

Use of the nicotine metabolite ratio as a genetically informed biomarker of response to nicotine patch or varenicline for smoking cessation: a randomised, double-blind placebo-controlled trial



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Summary

Background Substantial variability exists in therapeutic response and adverse effects with pharmacotherapies for tobacco dependence. Biomarkers to optimise treatment choice for individual smokers might improve treatment outcomes. We tested whether a genetically informed biomarker of nicotine clearance, the nicotine metabolite ratio (NMR; 3'-hydroxycotinine:cotinine), predicts response to nicotine patch or varenicline for smoking cessation.

Methods We undertook NMR-stratified multicentre, randomised, placebo-controlled clinical trial from Nov 16, 2010, to Sept 12, 2014, at four sites. Smokers seeking treatment were randomly assigned by baseline NMR status and study site, in blocks of 12 patients (1:1:1 ratio), to 11 weeks of placebo (placebo pill plus placebo patch), nicotine patch (active patch plus placebo pill), or varenicline (active pill plus placebo patch), plus behavioural counselling. Participants and investigators were masked to group allocation and NMR status. An intention-to-treat analysis was done. Participants were followed up for 12 months after the target quit date. The primary endpoint was biochemically verified 7 day point prevalence abstinence at the end of treatment to estimate the pharmacological effect of treatment by NMR. The trial is registered at ClinicalTrials.gov, number NCT01314001.

Findings 1246 participants (662 slow metabolisers of nicotine, 584 normal metabolisers of nicotine) were enrolled and randomly assigned to the three interventions (408 placebo, 418 nicotine patch, 420 varenicline). At end of treatment, varenicline was more efficacious than nicotine patch in normal metabolisers (OR 2.17, 95% CI 1.38–3.42; $p=0.001$), but not in slow metabolisers (OR 1.13, 0.74–1.71; $p=0.56$). In the longitudinal model including all timepoints, the NMR-by-treatment interaction was significant (ratio of odds ratios [ORR] 1.96, 95% CI 1.11–3.46; $p=0.02$). An NMR-by-treatment interaction showed that slow (vs normal) metabolisers reported greater overall side-effect severity with varenicline versus placebo ($\beta=-1.06$, 95% CI -2.08 to -0.03 ; $p=0.044$).

Interpretation Treating normal metabolisers with varenicline and slow metabolisers with nicotine patch could optimise quit rates while minimising side-effects.

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Introduction

Despite substantial reductions in tobacco use since the 1960s, rates of tobacco use have remained stable in the USA for the past decade¹ and have increased in low-income countries.² Worldwide, about 6 million annual deaths are attributable to tobacco use,² and US\$200 billion is spent on tobacco-related health-care costs.³ Smoking cessation treatments approved by the US Food and Drug Administration include nicotine replacement therapies, bupropion, and varenicline. Although transdermal nicotine patch is the safest and most widely used form of pharmacotherapy in the USA and Europe,⁴ end-of-treatment quit rates in clinical trials rarely exceed 30%.⁵ The efficacy of nicotine patch is comparable with bupropion,⁶ but could be lower than varenicline.^{7,8} However, varenicline's efficacy might be offset by the greater likelihood of side-effects.⁹ The substantial

individual variability in therapeutic response and side-effects provides a strong rationale to validate novel biomarkers to optimise pharmacotherapy choice.¹⁰

We identified a genetically informed biomarker of nicotine clearance, specifically the ratio of two metabolites derived from nicotine during smoking, 3'-hydroxycotinine and cotinine, referred to as the nicotine-metabolite ratio (NMR). The NMR reflects the activity of the liver enzyme CYP2A6, the major nicotine-metabolising and cotinine-metabolising enzyme. A substantial advantage of the NMR over CYP2A6 genotyping is that it incorporates both genetic and environmental (eg, oestrogen) effects on CYP2A6 activity and nicotine clearance.¹¹ Retrospective analyses of previous randomised trials have shown that slow metabolisers (lower NMR values and rates of nicotine clearance) respond well to nicotine patch, with no incremental benefit from the non-nicotine

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See [Comment](#) page 91

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replacement therapy medication bupropion; normal metabolisers do more poorly than slow metabolisers on nicotine patch, but benefit from bupropion.^{12–15} To date, no study has examined whether the NMR predicts the efficacy of varenicline, a widely used non-nicotine replacement therapy medication that is more efficacious than bupropion.^{16,17}

To translate these findings to practice, we did the first NMR-stratified placebo-controlled randomised trial of nicotine patch versus varenicline among 1246 smokers. Although CYP2A6 does not contribute to varenicline metabolism, previous bupropion trial data¹⁴ suggested that a non-nicotine replacement therapy medication would aid quitting among normal metabolisers. Among normal metabolisers, we expected varenicline to be more efficacious than nicotine patch, whereas among slow metabolisers, we expected nicotine patch and varenicline to be equally efficacious.

