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Smoking Cessation Pharmacotherapy Based on Genetically-Informed Biomarkers: What is the Evidence?

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Orestis A. Panagiotou, MD,^{1,2} Ewoud Schuit, PhD,^{3,4,5} Marcus R Munafò, PhD,⁶ Derrick A Bennett, PhD,⁷ Andrew W. Bergen, PhD,^{8,9} Sean P. David, MD, SM, DPhil^{5,10}

1. Department of Health Services, Policy and Practice, Brown University School of Public Health, Providence, RI
2. Center for Gerontology and Healthcare Research, Brown University School of Public Health, Providence, RI
3. Cochrane Netherlands, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands
4. Julius Center for Health Sciences and Primary Care, University Medical Center Utrecht, Utrecht University, Utrecht, Netherlands
5. Meta-Research Innovation Center at Stanford (METRICS), Stanford University, Stanford, CA
6. MRC Integrative Epidemiology Unit at the University of Bristol, and School of Psychological Science, University of Bristol, Bristol, UK
7. Clinical Trial Service Unit and Epidemiological Studies Unit, Nuffield Department of Population Health, University of Oxford, Oxford, UK.
8. Biorealm, Walnut, CA

9. Oregon Research Institute, Eugene, OR

10. Division of Primary Care and Population Health, Department of Medicine, Stanford University, Stanford, CA

Corresponding Author: Orestis A. Panagiotou, MD, PhD, Department of Health Services, Policy & Practice, Brown University School of Public Health, 121 South Main St., Providence, RI, 02912. Email: orestis_panagiotou@brown.edu; Tel: 401-863-3894.

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ABSTRACT

Introduction: Pharmacogenomic studies have used genetic variants to identify smokers likely to respond to pharmacological treatments for smoking cessation.

Methods: We performed a systematic review and meta-analysis of primary and secondary analyses of trials of smoking cessation pharmacotherapies. Eligible were trials with data on *a priori* selected single nucleotide polymorphisms (SNPs), replicated nonSNP polymorphisms, and/or the nicotine metabolite ratio (NMR). We estimated the genotype-by-treatment interaction as the ratio of risk ratios (RRR) for treatment effects across genotype groups.

Results: We identified 18 trials (n=9,017 participants), including 40 active (bupropion, nicotine replacement therapy [NRT], varenicline, or combination therapies) versus placebo comparisons and 16 active versus active comparisons. There was statistical evidence of heterogeneity across rs16969968 genotypes in *CHRNA5* with regard to both six-month abstinence and end-of-treatment abstinence in non-Hispanic black smokers and end-of-treatment abstinence in non-Hispanic white smokers. There was also heterogeneity across rs1051730 genotypes in *CHRNA3* with regard to end-of-treatment abstinence in non-Hispanic white smokers. There was no clear statistical evidence for other genotype-by-treatment combinations. Compared to placebo, NRT was more effective among non-Hispanic black smokers with rs16969968-GG with regard to both six-month abstinence (RRR for GG vs. GA or AA, 3.51; 95% CI 1.19-10.30) and end-of-treatment abstinence (RRR for GG vs. GA or AA, 5.84; 95% CI, 1.89 to 18.10). Among non-Hispanic white smokers, NRT effectiveness relative to placebo was comparable across rs1051730 and rs169969960 genotypes.

Conclusions: We did not identify widespread differential effects of smoking cessation pharmacotherapies based on genotype. The quality of the evidence is generally moderate.

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IMPLICATIONS

Although we identified some evidence of genotype-by-treatment interactions, the vast majority of analyses did not provide evidence of differential treatment response by genotype. Where we find some evidence, these results should be considered preliminary and interpreted with caution because of the small number of contributing trials per genotype comparison, the wide confidence intervals, and the moderate quality of evidence. Prospective trials and individual-patient data meta-analyses accounting for heterogeneity of treatment effects through modeling are needed to assess the clinical utility of genetically-informed biomarkers to guide pharmacotherapy choice for smoking cessation.

