

## ORIGINAL INVESTIGATION

# Organic Cation Transporter Variation and Response to Smoking Cessation Therapies

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Received February 16, 2014; accepted August 9, 2014

## ABSTRACT

**Introduction:** We evaluated chr6q25.3 organic cation transporter gene (SLC22A1, SLC22A2, SLC22A3) variation and response to smoking cessation therapies. The corresponding proteins are low-affinity transporters of choline, acetylcholine and monoamines, and smoking cessation pharmacotherapies expressed in multiple tissues.

**Methods:** We selected 7 common polymorphisms for mega-regression analysis. We assessed additive model association of polymorphisms with 7-day point prevalence abstinence overall and by assigned pharmacotherapy at end of treatment and at 6 months among European-ancestry participants of 7 randomized controlled trials adjusted for demographic, population genetic, and trial covariates.

**Results:** Initial results were obtained in 6 trials with 1,839 participants. Nominally statistically significant associations of 2 SLC22A2 polymorphisms were observed: (1) with rs316019 at 6 months, overall ([c.808T>G; p.Ser270Ala],  $OR = 1.306$ , 95% CI = 1.034–1.649,  $p = .025$ ), and among those randomized to nicotine replacement therapy (NRT) ( $OR = 1.784$ , 95% CI = 1.072–2.970,  $p = .026$ ); and (2) with rs316006 (c.1502-529A>T) among those randomized to varenicline ( $OR = 1.420$ , 95% CI = 1.038–1.944,  $p = .028$ ,  $OR = 1.362$ , 95% CI = 1.001–1.853,  $p = .04$ ) at end of treatment and 6 months. Individuals randomized to NRT from a seventh trial were genotyped for rs316019; rs316019 was associated with a nominally statistically significant effect on abstinence overall at 6 months among 2,233 participants ( $OR = 1.249$ , 95% CI = 1.007–1.550,  $p = .043$ ).

**Conclusions:** The functional OCT2 Ser270Ala polymorphism is nominally statistically significantly associated with abstinence among European-ancestry treatment-seeking smokers after adjustments for pharmacotherapy, demographics, population genetics, and without adjustment for multiple testing of 7 SNPs. Replication of these preliminary findings in additional randomized controlled trials of smoking cessation therapies and from multiple continental populations would describe another pharmacogenetic role for SLC22A2/OCT2.

## INTRODUCTION

chr6q25.3 polyspecific organic cation transporter gene variation may influence response to smoking cessation therapies. Public data supporting this hypothesis includes: linkage analyses of smoking behaviors; genome-wide association studies (GWAS) of metabolism; functional studies; Autosome and genome-wide linkage analyses of smoking intensity and duration, nicotine

dependence, and withdrawal identify significant markers or peaks in the chr6q23.2-q27 region (Supplemental Table 1). An autosome-wide meta-analysis of five linkage analyses<sup>1-4</sup> of the Fagerström Test for Nicotine Dependence (FTND)<sup>5</sup>, identified two chr6q23.2-q27 30 centiMorgan bins as the first two of six bins with nominal autosome-wide significance.<sup>6</sup> We searched the database of Genotypes and Phenotypes (dbGaP) to identify candidate GWAS SNPs in the chr6q23.2-q27 region. One study

doi:10.1093/ntr/ntu161

Advance Access publication August 20, 2014

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of human metabolic individuality<sup>7</sup> and two studies of kidney function measures<sup>8,9</sup> identified genome-wide significant SNPs within *SLC22A1* and *SLC22A2* (Supplemental Table 2). King *et al*<sup>10</sup> identified the *SLC22A2* single nucleotide polymorphism (SNP) rs595374 as associated with nausea in a candidate gene-based analysis of three RCTs randomizing individuals to varenicline, bupropion, or placebo.<sup>11–13</sup> Based on these public data, we examined *SLC22A1*, *SLC22A2*, and the flanking gene, *SLC22A3*.