Methods

Study design and participants

We randomly assigned participants by NMR group to one of three treatment groups: placebo (placebo pill plus placebo patch); nicotine patch (placebo pill plus active patch); or varenicline (active pill plus placebo patch) (figure 1). Our primary aim was to compare the efficacy of nicotine patch versus varenicline by NMR group (normal metabolisers *vs* slow metabolisers). A placebo condition was included to examine side-effects of treatment by NMR group.

We undertook the clinical trial at four academic medical centres (University of Pennsylvania, Centre for Addiction and Mental Health, University of Toronto, State University of New York at Buffalo, and MD Anderson Cancer Center); assessment of the NMR was done at the University of Toronto. From Nov 16, 2010, to Sept 16, 2013, we recruited participants through advertisements for a free smoking cessation programme. Eligible participants were 18–65 years old and reported smoking ten cigarettes or more per day for 6 months or longer (verified by carbon monoxide concentrations greater than 10 ppm).

Exclusion criteria included use of non-cigarette tobacco products, e-cigarettes, or current smoking treatment; history of substance misuse treatment, current use of cocaine or methamphetamine, or more than 25 alcoholic drinks per week; medical contraindications (pregnancy, history of cancer, kidney or liver disease, or transplant, clinically significant cardiac dysrhythmias, stroke, angina, heart attack, or uncontrolled hypertension); history of DSM-IV Axis 1 psychiatric disorder or suicide risk score on the MINI International Neuropsychiatric Interview (MINI) of more than 1, or current major depression; current use of antipsychotics, stimulants, opiate medications, anti-coagulants, rescue inhalers, antiarrhythmics, or medications altering CYP2A6 activity (eg, monoamine oxidase inhibitors, tricyclic antidepressants); and inability to provide informed consent or any condition that could

compromise safety. All patients provided written, informed consent.

Randomisation and masking

A biostatistician, independent of the study investigators, developed the randomisation procedure, which was integrated into a centralised data management system. We randomly assigned participants to the treatment groups in a 1:1:1 ratio. Randomisation was stratified by baseline NMR status and study site, and blocked in blocks of 12 patients (four per treatment per block) to ensure approximate balance. Participants, study investigators, and personnel (except for the biostatistician and senior data manager) were masked to treatment group allocation and NMR status. Data were unmasked for analysis after collection of all 6-month follow-up data.

Procedures

Participants eligible at telephone screening completed an in-person medical examination and psychiatric history, completed self-report measures of demographics and smoking history, and provided blood samples for the NMR assay. NMR results, reported within 7 days, were used for final eligibility determination.

We assessed demographics, smoking rate, and nicotine dependence (Fagerström Test for Nicotine Dependence).¹⁸ The MINI determined lifetime history of axis I psychiatric diagnoses. For the NMR assay, cotinine and 3'-hydroxycotinine were assessed by liquid chromatography–mass spectrometry; limits of quantification were 1 ng or less per mL of whole blood.¹⁹ Self-reported withdrawal symptoms were measured with the Minnesota Nicotine Withdrawal Scale (pre-quit and weeks 0, 1, 4).²⁰ A self-report checklist measured the severity of common side-effects (none [0] to severe [3]), at weeks 0 (target quit date), 1, and 4, and was summed to create a side-effects index (we refer to these as side-effects, rather than adverse events to distinguish these responses from the open-ended serious adverse events reported by participants).¹⁵ We assessed self-reported use of pills and patches at each visit and collected unused patches and pill blister-packages to confirm self-reports.²¹

Slow metabolisers were oversampled to create a study sample of roughly equal numbers of slow metabolisers and normal metabolisers. For the first 25% of participants enrolled, the NMR cutoff for stratification (and oversampling of slow metabolisers) was 0.26 based on a previous clinical trial of nicotine replacement therapy.¹⁵ However, to ensure achievement of recruitment goals, the data and safety monitoring board made a determination (masked to study outcomes) to increase the stratification cutoff to 0.31 (redefining slow metabolisers as NMR <0.31 and normal metabolisers [including rapid metabolisers] as those with NMR ≥0.31). This cut-off was based on graphical representation of the NMR in the screened population (appendix), and was used in all analyses.