INTRODUCTION

Tobacco smoking remains the leading cause of preventable death in the world. Successful smoking cessation substantially reduces risks for smoking-related diseases and many FDA-approved pharmacotherapies are available, including nicotine replacement therapy (NRT), sustained-release bupropion, and varenicline. Meta-analyses have confirmed the efficacy of these therapies on abstinence at six-month or longer follow-up.¹ Yet, most pharmacological treatments are ineffective for the majority treatment-seeking smokers.¹

The long-term abstinence rates are less than 40% even with the most efficacious smoking cessation treatments¹ and there is known to be substantial heritability for tobacco dependence. Investigators have explored associations between genetic polymorphisms for tobacco dependence and smoking cessation drug targets (many of which overlap) to better identify individuals who are more or less likely to successfully quit and abstain from smoking in response to specific medications. Such genomic analyses have the potential to improve the efficacy of smoking cessation drugs and “personalize” treatment.² As the number of pharmacogenomic studies for smoking cessation increases, synthesizing the emerging evidence can inform treatment decisions.³

We examined the state of the evidence as to whether genetic information could be used to identify smokers likely to respond to pharmacological treatments for smoking cessation. This report summarizes a large evidence synthesis conducted for the Cochrane Collaboration.⁴

METHODS

Data Sources and Searches

We searched the Cochrane Tobacco Addiction Group specialized register, which contains trials from MEDLINE, Embase, PsycINFO, and reference lists of previous trials and overviews. We also searched the WHO portal, the UK clinical trials gateway, the US, Australian, and New Zealand clinical trials registers, and genetic/genomic databases (Pharmacogenomics Knowledgebase, Pharmacogenomics of Nicotine Addiction Treatment).

Eligibility Criteria

We sought to identify, and deemed eligible, published and unpublished randomized and quasi-randomized controlled trials (RCTs) of adult smokers seeking nicotine-based (all forms of NRT, e.g. patch, gum, lozenge, inhaler, spray) and/or non-nicotine-based (e.g. bupropion, varenicline, cytisine, nortriptyline) smoking cessation pharmacotherapies, regardless of setting, nicotine dependence or ethnicity. The primary and secondary outcomes were point prevalence smoking abstinence at six months and at end of treatment (typically between 7 and 12 weeks), respectively. For each RCT, information on *a priori* selected genome-wide significant (P -value $< 5 \times 10^{-8}$) single nucleotide polymorphisms (SNPs) for cigarettes per day, nicotine dependence, or smoking cessation was recorded. Information on non-SNP polymorphisms associated with smoking cessation treatment response in at least two trials and the Nicotine Metabolite Ratio (NMR), which is highly influenced by the cytochrome p450 2A6 (*CYP2A6*) gene,⁵ was recorded.

Evidence Quality

We assessed included studies for risks of selection bias, performance and detection bias, and other threats to study quality.⁶ We also assessed sources of bias specific to genotype studies:

genotype frequencies for each study and polymorphism; deviation from Hardy-Weinberg equilibrium; source of DNA; genotyping protocol; and quality control methods.⁷ To rate the certainty in the overall findings of the systematic review, we used the Grading of Recommendations Assessment, Development and Evaluation (GRADE) framework.

Statistical Analysis

We calculated the cessation rate as the number of people abstinent at six months and at end of treatment divided by the total number of participants, separately in each genotype/treatment subgroup. We performed fixed-effect meta-analyses of the effects of active treatment versus placebo on abstinence separately in reference homozygote major (M/M), heterozygote (M/m), and homozygote minor (m/m) genotype strata. We generated risk ratios (RR) and 95% confidence intervals (CI) for each comparison and for each genotype.