*SLC22A1*, *SLC22A2*, and *SLC22A3* code for polyspecific organic cation transporter proteins (OCT1, OCT2, OCT3) of 554, 555, and 556 amino acids with 50% amino acid identity<sup>14</sup>, and with 12 transmembrane domain, intracellular amino terminal, and extracellular carboxy terminal, protein structure.<sup>15</sup> The proteins' primary function is to transport ligands into cells, but they can facilitate diffusion in either direction; OCT1 and OCT3 transport ligands across the basolateral hepatocyte membrane, OCT2 transports ligands across the basolateral renal proximal tubule cells, and OCT1 and OCT2 transport ligands across the luminal membrane of the brain circulatory endothelium.<sup>15</sup> Low affinity ligand binding sites are directly involved in transport, while high affinity binding sites are involved in allosteric regulation of transport.<sup>16</sup> The three genes/proteins are differentially expressed: predominantly in liver (*SLC22A1*/OCT1); predominantly in kidney (*SLC22A2*/OCT2); and in multiple peripheral organs and brain (*SLC22A3*/OCT3), respectively.<sup>17,18</sup> All three genes are expressed in multiple brain regions at lower levels than in the periphery.<sup>19–23</sup> All three proteins serve as low affinity Na<sup>+</sup> and Cl<sup>-</sup> independent transport systems for choline, acetylcholine, and monoamines.<sup>24</sup> OCT2 mediates post-synaptic transport of norepinephrine and serotonin<sup>25</sup> and synaptic vesicle transport in cholinergic neurons and neuromuscular junction neurons.<sup>26</sup> *SLC22A2* is expressed in dopaminergic areas of brain.<sup>27</sup> OCT3 is thought to represent the extraneuronal monoamine transport (uptake<sub>2</sub>) system,<sup>23,28–30</sup> and is also expressed in central neurons.<sup>31</sup> OCT1, OCT2, and OCT3 are expressed on the luminal membrane of ciliated bronchial epithelial cells, where acetylcholine transport by OCT1 and OCT2 was demonstrated.<sup>32</sup>

These studies suggest that OCT proteins could influence response to smoking cessation therapies: through their transport function in multiple organs and tissues, for example, of monoamines; directly, through transport of smoking cessation therapies; or indirectly, by regulation of OCT transport function by smoking cessation therapies. Nicotine inhibits tetraethylammonium (an OCTn model substrate) accumulation within a human embryonic kidney cell line (HEK-293) mediated by OCT1 with an IC<sub>50</sub> of 63 μM *in vitro*, and by OCT2 with an IC<sub>50</sub> of 50 μM.<sup>33</sup> Lips *et al*<sup>32</sup> demonstrated an IC<sub>50</sub> of 42 μM nicotine with OCT2 expressed in *Xenopus* oocytes. Feng *et al*<sup>34</sup> demonstrated that varenicline, excreted by the kidney predominantly without undergoing metabolism<sup>35</sup>, serves as an OCT2 substrate *in vitro*, and, at a much higher concentration, as an OCT2 inhibitor. A clinical study (*N* = 12) of joint administration of varenicline and cimetidine, a known OCT2 inhibitor, demonstrated reduced clearance and increased plasma concentration of varenicline of ~25% and 29%, changes that the authors did not consider clinically meaningful.<sup>34</sup> Haenisch *et al*<sup>36</sup> analyzed the influence of multiple antidepressants on 1-methyl-4-phenylpyridinium (another OCTn model substrate) uptake *in vitro* and demonstrated 18% inhibition of OCT2 at 29 μM

bupropion. These primarily *in vitro* studies provide evidence that multiple smoking cessation therapies influence OCT2 function, however the observed IC<sub>50</sub> values by nicotine (50 μM<sup>33</sup> or 42 μM<sup>32</sup>), varenicline (890 μM<sup>34</sup>), and bupropion (29 μM<sup>36</sup>) occur at much higher plasma drug concentrations (~100-fold–20,000-fold) than occur in patients taking prescribed doses of nicotine (~75 nM<sup>37</sup>), varenicline (~43 nM<sup>38</sup>) or bupropion (208–416 nM<sup>39</sup>). No published evidence suggests that smoking cessation pharmacotherapies influence OCT3 function.

To test whether OCT1, OCT2, or OCT3 are involved in clinical response to smoking cessation therapies, we analyzed seven SNPs from *SLC22A1*, *SLC22A2*, and *SLC22A3* in up to 2,233 treatment-seeking smokers randomized to multiple smoking cessation therapies from seven randomized controlled trials of treatment-seeking smokers. We report nominally statistically significant associations of two linked *SLC22A2* SNPs with prospective abstinence, without multiple test correction for seven SNPs.

## METHODS

### Human Subjects

Participant informed written consent was obtained by the Principal Investigators of each randomized controlled trial and of each laboratory trial of nicotine and cotinine metabolism. Institutional Review Board approval for this research was obtained by Principal Investigators at the University of California San Francisco and at SRI International.