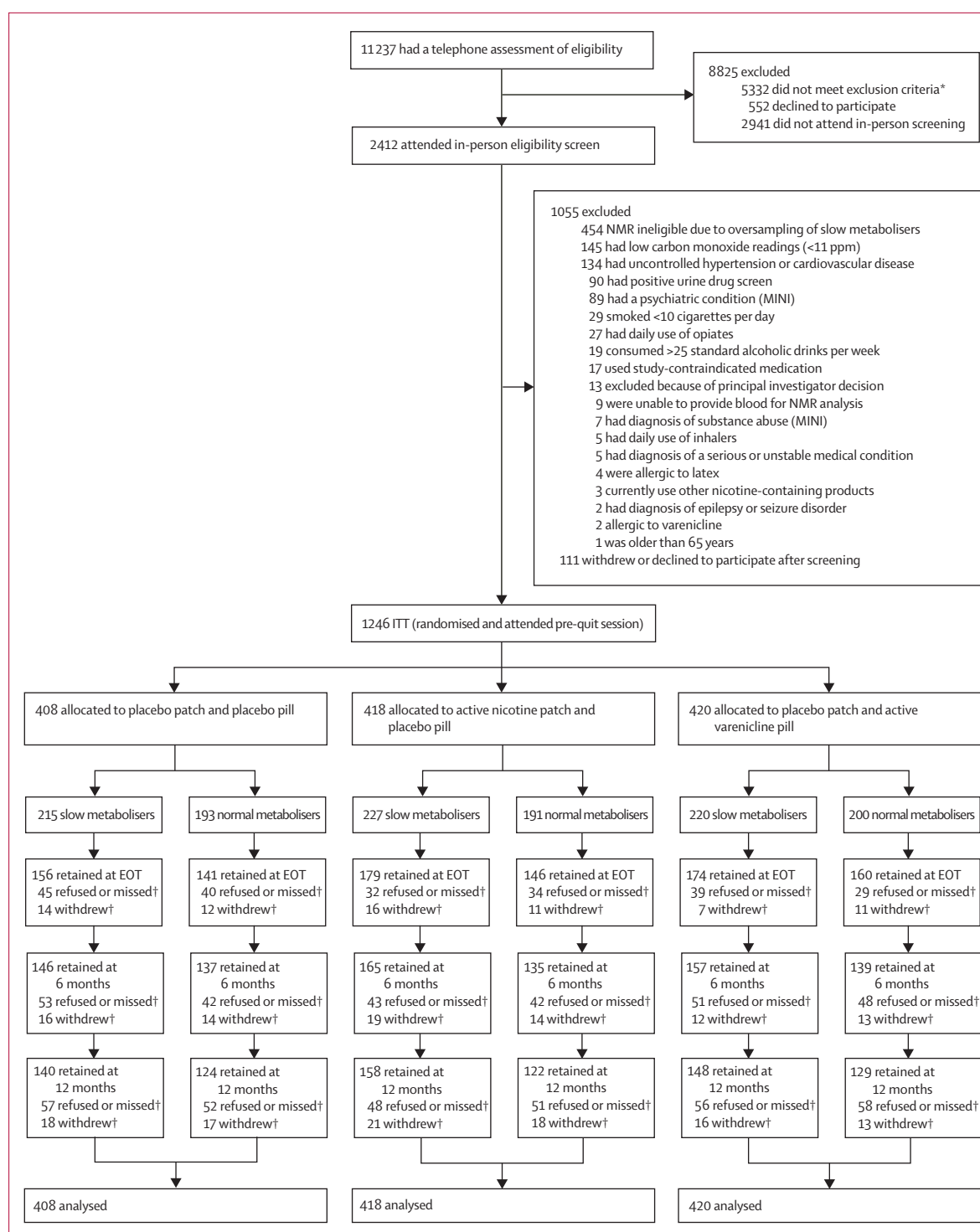


Figure 1: Trial profile

Inclusion and exclusion criteria were identical for phone and in-person eligibility assessments; participants who were ineligible or who withdrew or declined enrolment before randomisation were not analysed or followed. Between the end of treatment and 12-month follow-up assessments, 256 participants reported use of some form of smoking cessation medication (nicotine replacement therapy, varenicline, or bupropion). Reported use was not associated with 6-month or 12-month cessation rates, treatment group, or NMR group. NMR=nicotine metabolite ratio. MINI=Mini International Neuropsychiatric Interview. ITT=intention-to-treat. EOT=end of treatment. *Reasons for participant ineligibility at phone screening are available from the authors on request. †Those included in an intention-to-treat analysis.