We assessed heterogeneity⁸ in treatment effects across genotype strata using the τ^2 , I^2 , and Cochran's Q statistics. For analyses with statistically significant heterogeneity across genotype subgroups, we estimated the difference in treatment effects in M/M versus m/m and in M/m versus m/m to capture the interaction effects of genotype x treatment. As estimates of treatment effects were expressed in the log-scale, we expressed the genotype-by-treatment interaction as the ratio of risk ratios (RRR) for treatment effect in one genotype group over treatment effect in the other genotype group. An RRR > 1 means that the treatment effect is greater in individuals with the M/M versus non-M/M genotype.

RESULTS

We included data from 18 trials of smoking cessation pharmacotherapies (N=9,017 participants).⁴ Data were available for nine SNPs in five genes (rs215605 in *PDE1C*; rs1329650 and rs1028936 in *HECTD2-ASI*; rs2036527, rs588765, rs16969968 and rs1051730 in the *CHRNA3-A5-B4* cluster; and rs7937 and rs3733829 in *EGLN2* near *CYP2A6*), two variable number of tandem repeat polymorphisms (VNTRs) in two genes (*DRD4* and *SLC6A4*), and the NMR. For two ethnic groups with sufficient data, fixed effect meta-analyses were performed for active vs. placebo (N=40), and active vs. active (N=16) pharmacotherapy trial arms within genotype groups separately for non-Hispanic black (n=2,093) and non-Hispanic white (n=6,924) smokers. Except for NRT vs. placebo and for those ethnicity groups, outcomes, and biomarkers shown in **Table 1**, we found no evidence of statistical between-genotype heterogeneity for any other biomarker (including NMR which is one of the most extensively studied biomarkers) and treatment comparisons.

For rs16969968 within the alpha-5 nicotinic acetylcholine receptor (*CHRNA5*) gene, NRT efficacy on six-month abstinence and end-of-treatment abstinence differed by rs16969968 genotype in non-Hispanic black smokers (P=0.03 and P=0.003 for heterogeneity between genotype groups for each outcome, respectively) as compared to placebo. Both six-month abstinence and end-of-treatment abstinence were increased in the NRT group as compared with the placebo group among participants with a GG (reference) genotype (**Table 1**). As shown in **Figure 1**, the relative efficacy of NRT vs. placebo was higher among non-Hispanic black smokers with rs16969968-GG genotype for both six-month abstinence (RRR for GG vs. GA or AA genotypes, 3.51; 95% CI 1.19 to 10.3) and end-of-treatment abstinence (RRR for GG vs. GA or AA, 5.84; 95% CI, 1.89 to 18.1). Further, end-of-treatment abstinence was higher with active NRT vs. placebo among in non-Hispanic white smokers with the rs16969968-GA genotype

($P=0.02$ for heterogeneity between genotype groups; **Table 1**). However, NRT effectiveness relative to placebo was comparable across rs16969968 genotypes (RRR for GG vs. AA, 0.78, 95% CI, 0.33 to 1.47; RRR for GA vs. AA, 1.04; 95% CI, 0.45 to 2.41) (**Figure 1**).

The effect of NRT vs. placebo also differed by genotypes of rs1051730 in the alpha-3 nicotinic acetylcholine receptor (*CHRNA3*) gene in non-Hispanic white smokers with regard to end-of-treatment abstinence ($P=0.004$ for heterogeneity between genotype groups; **Table 1**) as NRT was more effective than placebo among participants with GA or AA genotypes. However, as shown in **Figure 1**, NRT effectiveness relative to placebo was comparable across rs1051730 genotypes with no evidence of genotype-by-treatment interaction (RRR for GG vs. GA, 0.54, 95% CI 0.26 to 1.14; RRR for GA vs. AA, 0.96, 95% CI 0.44 to 2.11).