### Randomized Controlled Trial Participant Clinical and Genetic Data

We utilized data from seven randomized controlled trials (Table 1 and Supplementary Table 3). These trials were conducted in Washington DC and Philadelphia PA,<sup>40</sup> Washington,<sup>41</sup> California,<sup>42,43</sup> and Wisconsin.<sup>44–46</sup> These were treatment efficacy trials, comparing pharmacotherapies with either group or individual behavioral counseling sessions,<sup>40,42–46</sup> or, a behavioral effectiveness trial comparing behavioral therapy delivery modes.<sup>41</sup>

For this analysis, we nominated SNPs (Supplementary Table 4) based on one or more of the following criteria: (a) evidence for genome-wide association with a metabolic phenotype, (b) non-synonymous substitutions or variants likely to be functional, (c) evidence from a candidate gene association study of response to smoking cessation therapies,<sup>10</sup> (d) genotypes from our database,<sup>46</sup> and (e) minor allele frequency ≥ 0.01 in 1000 Genomes or HapMap Utah residents with ancestry from northern and western Europe (CEU). Seventy-seven SNPs at the three gene loci (±50kbp) were previously chosen for Illumina GoldenGate® assay synthesis, 68 were genotyped and met quality control criteria, and 674 SNPs were imputed as described<sup>47</sup> in six randomized controlled trials.<sup>41–46</sup> We restricted our analyses to self-identified European ancestry individuals to reduce potential heterogeneity of genetic effects across continental population groups. Assessment of genotype completion rates, genotyped or imputed genotypes from

**Table 1.** Randomized Controlled Trial Participant Demographic, Dependence, Pharmacotherapy, and Abstinence Characteristics

PNAT trial ID	3A	5	6A	6B	9A	9B	9C
Investigator	Lerman	Swan	Hall	Hall	Baker	Baker	Baker
NCT Trial ID <sup>a</sup>	00326781	00301145	00087880	00086385	01621009	01621022	00332644
N <sup>b</sup>	394	487	150	174	173	171	684
Age (years), mean (SD)	46.6 (11.3)	49.1 (11.5)	41.8 (9.6)	57.3 (5.9)	37.7 (11.2)	41.3 (10.8)	44.4 (11.8)
BMI, mean (SD)	27.5 (5.4)	27.8 (5.8)	26.5 (4.7)	26.5 (5.9)	26.6 (5.7)	26.7 (5.5)	28.8 (6.7)
College (%)	51.5	25.5	51.7	58.6	19.7	18.2	22.8
Female (%)	46.7	68.8	38.0	41.4	53.8	58.5	60.0
Married (%)	48.7	69.0	24.7	28.7	44.8	48.0	47.2
FTND, mean (SD)	5.55 (2.23)	5.15 (2.1)	4.82 (2.1)	4.87 (2.1)	5.13 (2.4)	5.76 (2.1)	5.21 (2.2)
CPD, mean (SD)	23.6 (9.2)	20.2 (8.3)	19.1 (7.4)	20.8 (8.8)	21.5 (8.3)	24.1 (9.7)	21.5 (8.8)
Pharmacotherapies <sup>c</sup>	NRT, patch vs. spray	VAR	Combined NRT and BUP	Combined NRT and BUP	BUP, PLA	BUP, PLA	NRT, BUP, PLA
Randomization arms	2	3	5	4	4	2	4
EOT ABS <sup>d</sup>	0.325	0.554	0.642	0.672	0.272	0.216	0.418
6MO <sup>e</sup> ABS	0.190	0.431	0.460	0.626	0.145	0.205	0.317

Note. BMI = body mass index; CPD = cigarettes per day; EOT = end of treatment; FTND = Fagerstrom Test for Nicotine Dependence [clinicaltrials.gov](http://clinicaltrials.gov) ID.

<sup>b</sup>N of self-identified White participants with DNA.

<sup>c</sup>Nicotine replacement therapy (NRT), Bupropion (BUP), placebo (PLA), Varenicline (VAR), combined NRT and BUP (NRT + BUP).

<sup>d</sup>End of treatment 7-day point prevalence abstinence (abstinence).

<sup>e</sup>6 months.

nominated or proxy SNPs, linkage disequilibrium, and minor allele frequencies reduced this to six SNPs with genotyped genotype data (Table 2 and Supplementary Table 4). The non-synonymous *SLC22A1* SNP, rs12208357<sup>48,49</sup> was genotyped via TaqMan® SNP Genotyping Assay (Table 2 and Supplementary Table 4). Among the expected 12,873 genotypes, the missing genotype rate was 0.05%. After initial analyses of six trials, we genotyped rs316019 via TaqMan® SNP Genotyping Assay in DNA samples in 598 DNA samples from one additional trial, with a missing genotype rate of 0.05%.<sup>40</sup> SNP allele frequencies did not differ significantly across the 24 arms of the seven trials (all *p* values > .08). We observed three arm-by-SNP strata with Hardy-Weinberg equilibrium *P* < .05, versus eight expected by chance (Supplementary Table 5). Linkage disequilibrium<sup>50</sup> between these seven SNPs is modest, with the largest *r*<sup>2</sup>-values among the *SLC22A2* SNPs rs316006 and rs3798156 (*r*<sup>2</sup> = 0.44), and rs316006 and rs316019 (*r*<sup>2</sup> = 0.37), and all remaining *r*<sup>2</sup>-values ≤ 0.18 (Supplementary Table 6).