After an in-person 1 h pre-quit group counselling session at the local clinical site (week -1), varenicline (or matching placebo) was initiated using the guidelines for 1 week pre-cessation dose titration. Nicotine patch (or matching placebo) was initiated on the morning of the target quit date. Brief (about 15 min), protocol-driven, telephone counselling was delivered by two counsellors at University of Pennsylvania (weeks 0 [target quit date], 1, 4, 8). Counselling focused on skills to quit and avoid relapse, instructions on the use of medication, and medication compliance. Staff at University of Pennsylvania collected mid-treatment data (eg, withdrawal, side-effects) by telephone. Self-reported smoking status was assessed using a standard timeline follow-back procedure,²² and biochemically verified.

The University of Pennsylvania Investigational Drug Service distributed medication to the clinical sites. Active patches were purchased from GlaxoSmithKline (Nicoderm CQ) and identical placebo patches were purchased from Rejuvenation Labs (Salt Lake City, UT, USA). Participants received 11 weeks of patches to match the duration of varenicline after the target quit date: 21 mg (6 weeks), 14 mg (2 weeks), and 7 mg (3 weeks). Pfizer manufactured active varenicline and matching placebo pills. Varenicline was delivered for 12 weeks (1 week before the target quit date) as in previous trials:¹⁷ days 1–3 (0.5 mg once daily); days 4–7 (0.5 mg twice daily); and days 8–84 (1.0 mg twice daily). The institutional review boards at all sites approved the protocol.

Outcomes

The primary endpoint was 7-day point prevalence abstinence at end of treatment (week 11) to estimate the pharmacological effect by NMR group during the medication period. This outcome was chosen based on guidelines for smoking cessation trials.²³ Abstinence was defined as no self-reported smoking (not even a puff) for at least 7 days before the telephone assessment, with in-person verification for those self-reporting abstinence (carbon monoxide 8 ppm or less).²³ Participants lost to follow-up were considered smokers.²³ To estimate the pharmacological effect by NMR, participants should be on medication; therefore, the end of treatment quit rate was the primary endpoint. Secondary endpoints were side-effects, withdrawal symptoms, and 6-month and 12-month quit rates.

Statistical analysis

To test the primary hypothesis of an NMR-by-treatment interaction at end of treatment, we estimated a logistic regression model using all data. This model included variables for the odds ratios (ORs) for the treatment effects (nicotine patch *vs* varenicline) within slow metabolisers and normal metabolisers. We measured the interaction effect as the ratio of the odds ratios (ORRs), calculated by exponentiating the coefficients corresponding to the

interaction terms in the logistic model. These models were repeated for 6-month and 12-month cessation outcomes. We also used longitudinal logistic regression (general estimating equations) to examine the NMR-by-treatment interaction incorporating all timepoints. All models controlled for study site; we also tested our multivariate models controlling for sex, nicotine dependence, and race. Self-reported side-effects (continuous measure from the checklist) were examined for each active treatment (*vs* placebo) within each NMR group using general estimating equation models including timepoint, and adjusted for study site and pre-quit levels of side-effects; the NMR-by-treatment interaction was estimated as a difference of differences (β coefficients, which estimate mean differences in side-effect severity).²⁴ The correlation structure used in the general estimating equation model for side-effects used subject-specific random effects. The model was linear and the measures were continuous. Assumptions were assessed and the distribution was non-normal. However, we verified that we obtained the same results using bootstrap methods (with exact CIs) that are not sensitive to violations of normality. We did receiver operating curve (ROC) analyses with abstinence as the binary response and NMR as the continuous predictor, separately in the three treatment groups. We tested for heterogeneity among ROC values using the methods of DeLong and colleagues²⁵ as implemented in Stata ROCCOMP.

Our original target sample was 1350. The data safety and monitoring board did a masked interim futility analysis, based on the conditional power method,²⁶ in February, 2013. Additionally, we recalculated the power and determined that a sample size of 1200 would provide adequate power. Specifically, the sample size of 1200 provided 80% power to detect an ORR of 3.2 for the NMR-by-treatment (nicotine patch *vs* varenicline) interaction at end of treatment. In models that included the placebo group, the full intention-to-treat sample of 1246 participants (662 slow metabolisers, 584 normal metabolisers) was used, including the placebo group. In models that compared varenicline with nicotine patch, the intention-to-treat sample of 838 was used, excluding the placebo group. Stata (version 13) was used for the analyses. The trial is registered at ClinicalTrials.gov, number NCT01314001.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Retention rates at end of treatment exceeded 70% and retention did not vary across treatment groups or NMR group (figure 1). The treatment groups did not differ on