DISCUSSION

We found evidence that the efficacy of NRT vs. placebo on smoking abstinence differs by rs16969968 genotypes but the effect modification conferred by this SNP varies across ethnicity groups. Non-Hispanic black smokers with rs16969968-GG genotypes are 3.5-fold and 5.8-fold more likely to abstain at 6 months and at end of treatment, respectively. We found no evidence of effect modification for non-Hispanic white smokers except for short-term outcomes. Nevertheless, sample sizes for some genotype groups were small and the frequency of rs16969968 alleles differs between non-Hispanic black and non-Hispanic white smokers, perhaps explaining the observed results. The non-synonymous variant rs16969968 in *CHRNA5* results in an aspartic acid to asparagine change (D398N) in the nicotinic acetylcholine receptor $\alpha 5$ subunit protein, reducing $\alpha 4\beta 2\alpha 5$ response in an *in vitro* cellular model expressing the human allele and increasing nicotine self-administration in a murine self-administration paradigm.^{9,10}

The rs1051730 variant does not represent a functional variant and its associations with smoking behaviors are due to the high linkage disequilibrium with rs16969968 in European-ancestry populations.³ Both SNPs have the largest effects for smoking-related phenotypes in epidemiological studies,¹¹ which could explain the gene-by-treatment interactions observed here. One hypothesis for the differences observed between non-Hispanic black and non-Hispanic white smokers at rs16969968 could reflect the distribution of the rs16969968 minor allele across ancestries, where it is rare in African population samples (minor allele frequency, MAF<1%), at low frequency in African American populations (MAF=7%) and at moderate frequency in European ancestry populations (MAF=37%).¹⁰ We obtained genotype counts and ethnicity labels from investigators, but we did not have access to ancestry informative markers to adjust results for ancestry proportions; thus, we cannot exclude confounding by ancestry contributing to observed results at the rs16969968 variant. While these findings suggest superior short-term efficacy of NRT in specific rs1051730 and rs16969968 genotype subgroups among NHWs, the small number of available studies and the large uncertainty around effect estimates limit confidence in the validity of these findings.

Overall Completeness and Applicability of Evidence

Detection of gene-by-treatment interactions requires large sample sizes,¹² which are not currently available. Traditionally, RCTs are designed to address the comparative effectiveness of interventions but genomic analyses are usually conducted retrospectively after trial completion and are rarely taken into account at the design stage. Although we aimed to overcome sample size limitations of individual studies by combining data through meta-analysis, for most

genotype-by-treatment comparisons we were not able to obtain summary-level data from two or more trials.

We synthesized evidence on the effects of smoking cessation treatments according to specific genotypes. Therefore, our results relate to heterogeneity of treatment effects¹³ according to specific variants. Although treatment effect heterogeneity is critical for clinical and policy decisions, it provides only indirect evidence of the clinical utility of these genetic markers (i.e., whether measuring a particular biomarker and guiding treatment with this information will result in better or worse clinical outcomes). RCTs included in our analyses used biomarkers chosen on the basis of biological plausibility or prior genome-wide significance with regards to smoking behaviors. However, treatment response is likely to be under polygenic influences of multiple variants with small and large effects.

Evidence Gaps

The quality of available evidence is generally moderate with regard to modifying effects of genomic variants for smoking cessation pharmacotherapies. The quality of the evidence was most often downgraded because of imprecision and risk of bias due to potential selection bias in genotyping trial participants. Although these analyses suggest that some genetic subgroups respond more favorably to NRT, research participants were not assigned treatment based on their genotypes in the eligible studies. Additionally, none of the meta-analyses that provided evidence of differential treatment effects by genotype included more than two trials per genotype comparison resulting in wide confidence intervals reflecting the uncertainty of treatment effects. Moreover, owing to the large numbers of biomarkers, interventions, endpoints, and ancestry subgroups, the systematic review includes numerous analyses, which should be interpreted

cautiously due to multiple testing. Finally, confidence in these findings is further limited by the lack of correction for genetic ancestry.