### Logistic Modeling of the Effect of SNPs on EOT and 6MO Abstinence

Our primary regression analysis models were conducted for each SNP using additive models, adjusting for demographics (age [age and age squared], education [presence or absence of college degree], gender, marital status [married or other]), the first 10 principal components of population genetic variation, and indicator variables for the pharmacotherapy randomization groups. We imputed missing values 20 times for age (*n* = 2), education (*n* = 6), marital status (*n* = 3), cigarettes per day (CPD) (*n* = 3), Fagerström Test for Nicotine Dependence score (*n* = 37) and BMI (*n* = 40).<sup>51</sup> Regression analyses were performed on each imputed data set and the results were combined

with adjustment to the variance of regression parameters, to reflect the additional variance attributable to the imputations.<sup>52</sup> Pharmacotherapy group sample sizes for NRT, bupropion, placebo, varenicline, and combined therapy (NRT and bupropion) at EOT were 370, 373, 285, 487, and 324 respectively. At EOT through 6MO, the combined therapy group was randomized to combined therapy, to chronic bupropion, and to chronic NRT, resulting in sample sizes of 161, 98, and 65 respectively. NRT patch and NRT spray pharmacotherapy group sample sizes within all seven trials were 563 and 201; within Cohort 3A alone, sample sizes were 193 and 201. Regression analyses were performed including all individuals simultaneously, thus the number of variables is a small fraction of the number of individuals. Regression analysis sample sizes are: 1,834 for rs12208357; 1,838 for rs316006 and rs3088442; and 1,839 for rs662138, rs316019, rs3798156, and rs2504934. After the addition of the seventh trial, the total sample size for rs316019 analysis was 2,233.

For each SNP, regression analyses were performed using end of treatment and 6 month seven day point prevalence abstinence to: (a) quantify SNP effects on abstinence across all individuals, adjusting for pharmacotherapy randomization; (b) quantify SNP effects on abstinence within each pharmacotherapy randomization group; (c) test the homogeneity of SNP effects on abstinence between groups. We do not correct for each SNP by pharmacotherapy group model because these models are analyzing subsets of the entire sample of individuals. We interpret nominally statistically significant results from this analysis of multiple SNPs using *a priori* association findings and knowledge of SNP function, and we applied a conservative Bonferroni multiple test correction for seven SNPs to identify which nominally statistically significant results retained statistical significance. Non-exclusive reasons for

**Table 2.** *SLC22A1*, *SLC22A2*, and *SLC22A3* SNPs Analyzed in Six Randomized Controlled Trials

SNP rsID	Gene	Coor(37.4)	Transcript annotation <sup>a</sup>	Gen/prot <sup>b</sup>	CEU <sub>MAF</sub> <sup>c</sup>	HapMap <sup>d</sup>	Geno <sup>e</sup>	Imputed <sup>f</sup>
rs12208357 <sup>g</sup>	<i>SLC22A1</i>	160543148	NM_003057.2:c.181C>T	R61C	0.075 (T)	No	TaqMan®	No
rs662138 <sup>h</sup>	<i>SLC22A1</i>	160564476	NM_003057.2:c.1277-97C>G	IVS7	0.225 (G)	Yes	Yes	Yes
rs316006 <sup>i</sup>	<i>SLC22A2</i>	160646365	NM_003058.3:c.1502-529A>T	IVS9	0.195 (T)	Yes	Yes	Yes
rs316019 <sup>g</sup>	<i>SLC22A2</i>	160670282	NM_003058.3:c.808T>G	S270A	0.097 (T)	Yes	Yes	Yes
rs3798156 <sup>h</sup>	<i>SLC22A2</i>	160676216	NM_003058.3:c.518+1430G>A	IVS2	0.128 (A)	Yes	Yes	Yes
rs2504934 <sup>j</sup>	<i>SLC22A3</i>	160781666	NM_021977.3:c.429+11786G>A	IVS1	0.243 (A)	Yes	Yes	Yes
rs3088442 <sup>g,h</sup>	<i>SLC22A3</i>	160872652	NM_021977.3:c.*564G>A	3'UTR	0.333 (A)	No <sup>k</sup>	Yes	Yes

<sup>a</sup>*SLC22A1* and *SLC22A3* are transcribed on the + strand, and *SLC22A2* is transcribed on the – strand.

<sup>b</sup>Gene or protein annotation.

<sup>c</sup>Minor allele in Utah residents with ancestry from northern and western Europe.

<sup>d</sup>HapMap V3R2.

<sup>e</sup>Genotypes available from Illumina GoldenGate panel or TaqMan® assay.

<sup>f</sup>Imputed genotypes available.

<sup>g</sup>Functional variant.

<sup>h</sup>GWAS.