	Placebo			Nicotine patch			Varenicline		
	Slow metaboliser (n=215)	Normal metaboliser (n=193)	All (n=408)	Slow metaboliser (n=227)	Normal metaboliser (n=191)	All (n=418)	Slow metaboliser (n=220)	Normal metaboliser (n=200)	All (n=420)
Ethnic origin									
White	97 (45%)	128 (66%)	225 (55%)	117 (52%)	119 (62%)	236 (56%)	99 (45%)	133 (66%)	232 (55%)
Black	95 (44%)	56 (29%)	151 (37%)	94 (41%)	57 (30%)	151 (36%)	107 (49%)	53 (27%)	160 (38%)
Other	23 (11%)	9 (5%)	32 (8%)	16 (7%)	15 (8%)	31 (7%)	14 (6%)	14 (7%)	28 (7%)
Female	83 (39%)	91 (47%)	174 (43%)	88 (39%)	94 (49%)	182 (44%)	88 (40%)	99 (49%)	187 (45%)
High school education or lower	68 (32%)	57 (30%)	125 (31%)	78 (34%)	49 (26%)	127 (30%)	74 (34%)	61 (30%)	135 (32%)
Income US\$50 000 or more	65 (31%)	79 (41%)	144 (36%)	82 (37%)	75 (39%)	157 (38%)	74 (34%)	71 (36%)	145 (35%)
Age (years)	44 (11)	47 (11)	46 (11)	46 (11)	46 (11)	46 (11)	44 (12)	46 (12)	45 (12)
Not employed	79 (37%)	84 (44%)	163 (40%)	69 (30%)	71 (37%)	140 (33%)	88 (40%)	75 (37%)	163 (39%)
FTND score	5.3 (1.92)	5.4 (2.00)	5.4 (2.00)	5.2 (2.00)	5.3 (1.89)	5.2 (1.90)	5.1 (2.00)	5.1 (2.02)	5.1 (2.01)
Cigarettes per day	17.6 (7.0)	19.6 (8.7)	18.5 (7.9)	17.6 (7.0)	18.5 (7.0)	18.0 (7.0)	16.7 (5.4)	18.4 (6.3)	17.5 (5.9)

Data are n (%) or mean (SD). There were no significant differences between treatment groups. Data are complete except 17 participants refused to provide data on income. NMR=nicotine metabolite ratio. FTND=Fagerström Test for Nicotine Dependence.

Table 1: Baseline demographic and smoking history by treatment group and NMR group

demographic and smoking history variables (table 1). Slow metabolisers were younger ($p=0.02$), less likely to be white ($p<0.0001$), more likely to be male ($p=0.001$), and smoked fewer cigarettes per day ($p<0.001$), versus normal metabolisers, as reported previously.²⁷ Sex differences in the NMR groups are expected because of oestrogen effects on nicotine metabolism rate,²⁷ and the difference in ethnicity is expected because of differences in the frequency of reduced or inactive CYP2A6 alleles. The average NMR for slow metabolisers was 0.20 (SD 0.07) and for normal metabolisers was 0.50 (0.18). The distribution of NMR in the screened population ($n=1733$) and in the intention-to-treat sample ($n=1246$) where slow metabolisers were oversampled is shown in the appendix.

The NMR-by-treatment interaction was significant in the general estimating equation model that included all timepoints (ORR 1.96, 95% CI 1.11–3.46; $p=0.02$). The 12-month effect for timepoint (OR 0.58, 0.46–0.72; $p<0.001$) and the corresponding timepoint-by-treatment interaction (ORR 0.58, 0.42–0.79; $p=0.001$) showed that the efficacy of varenicline decreased significantly over time (compared with nicotine patch). We also did a validation analysis in which we re-ran the analyses in four different ways: by considering all dropouts to be smoking (analysis by general estimating equation); by including only cases who completed end of treatment, 6-month, and 12-month follow-up (general estimating equation); by considering dropouts at any timepoint as missing (general estimating equation); and by considering dropouts as missing data (analysis by mixed model, valid under missing-at-random dropout). The results were similar under all four models, with ORRs ranging from 2.00 to 2.07, and p values ranging from 0.01 to 0.04.

At end of treatment, varenicline was more efficacious than nicotine patch in normal metabolisers (OR 2.17, 95% CI 1.38–3.42; $p=0.001$), but not in slow metabolisers