Future Directions

Prediction of response to smoking cessation pharmacotherapies based on genomic information is complex posing challenges for the design and analysis of trials. Access to individual participant data (IPD) may improve the quantity and quality of data, enabling standardization of outcomes across trials and detailed data checking.¹⁴ Analysis of IPD offers flexibility in investigating effect modifiers and is particularly useful for addressing genotype-treatment interactions. Increases in data sharing by RCTs reporting response to treatment along with genetic association summary statistics may be the catalyst for future replication and meta-analytic studies.¹⁵

Recent methodological advances may facilitate the design and analysis of predictive medicine trials.¹⁶ Genome-wide data permit examination of multiple *a priori* SNPs in a genetic risk score for treatment response, along with the identification of SNPs associated with response to smoking cessation treatments. This may lead to the development and validation of predictive genomic signatures for response to smoking cessation therapies.¹⁷ As genotyping costs fall, it is important that trials interrogate genome-wide variation to identify biomarkers that predict treatment response for future assessment of clinical utility. Polygenic risk scores, combining effects of many individual **markers** that are strong predictors of outcomes, are likely to be helpful in this regard. Because thousands or millions of variants across the genome may confer useful predictive information, RCTs should routinely collect and analyze DNA samples at the genome-

wide level.^{2,3} The value of this practice is best exemplified in analyses of response to treatment for tobacco dependence, major depression, and schizophrenia.¹⁸⁻²⁰

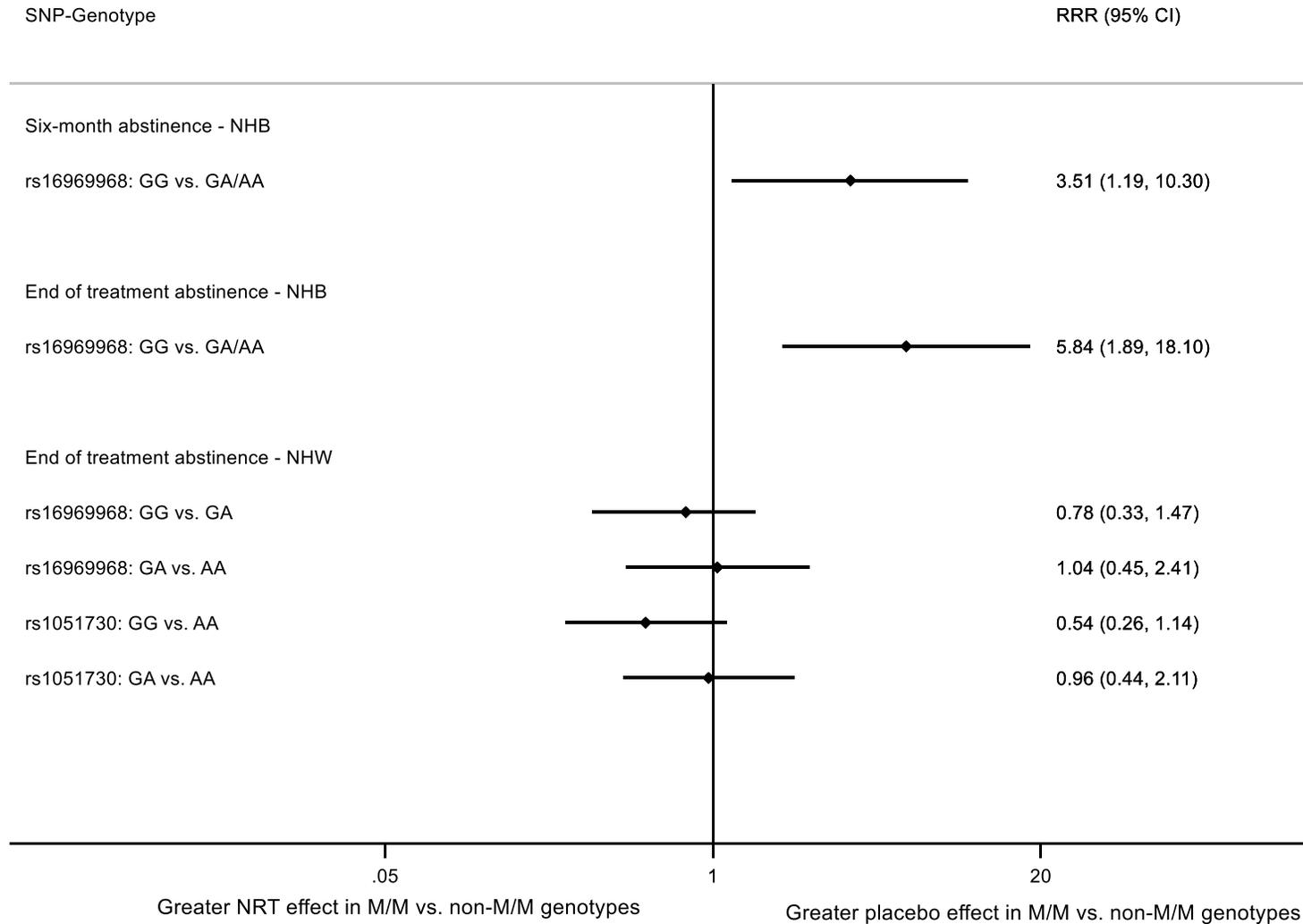
Table 1. Effectiveness of nicotine replacement therapy compared to placebo for genetic polymorphisms in the nicotinic acetylcholine receptor *CHRNA5/CHRNA3* locus, by ethnicity group. Shown are the meta-analyses with evidence of heterogeneity across genotype groups.