<sup>i</sup>Proxy for rs595374.

<sup>j</sup>Proxy for rs2504954.

<sup>k</sup>HapMap V28.

exclusion in this analysis of 2,233 individuals from 24 arms of seven trials randomizing 5,196 individuals include: biospecimens not collected (2,507 [48%]); randomization to a treatment arm not selected for analysis (757 [15%]); not self-identifying as White (392 [7.5%]); (d) sample completion rate <95% (45 [0.9%]); and/or (e) chromosomal sex and clinical gender mismatch (18 [0.4%]).

### Post-hoc Analyses Performed

We performed additional regression analyses to evaluate the influence of BMI and of the baseline nicotine dependence measures CPD and FTND on SNP association to abstinence. We tested whether dependence measures mediated nominally statistically significant associations with abstinence overall or by pharmacotherapy randomization group. We tested whether nausea or medication adherence mediated the statistically significant association of rs316006 with abstinence in individuals randomized to varenicline. We assessed association of rs316019 with CPD and FTND, adjusting for demographic variables, principal components of genetic variation, and trial arm. We estimated haplotypes and diplotypes formed by the three *SLC22A2* SNPs among 1,839 individuals from six trials. We performed analyses to quantify the effect of *SLC22A2* diplotype category relative to the reference diplotype on abstinence overall, and in the sample of participants prescribed varenicline or randomized to NRT. Power analyses were performed using the CEU minor allele frequency of rs316019, the mean 6 month abstinence rate observed in 80 randomized controlled trial placebo arms by the Clinical Practice Guideline,<sup>53</sup> and the observed 6 months abstinence ratio. We assessed association of rs316019 with measures of nicotine renal clearance in two laboratory studies of nicotine and cotinine metabolism.<sup>54,55</sup>

We performed genotype QC in SAS Genetics Version 9.2 (Cary), phenotype imputation and phenotype genotype regression analyses using STATA 12.0 (StataCorp), principal components analyses using EIGENSTRAT<sup>56</sup> and GCTA,<sup>57</sup> LD analyses using Haploview<sup>58</sup> and SNAP,<sup>59</sup> haplotype and diplotype analyses using PHASE,<sup>60</sup> and power analyses using Quanto.<sup>61</sup> All tests were two-sided, and alpha for all tests was 0.05.

## RESULTS

SNP association with abstinence over all individuals and by pharmacotherapy randomization group in six randomized controlled trials identified four nominally statistically significant models: rs316006 association with abstinence at end of treatment (EOT) and at 6 months (6MO) in individuals prescribed varenicline ( $OR_{EOT} = 1.420$ , 95% CI = 1.038–1.943,  $p = .028$ ;  $OR_{6MO} = 1.363$ , 95% CI = 1.001–1.855,  $p = .049$ ); rs316019 association with abstinence at 6MO in all individuals ( $OR_{6MO} = 1.306$ , 95% CI = 1.034–1.650,  $p = .025$ ) and in individuals randomized to NRT ( $OR_{6MO} = 1.784$ , 95% CI = 1.073–2.968,  $p = .026$ ) (Supplemental Table 7). Analyses including BMI or nicotine dependence measures as covariates resulted in very minor changes in the statistical significance of these models (Supplementary Tables 8 and 9). We did not observe statistically significant heterogeneity of SNP effects across pharmacotherapy randomization groups or mediation of association with abstinence with nicotine dependence, nausea, or medication compliance (data not shown).

From three *SLC22A2* SNPs in 1,839 individuals, three common (frequency > 0.01) haplotypes and six common diplotypes were observed (Supplementary Table 10). The reference haplotype and diplotype are present in 78.0% and 60.6% of individuals, with two diplotypes of intermediate prevalence, three diplotypes with prevalence >1% and <2%, and six rare diplotypes with cumulative prevalence <2.5% (Supplementary Table 10). Most (93.8%) non-reference haplotypes include the rs316006 minor allele linked either to the minor allele of rs3798156 or of rs316019, and most (97.3%) rs316019 minor alleles are linked to the rs316006 minor allele (Supplementary Table 10). Borderline ( $p = .05$ ) and nominally statistically significant ( $p = .034$ ) associations were observed for the diplotype (frequency 14.4%) comprised of one reference haplotype and one haplotype with the minor alleles of 316006 and with rs316019 in multivariate analyses of abstinence at 6 months in the entire sample, and, in those randomized to NRT or prescribed varenicline (Supplementary Table 11). We did not observe significant association of rs316019 with renal nicotine clearance in two laboratory-based metabolic studies of labeled nicotine and labeled cotinine (Supplementary Table 12).