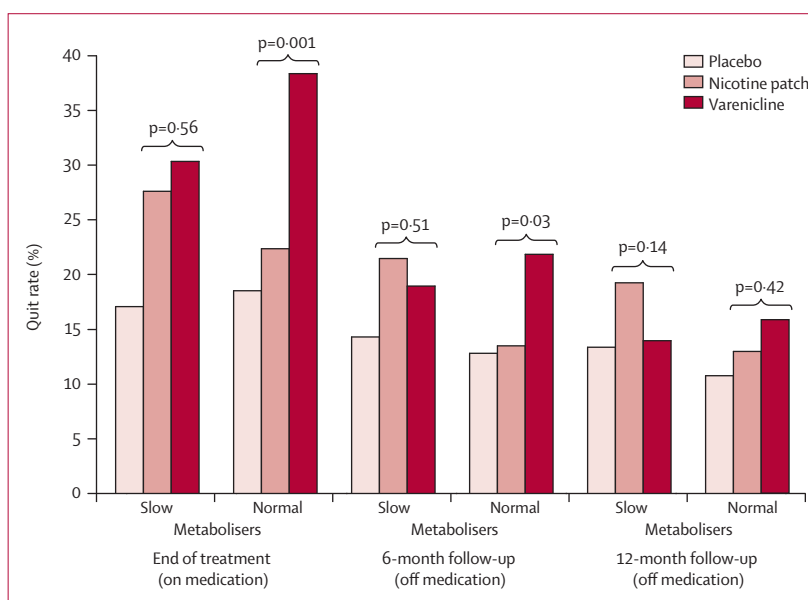


Figure 2: Quit rates by treatment group and NMR group

Significant interaction for the head-to-head comparison of nicotine patch versus varenicline in the longitudinal (general estimating equation) model (ORR 1.96, 95% CI 1.11–3.46, $p=0.02$). Placebo shown for comparison. Individual regression model values were: ORR 1.89, 95% CI 1.02–3.45, $p=0.04$ (end of treatment); ORR 2.07, 1.01–4.22, $p=0.05$ (6 months); ORR 1.78, 0.83–3.80, $p=0.14$ (12 months). Individual p values on graph correspond to regression models comparing nicotine patch with varenicline within metaboliser group. NMR=nicotine metabolite ratio. ORR=ratio of odds ratios.

(OR 1.13, 0.74–1.71; $p=0.56$; figure 2), yielding a significant NMR-by-treatment interaction (ORR 1.89, 1.02–3.45; $p=0.04$). In a model including the placebo group as a reference, the interaction effect was similar ($p=0.05$). Of the covariates, only nicotine dependence score predicted quitting significantly (OR 0.83, 95% CI 0.76–0.90; $p<0.001$). At 6 months, varenicline was more efficacious than nicotine patch in normal metabolisers (OR 1.81, 95% CI 1.05–3.11; $p=0.03$), but not in slow

	Placebo		Nicotine patch		Varenicline	
	Slow metaboliser (n=215)	Normal metaboliser (n=193)	Slow metaboliser (n=227)	Normal metaboliser (n=191)	Slow metaboliser (n=220)	Normal metaboliser (n=200)
Week -1 (pre-quit)	3.95 (4.84)	3.40 (4.34)	3.26 (3.97)	3.97 (4.74)	3.05 (3.93)	3.57 (4.19)
Week 0 (target quit date)	4.22 (4.37)	4.27 (4.57)	3.98 (3.59)	4.28 (4.96)	4.68 (4.66)	4.06 (4.01)
Week 1	5.58 (5.08)	5.46 (5.00)	5.44 (5.03)	5.58 (5.48)	6.04 (5.23)	5.26 (4.98)
Week 4	5.33 (5.70)	4.93 (5.68)	4.24 (4.40)	4.52 (5.46)	4.97 (4.88)	4.39 (3.96)

Data are mean (SD). NMR=nicotine metabolite ratio.

Table 2: Total side-effect severity index by treatment group and NMR group

metabolisers (OR 0.85, 95% CI 0.53–1.37; $p=0.51$); the NMR-by-treatment interaction was statistically significant (ORR 2.07, 95% CI 1.01–4.22; $p=0.05$). For this model, nicotine dependence score also predicted quitting significantly (OR 0.83, 95% CI 0.76–0.91; $p<0.001$). The interaction effect at 12 months was not significant (ORR 1.78, 95% CI 0.83–3.80; $p=0.14$). At end of treatment, slow metabolisers had quit rates of placebo 17.2%, nicotine patch 27.7%, and varenicline 30.4% compared with normal metabolisers, placebo 18.6%, nicotine patch 22.5%, and varenicline 38.5%. At 6 months, slow metabolisers had quit rates of placebo 14.4%, nicotine patch 21.6%, and varenicline 19.1%, compared with normal metabolisers, placebo 12.9%, nicotine patch 13.6%, and varenicline 22.0%. At 12 months, slow metabolisers had quit rates of placebo 13.4%, nicotine patch 19.4%, and varenicline 14.1%, compared with normal metabolisers, placebo 10.9%, nicotine patch 13.1%, and varenicline 16.0%.