Ethnicity Group	Outcome	SNP-Genotype	RR (95% CI)	Between-genotype heterogeneity	Trials, N	Sample size, N
Non-Hispanic black or African American	Six-month abstinence	rs16969968-GG	1.47 (1.07-2.03)	<i>P</i> =0.03	2	709
		rs16969968-GA or AA	0.43 (0.15-1.26)			
	End of treatment abstinence	rs16969968-GG	1.57 (1.15-2.15)	<i>P</i> =0.003	2	709
		rs16969968-GA or AA	0.29 (0.10-0.86)			
Non-Hispanic white	End of treatment abstinence	rs16969968-GG	1.01 (0.77-1.33)	<i>P</i> =0.02	2	1,127
		rs16969968-GA	1.85 (1.33-2.59)			
		rs16969968-AA	1.80 (0.45-7.23)			
		rs1051730-GG	1.09 (0.85-1.41)	<i>P</i> =0.004	2	1,391
		rs1051730-GA	2.13 (1.52-2.97)			
rs1051730-AA	2.18 (1.04-4.58)					

Abbreviations: CI, confidence interval; SNP, single nucleotide polymorphism; RR, risk ratio.

For both ethnicity groups and for both rs16969968 and rs1051730, G is the major allele and A is the minor allele; linkage disequilibrium (R^2) between rs16969968 in *CHRNA5* and rs1051730 in *CHRNA3* is 0.46 in the African American ancestry sample “ASW” and 1.0 in the European American ancestry sample “CEU” from the 1000 Genomes Project.

Figure 1. Differential effectiveness of nicotine replacement therapy vs. placebo across genetic polymorphisms in the nicotinic acetylcholine receptor *CHRNA5/CHRNA3* locus, by ethnicity.



Abbreviations: CI, confidence interval; NHB: non-Hispanic black; M/M: homozygote major genotype; M/m: heterozygote genotype; m/m: homozygote minor genotype NHW: non-Hispanic white; SNP, single nucleotide polymorphism; RRR, relative risk ratio.

DECLARATION OF INTERESTS

Dr. Bergen is an employee of BioRealm, LLC, which intends to commercialize an analysis platform for substance use disorder studies. Dr. David has received funding for study medication from Pfizer through its Investigator Initiated Research Program and is a scientific advisor for BaseHealth. No other conflicts are reported.

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