In mega-regression analyses including data from a seventh randomized controlled trial added *post-hoc* to increase the NRT pharmacotherapy randomization group sample size, we observed nominally statistically significant effects of rs316019 at 6 months over all 2,233 participants. We did not observe significant association at end of treatment, or by pharmacotherapy randomization group at either end of treatment or at 6 months (Supplementary Table 13).

### DISCUSSION

Linkage evidence suggests that the chr6q23.2-q27 region contains multiple genes that influence smoking behavior phenotypes. Review of chr6q23.2-q27 genome-wide significant associations with metabolic phenotypes identified *SLC22A1* and *SLC22A2* as candidates for analysis of response to smoking cessation therapies due to potential influence on hepatic and renal function. Based on *a priori* criteria, we selected and analyzed seven SNPs at the three chr6q25.3 solute carrier family 22 genes, and identified one functional *SLC22A2* SNP (rs316019) nominally statistically significantly associated with abstinence in 1,839 individuals randomized to smoking cessation therapies in six randomized controlled trials, and in 370 individuals randomized to NRT patch, both at 6 months. We observed a linked intronic SNP nominally statistically significantly associated with abstinence at end of treatment and at 6 months in 487 individuals prescribed varenicline. In *post-hoc* diplotype analyses, we observed statistically significant associations of a diplotype comprising the referent haplotype and the haplotype with the minor alleles of both of these *SLC22A2* SNPs with abstinence at six months in 854 individuals randomized to NRT or prescribed varenicline. In these planned analysis of six trials and in the *post-hoc* diplotypes analysis, the minor alleles were associated with increased abstinence, suggesting that a reduced function OCT protein increases abstinence. Our findings add to the chr6q23.2-q27 candidate gene literature associated with smoking behaviors (Table 3), and support the International Transporter Consortium's guidance to consider OCT2 as a transporter of emerging clinical importance.<sup>62-64</sup>

In *post-hoc* analyses designed to further explore the association of rs316019 with abstinence within individuals randomized to NRT, we again observed nominally statistically significant association of rs316019 with abstinence at 6 months in 2,233 individuals randomized to smoking cessation therapies. In an enlarged group of 563 individuals randomized to NRT we observed a reduced odds ratio that was not statistically significant.

The sample of treatment-seeking smokers included in this analysis does not overlap with, and is ~1.6- to 1.9-fold larger than the sample of 1,175 treatment-seeking smokers included in a candidate gene-based analysis of three RCTs randomizing individuals to varenicline, bupropion, or placebo.<sup>10</sup> King *et al*<sup>10</sup> identified a statistically significant association between rs595374 and nausea overall ( $OR = 0.64$ , 95% CI = 0.48–0.87,  $p = .004$ ,  $Q = 0.099$ ) and among those randomized to varenicline ( $OR = 0.59$ , 95% CI = 0.41–0.86,  $p = .006$ ), but not with abstinence. We used rs316006 as a proxy for rs595374, and we did observe nominally statistically significant association between rs316006 and abstinence in individuals prescribed varenicline. Differences in results between this analysis and King *et al*<sup>10</sup> may be due to differences in trial design,

participant characteristics, SNP selection, regression modeling approaches, and alpha thresholds. We included participant behavioral, demographic, dependence, and population genetic variables as covariates in our chr6q23.2-q27 analyses because we observed significant associations of these variables with abstinence in prior analyses.<sup>47,71</sup>

rs316019 codes for a functional amino acid substitution (Ala270Ser) in OCT2 (Table 2), with minor allele frequencies of ~10%–16% in: Utah residents with ancestry from northern and western Europe (CEU); Japanese in Tokyo, Japan (JPT); Han Chinese in Beijing, China (CHB); and in Yoruba in Ibadan, Nigeria (YRI). The *in vivo* human clinical literature provides evidence that, compared to individuals with the rs316019 major allele homozygote genotype: rs316019 minor allele carriers exhibit significantly reduced metformin renal clearance in a laboratory study of 15 Chinese;<sup>72</sup> rs316019 heterozygotes exhibit significantly reduced plasma levels of metformin in a clinical administration study of 23 individuals;<sup>73</sup> rs316019 heterozygotes exhibit significantly reduced cisplatin treatment-related nephrotoxicity among 78 Dutch and 53 Japanese cancer patients;<sup>74,75</sup> diabetic rs316019 minor allele homozygotes treated with metformin exhibit significantly increased plasma lactate than diabetic individuals not so treated (total strata study sample size = 11 Chinese diabetic patients);<sup>76</sup> and healthy rs316019 minor allele carriers exhibit significantly decreased levels of tryptophan (total study sample = 21 Koreans).<sup>77</sup> These studies suggest the effect of the minor allele is to: reduce metformin clearance;<sup>72,76</sup> increase transport of metformin;<sup>73</sup> reduce the transport of cisplatin;<sup>74,75</sup> and reduce tryptophan clearance.<sup>77</sup> Consistent with most of the clinical data except the study of Chen *et al*<sup>73</sup>, the *in vitro* functional literature suggests that the effect of the rs316019 minor allele is associated with significant reductions in the transport of model OCT2 substrates.<sup>78,79</sup> Thus ~19%–28% of European, East Asian, and Yoruban ancestry populations may be carriers of a reduced function OCT2 protein. Further *in vivo* and *in vitro* research on OCT2 variation and effects on drug disposition is needed.