The number needed to treat was calculated using conventional methods,²⁸ comparing nicotine patch with placebo, and varenicline with placebo, at end of treatment. Among normal metabolisers, nicotine patch yielded a number needed to treat of 26.0 (95% CI 19.7–32.3), whereas varenicline yielded a number needed to treat of 4.9 (4.7–5.1). For slow metabolisers, the numbers needed to treat were 10.3 (95% CI 9.4–11.2) for nicotine patch and 8.1 (7.5–8.7) for varenicline.

The ROC area was 0.51 (95% CI 0.43–0.58) for placebo, 0.54 (0.47–0.60) for nicotine patch, and 0.54 (0.48–0.60) for varenicline. Although effects were in the predicted direction, comparisons of ROC areas between treatment groups were not significant.

For varenicline (*vs* placebo), a significant NMR-by-treatment interaction was observed in side-effects (summary from the self-report checklist; $\beta=-1.06$, 95% CI -2.08 to -0.03; $p=0.044$). This reflected greater summary side-effects reported on varenicline (*vs* placebo) for slow metabolisers ($\beta=0.61$, 95% CI -0.10 to 1.32; $p=0.09$), but not for normal metabolisers ($\beta=-0.44$, 95% CI -1.19 to 0.30; $p=0.24$). Descriptive (post-hoc) item-level analysis showed that, in slow metabolisers, varenicline led to

significant increases in nausea ($\chi^2=18.7$, $p=0.0003$) and abnormal dreams ($\chi^2=13.0$, $p=0.005$); in normal metabolisers, varenicline led to significant increases in nausea ($\chi^2=15.7$, $p=0.01$), but decreases in irritability ($\chi^2=15.4$, $p=0.001$), anxiety ($\chi^2=11.2$, $p=0.01$), and attentional disturbance ($\chi^2=11.3$, $p=0.01$). For side-effects on nicotine patch (*vs* placebo), the NMR-by-treatment interaction was not significant ($\beta=-0.17$, 95% CI -1.21 to 0.86; $p=0.74$).

Serious adverse events, defined as any adverse event irrespective of causality that resulted in death, was life-threatening, required hospitalisation, or resulted in disability or incapacity, were determined by site physicians. There were 16 (3.9%), 22 (5.3%), and 11 (2.6%) serious adverse events on placebo, nicotine patch, and varenicline, respectively. Treatment group effects or NMR-by-treatment interactions on serious adverse events counts were not significant (see appendix for side-effect counts by treatment and NMR groups, and table 2 for summary side-effect values). There were no NMR-by-treatment interactions for withdrawal symptoms or medication adherence ($p>0.10$). On average, 62% of participants used 80% or more of the pill dose recommended and 63% used 80% or more of the patches recommended, comparable with previous reports.^{21,29}

Discussion

In this biomarker-stratified randomised clinical trial, varenicline was better than nicotine patch for normal metabolisers, but had equivalent efficacy for slow metabolisers. Slow metabolisers, but not normal metabolisers, reported more overall side-effects on varenicline (*vs* placebo). Although slow metabolisers were oversampled to be about 50% of the intention-to-treat sample, 40% of smokers attending the study intake were classified as slow metabolisers by the 0.31 cutpoint (appendix). Thus, matching treatment choice based on the NMR could provide a viable clinical strategy for optimising quit rates for all smokers, whilst minimising side-effects for slow metabolisers.

As expected, the association of the NMR with treatment response decreased after treatment was discontinued. A large decrease in quit rates on varenicline was observed over time, consistent with previous reports.¹⁷ As a result of the relapsing nature of tobacco dependence, the current paradigm of short-term treatment has been challenged by clinical trials of extended duration therapy.²¹ A placebo-controlled trial comparing 6 months of extended-duration nicotine patch versus the standard 8 weeks duration of nicotine patch showed that slow metabolisers achieve significant benefit from extended therapy with 6 month quit rates of about 50%.³⁰ An important question is whether normal metabolisers would benefit from extended varenicline therapy.

An improved understanding of the mechanisms underlying NMR associations with treatment response could help to refine the use of this biomarker in clinical

practice. The higher quit rates with nicotine patch among slow metabolisers than normal metabolisers are not because of differences in plasma nicotine concentrations,¹³ nor are these differences because of nicotine withdrawal as shown here. Moreover, the NMR is not associated with nicotine dependence in most studies,¹⁵ and controlling for baseline cigarettes per day or dependence did not alter our findings.

