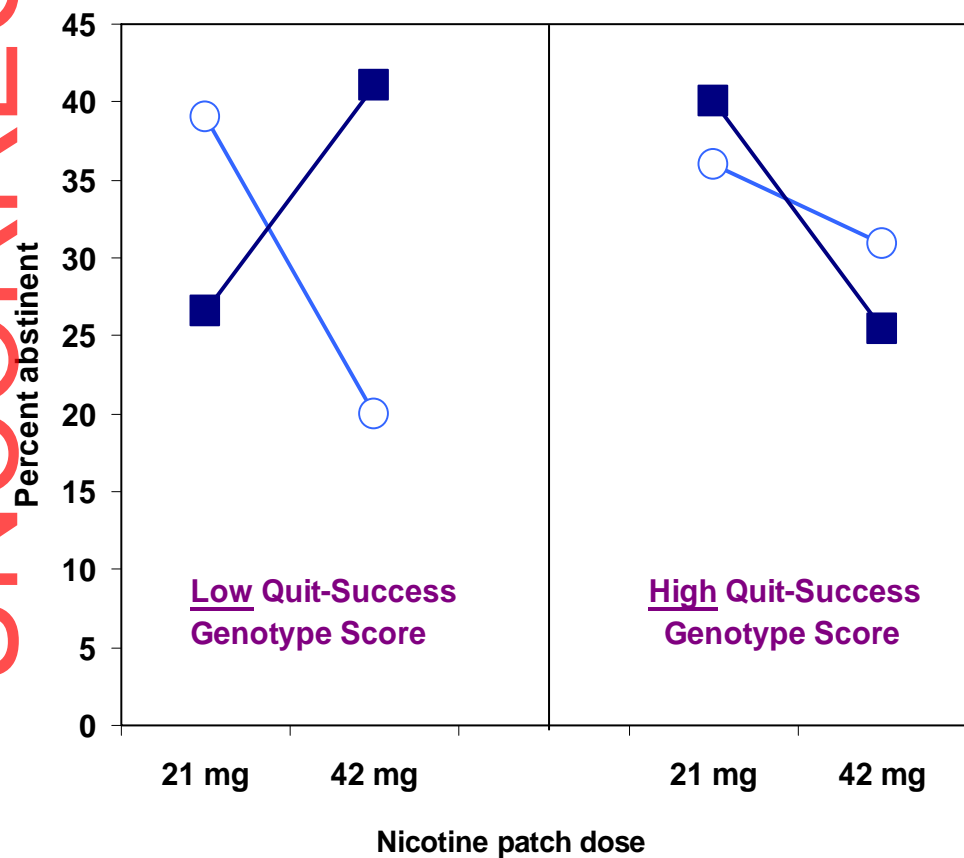
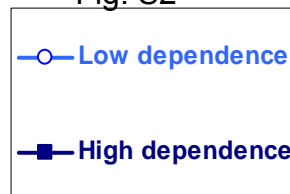
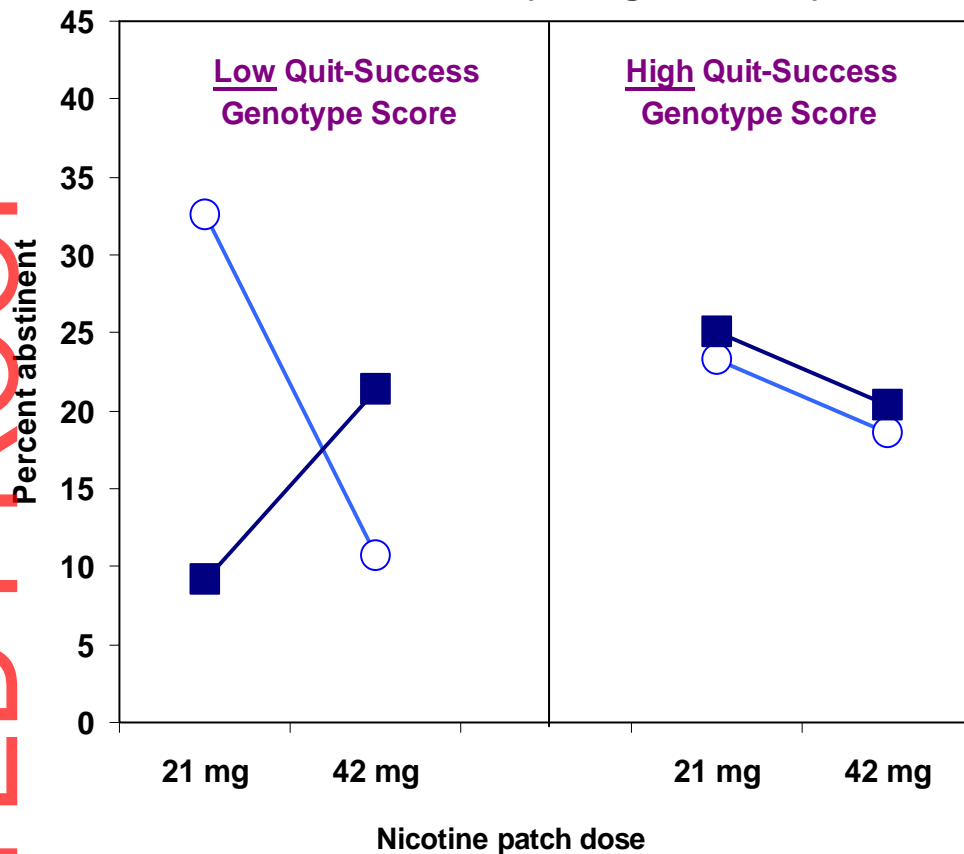
Fig. S2. Smoking abstinence as a function of nicotine patch dose (NRT dose), dependence (FTND) and quit success genotype score. Upper panel depicts percent 10-week continuous abstinence after the quit date; lower panel depicts percent 4-week continuous abstinence from week 7 to week 10 after the quit date.

Fig. S3. Six-month point (7-day) abstinence at 6 months after the quit date, as a function of nicotine patch dose (NRT dose), dependence (FTND) and quit success genotype score.



# Continuous abstinence (through week 10)

Fig. S2



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