Nominally statistically significant associations with abstinence at 6 months were observed with rs316006 in individuals prescribed varenicline, where individuals with the minor allele are ~36%–50% more likely to be abstinent than the referent genotype at both end of treatment and at 6 months. Given the modest linkage disequilibrium between rs316006 and rs316019, the rs316006 findings suggest that nicotinic acetylcholine receptor partial agonist pharmacotherapies may be more effective in individuals with the reduced function OCT2 allele. We hypothesize that an allele resulting in reduced OCT2 function might result in increased varenicline plasma concentrations, as occurs with an OCT2 inhibitor.<sup>34</sup> A reduced function OCT2 allele might result in reduced blood-brain barrier transport of smoking cessation pharmacotherapies.<sup>19</sup> The reduced function allele might also result in increased extracellular monoamines in brain in the post-quit period when extracellular monoamine concentrations may be reduced in the context of nicotine withdrawal.<sup>80</sup> Each of these potential effects of a reduced function OCT2 allele in specific organs and tissues might exert effects on smoking cessation pharmacotherapy effectiveness.

Some of the results of this analysis generate testable hypotheses. For example, plasma concentrations of varenicline could be measured in individuals prescribed this medication<sup>34</sup> to test for the effect of functional OCT2 alleles on medication

**Table 3.** chr6q23.2-q27 Candidate Genes Associated With Smoking Behaviors and Substance Dependence

Gene name	Location	Measure or diagnosis	Analysis method	Size and ancestry	N SNPs
Regulator of G-protein signaling 17 protein ( <i>RGS17</i> )	153,332,032–153,452,389	Four substance dependence diagnoses, and smoking initiation	Regression	3,237 EA and AA <sup>a</sup> , and 4,183 Korean males <sup>b</sup>	21 in 2 candidate genes, and GWAS
mu opioid receptor ( <i>OPRM1</i> )	154,360,443–154,568,001	Multiple and general substance dependence	Meta-analysis of case:control regression	28,693 EA <sup>c</sup>	rs1799971
Villin 2 ( <i>VIL2</i> )	159,106,761–159,160,444	Smoking cessation, NRT versus PLA	Case:control <i>t</i> test	550 EA treatment-seeking smokers <sup>d</sup>	Autosome-wide
Organic cation transporter 2 ( <i>SLC22A2</i> )	160,637,794–160,679,963	Nausea, and 6 month prospective abstinence	Case:control regression	1,175 <sup>e</sup> , and 1,839 <sup>f</sup> EA treatment-seeking smokers	254 tagSNPs in 24 candidate genes, and 7 SNPs in 3 candidate genes
Mitogen-activated protein kinase kinase 4 ( <i>MAP3K4</i> )	161,412,964–161,530,945	FTND score	Regression, and case:control genotype and haplotype	1,603 EA <sup>g</sup> , and 660 Han Chinese ever-smokers <sup>h</sup>	3,369 SNPs in 348 candidate genes, and 360 SNPs in 45 candidate genes
Parkin isoform 1 ( <i>PARK2</i> )	161,886,134–163,068,824	Smoking cessation, over all smoking cessation therapies	Case:control <i>t</i> test	550 EA treatment-seeking smokers <sup>d</sup>	Autosome-wide

<sup>a</sup>EA = European-American ancestry, AA = African-American ancestry, H. Zhang, Wang, Kranzler, Anton, and Gelernter.<sup>65</sup>

<sup>b</sup>Yoon et al.<sup>66</sup>

<sup>c</sup>Saccone, Schwantes-An, and Group.<sup>67</sup>

<sup>d</sup>Uhl et al.<sup>68</sup>

<sup>e</sup>King et al.<sup>10</sup>

<sup>f</sup>This study.

<sup>g</sup>Gruzca et al.<sup>69</sup>

<sup>h</sup>Wei et al.<sup>70</sup>

plasma levels, to test association between medication plasma levels and prospective abstinence, and on the influence of the functional OCT2 alleles on prospective abstinence, as has been reported for the bupropion metabolite hydroxybupropion and *CYP2B6* variation.<sup>81</sup> Effects of the functional OCT2 allele on brain structure and function may be observable, given prior studies that have identified effects on brain structure<sup>82</sup> and function<sup>83</sup> of cholinergic<sup>47,84</sup> and dopaminergic<sup>40,85</sup> alleles that influence smoking cessation.