Other neuropharmacological mechanisms can be considered. Because normal metabolisers smoke more cigarettes per day than slow metabolisers,³¹ conditioned smoking responses might be stronger in this group. Normal metabolisers have enhanced responses in the brain's dopamine reward circuitry when viewing smoking cues, compared with slow metabolisers.³² Furthermore, normal metabolisers have greater daily fluctuation in blood (and presumably brain) nicotine concentrations than slow metabolisers, which could contribute to greater reward from smoking.³³ This finding could explain why, for normal metabolisers, both varenicline and bupropion are more efficacious than nicotine patch. Although bupropion and varenicline have differing mechanisms of action, both are non-nicotine replacement therapy medications that increase dopamine concentrations in brain reward pathways.

Our study had strengths and limitations. It is the largest pharmacogenetic study of tobacco dependence treatment and the first to use prospective stratification. The rate of loss to follow up did not differ by treatment group or NMR group. The mixed ethnicity of participants and the absence of sex or race interactions with NMR and treatment suggest that the NMR works well in both white and African-American smokers; however, a limitation is that few Hispanics or Asians were included. As in most smoking cessation trials, we excluded individuals with major psychiatric and medical comorbidities, restricting the generalisability of our findings. The quit rates are lower than some previous studies,^{17,21} which might be due to the high unemployment rate in our sample (37%). Tobacco use is associated with unemployment, and was reported to increase during the recent economic decline in the USA,³⁴ coinciding with our study.

With respect to the clinical implications, it could be argued that varenicline might be superior to the nicotine patch for smokers overall and that the side-effects of varenicline are generally mild and tolerable. However, varenicline does have a black-box warning in the USA. Furthermore, our data show that slow metabolisers do not achieve greater benefit from varenicline relative to nicotine patch, and are vulnerable to more side-effects than normal metabolisers.

Thus, in addition to the imperative of increasing the utilisation of treatments for nicotine dependence,³⁵ our data suggest that treating normal metabolisers with varenicline, and slow metabolisers with nicotine patch could provide a viable clinical approach. Extending the duration of use of these treatments could potentially

Panel: Research in context

Systematic review

We searched PubMed for English language publications using the terms “nicotine metabolite ratio” and “smoking cessation” (or “tobacco/nicotine dependence treatment”), without date restrictions. We located five retrospective analyses of data collected from randomised trials testing associations of 3'-hydroxycotinine to cotinine ratio with response to smoking cessation treatments. None of these studies prospectively stratified randomisation to alternative treatments, nor did any include the newer medication varenicline. All five papers are cited.^{12–15,30}

Interpretation

Our findings showed that varenicline was better than nicotine patch for normal metabolisers of nicotine, but had equivalent efficacy for slow metabolisers. Slow metabolisers, but not normal metabolisers, reported more overall side-effects on varenicline (vs placebo). These results support the potential clinical validity of the nicotine metabolite ratio as a biomarker to guide the choice of therapy for individual smokers. To optimise quit rates for all smokers while minimising side-effects, our data support treating normal metabolisers (60% of smokers in the population) with varenicline, and slow metabolisers with nicotine patches.

sustain the benefits of tailored treatment.³⁰ The NMR is practical for clinical use because it is unaffected by time of day of sampling, is stable at room temperature, is not dependent on time since last cigarette among ad-libitum smokers, and is stable within a smoker over time.^{11,19,36} Although, the assay results were reported in under 1 week at US\$50 per sample, it is conceivable that a point-of-care test could be developed and implemented in clinical practice. However, using the NMR to individualise treatment might incur additional costs and time. Our findings also underscore the notion that tobacco dependence is a heterogeneous condition and pharmacotherapies are not equally effective for all smokers (panel).

Contributors

CL and RFT designed the study and obtained funding. RAS, EPW, NIB, GES, and DFH assisted with design of the study, and preparation of the application for funding. RAS, LWH, PC, and TPG supervised data collection at the four sites. EPW and DFH supervised data analysis. Members of the PGRN-PNAT Research Group were involved in data collection and study implementation. All authors contributed to the writing. All authors have read and approved the manuscript.

Declaration of interests

CL received study medication and placebo, and support for medication packaging, from Pfizer; she has also consulted to Gilead, and has been a paid expert witness in litigation against tobacco companies. PC served on the scientific advisory board of Pfizer Pharmaceuticals, did educational talks sponsored by Pfizer on smoking cessation from 2006 to 2008, and has received grant support from Pfizer. RAS received medication and placebo free of charge from Pfizer for a different project, and has consulted to Pfizer and GlaxoSmithKline. TPG has had both investigator-initiated and industry-sponsored grants from Pfizer in the past 12 months, and serves on a data monitoring committee for Novartis. NIB has served as a consultant to several pharmaceutical companies that market smoking cessation medications and has been a paid expert witness in litigation against tobacco companies. RFT has acted as a consultant to pharmaceutical companies, primarily on smoking cessation. The remaining authors declare no competing interests.

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