We did not observe significant associations between the OCT2 functional SNP and prospective abstinence in individuals randomized to bupropion, combined, or placebo treatments, nor did we observe significant associations with *SLC22A1* and *SLC22A3* SNPs and abstinence. Statistical power for association analysis of the combined and placebo treatment groups was lower due to smaller sample sizes and may be responsible for these null results. Insufficient coverage of multiple functional polymorphisms in *SLC22A1* and *SLC22A3* that would have enabled haplotype-based or burden-based tests may be responsible for the second set of null results.

In summary, we performed pharmacogenetic analyses of chr6q25.3 OCT gene variation and prospective abstinence in a large sample of treatment-seeking smokers. The genes and SNPs were chosen based on *a priori* hypotheses including prior pharmacological and genetic studies. We identified nominally statistically significant associations of one functional and one linked *SLC22A2* SNP with 6 month abstinence in individuals randomized to either NRT patch or varenicline, respectively. There were no association results that were robust to multiple test correction for seven SNPs. The association results require testing in larger samples sizes to increase power to detect

statistically significant associations with abstinence overall, with the individual pharmacotherapies identified in this analysis and with other pharmacotherapies, and in additional population samples of different ancestries to evaluate potential population-specific effects. Larger samples would also permit well-powered tests of moderation of gene-abstinence association as a function of pharmacotherapy condition. If replicated, the known physiological functions of OCT2 in kidney and emerging functions in brain and in peripheral tissues suggest that analyses of *SLC22A2* expression and function in multiple human tissues may provide insight into potential pharmacogenetic mechanisms responsible for associations with prospective abstinence.

## SUPPLEMENTARY MATERIAL

Supplementary Tables 1–13 can be found online at <http://www.ntr.oxfordjournals.org>

## FUNDING

This work was supported by the National Institutes of Health of the United States Department of Health and Human Services (PGRN U01 DA20830 to RFT and C. Lerman; R01s CA71358 and DA11170 to GES; R01s DA16752, DA18691, DA15732, and DA9253 to SH; P50 CA84724, K05 CA139871 and P50 DA19706 to TBB; and R21 DA33813 to AWB), by the University of California Tobacco-Related Diseases Research Program (7PT2004 to NLB), and by a Research Agreement

between Medco Health Solutions, Inc., Affymetrix, Inc., and SRI International. This work was supported in part by the Centre for Addiction and Mental Health (RFT), an endowed Chair in Addictions (RFT), and the Wolfe Medical Research Chair in Pharmacogenomics (RBK). Randomized controlled trials 5, 9A, 9B, and 9C were funded by the National Cancer Institute, trials 6A and 6B were funded by the National Institute of Drug Abuse, and trial 3A was funded by the National Cancer Institute and by the National Institute of Drug Abuse. Varenicline and nominal support for recruiting trial 5 participants was provided by Pfizer. GlaxoSmithKline provided medication for trials 9B and 9C. The above sponsors did not have a role in the design of the trials, the collection, analysis, or interpretation of data, the writing of, or the decision to submit, the manuscript.

### DECLARATION OF INTERESTS

AWB reports employment at SRI International and honoraria from the National Institutes of Health and multiple Universities for reviews of proposals and for participation in symposia and trainee events involving nicotine and tobacco research. HSJ, RK, MM, DN and DVC report no conflicts of interest. CKE reports employment at the University of Southern California and BioRealm, LLC. PYK reports no conflicts of interest. JBM is employed by Group Health Cooperative. She has received honoraria from the National Institutes of Health for serving as a peer reviewer and from the University of Washington and Fred Hutchinson Cancer Research Center for serving as a consultant on extramurally-funded projects. RBK and SH report no conflicts of interest. RFT has acted as a consultant to pharmaceutical companies, primarily concerning smoking cessation medications. She also receives an honorarium for her editorial work for *Clinical Pharmacology and Therapeutics*. TBB and GES report no conflicts of interest.

### ACKNOWLEDGMENTS

We thank the participants of the randomized controlled trials and of the laboratory studies of nicotine and of cotinine metabolism for their participation in research. We thank C. Lerman for permission to include Cohort 3A data in this analysis. We thank L. Jack (SRI International) for management of randomized controlled trial data, F. Allen (University of California San Francisco) for management of Pharmacogenetics of Nicotine Addiction Treatment (PNAT) data, J. Woo (University of California San Francisco) for Illumina GoldenGate genotyping, and W. Lee and J. Liu (University of Southern California) for genotype data quality control and ancestry informative marker analysis of ancestry and population genetic variation. Drs. Bergen and Javitz had full access to all of the data in the study and take responsibility for the integrity and the accuracy of the data analysis. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

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