Published in final edited form as:

Subst Use Misuse. 2013 May ; 48(7): 490–494. doi:10.3109/10826084.2013.778280.

CYP2A6 Genotype and Smoking Behavior in Current Smokers Screened for Lung Cancer

Mindi A. Styn^{1,2}, Tomoko Nukui³, Marjorie Romkes^{3,4}, Kenneth A. Perkins⁵, Stephanie R. Land⁶, and Joel L. Weissfeld^{2,4}

¹Department of Health and Community Systems, University of Pittsburgh School of Nursing, Pittsburgh, Pennsylvania, USA

²Department of Epidemiology, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania, USA

³Center for Clinical Pharmacology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

⁴University of Pittsburgh Cancer Institute, Pittsburgh, Pennsylvania, USA

⁵Department of Psychiatry, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, USA

⁶Tobacco Control Research Branch, Behavioral Research Program, National Cancer Institute, Washington, District of Columbia, USA

Abstract

Functional CYP2A6 genetic variation partially determines nicotine metabolism. In 2005, we examined functional CYP2A6 variants associated with reduced metabolism (CYP2A6*2, CYP2A6*9, CYP2A6*4), smoking history, and change in smoking in 878 adult smokers undergoing lung cancer screening in an urban setting. At one year, 216 quit smoking for more than 30 days while 662 continued smoking. Compared to subjects who smoked 30 cigarettes per day at baseline, the odds of a reduced metabolism genotype was 52% higher in subjects smoking 20–29 cigarettes per day and 86% higher in subjects smoking less than 20 cigarettes per day (p -trend = 0.016). Reduced metabolism genotypes appeared unrelated to quitting. Though related to smoking dose, CYP2A6 may not influence cessation.

Keywords

smoking cessation; smoking initiation; cigarette smoking; genetics; cytochrome P450; nicotine metabolism

NOTES ON GENOTYPES AND ALLELES

Our chromosomes carry genetic material, or genes, that influence traits such as eye color or smoking behaviors. We have two copies of each chromosome and thus can have two forms

Copyright © 2013 Informa Healthcare USA, Inc.

Address correspondence to Mindi A. Styn, Ph.D., Department of Health and Community Systems, University of Pittsburgh School of Nursing, 415 Victoria Building, 3500 Victoria Street, Pittsburgh, PA 15261; mimst31@pitt.edu.

Declaration of Interest

Dr. Perkins has served as a consultant for Cypress Bioscience and Embera Neurotherapeutics. All other authors report no conflicts of interest. The authors alone are responsible for the content and writing of the article.

of the gene, one on each chromosome. Different forms of a gene are called alleles. The combination of alleles that a person has makes up that person's genotype. For example, in this paper we studied three of the most common *CYP2A6* variant alleles, *2, *4, and *9, plus the major allele, sometimes called "wild type," *1. If a person has the *2 allele on one chromosome and the *9 allele on the other chromosome, their genotype is *2/*9.

INTRODUCTION

Despite an abundance of data confirming the health risks of smoking, many individuals continue to smoke. A majority of smokers want to quit smoking and over half have attempted to quit at least once (Malarcher, Dube, Shaw, Babb, & Kaufmann, 2011). Understanding underlying genetic differences between individuals who have successfully quit smoking and those who have not may lead to important breakthroughs in smoking cessation treatment.

The hepatic cytochrome P450 *CYP2A6* pathway is primarily responsible for nicotine metabolism. Genetic variation in *CYP2A6*, the gene coding the *CYP2A6* enzyme, accounts for some inter-individual variation in nicotine metabolism (Benowitz, Swan, Jacob, Lessov-Schlaggar, & Tyndale, 2006). Findings from a recent twin study suggest that a few *CYP2A6* variant alleles (*1B, *1 × 2, *2, *4, *9, and *12) may explain 19.0% of the genetic variation in nicotine metabolism (Swan et al., 2009).

Inherited differences in nicotine metabolism appear to affect smoking behavior, including treatment response. In particular, smokers who inherit a metabolically deficient *CYP2A6* allele smoke fewer cigarettes a day and exhibit less nicotine dependence (Wassenaar et al., 2011). Smokers who metabolize nicotine slowly exhibit smaller puff volumes (Strasser et al., 2011) and appear to respond more favorably to nicotine replacement (Lerman et al., 2010).

To our knowledge, no studies have examined associations between *CYP2A6* genotype and smoking cessation in a nontreatment-seeking population. In a unique population of smokers volunteering for low-dose computed tomography (CT) lung cancer screening study, we examined associations between *CYP2A6* genetic variation and smoking behavior. We hypothesized lower initial intensity of cigarette smoking and higher rates of subsequent smoking cessation in persons with metabolically deficient *CYP2A6* genotypes.

METHODS

As described previously (Styn et al., 2009), this nested case-control study included smokers who had enrolled in a lung cancer screening study between 2002 and 2005. Based on data collected at the one-year follow-up interview, we selected for genotyping 225 individuals not smoking for more than 30 days and 675 individuals still smoking; the latter frequency matched three to one to the former according to sex, age, and month of enrollment. We conceived our genetic studies too long after the follow-up to permit biochemical confirmation of smoking status. Subjects provided signed informed consent, as approved by our Institutional Review Board.

We used a Nanogen NanoChip® Electronic Microarray-based multiplexed assay (Nanogen®, San Diego, CA) to genotype subjects for Leu160His (rs1801272) and TATA box -48T>G (rs28399433) single nucleotide polymorphisms that define the *CYP2A6**2 and *9 alleles, respectively (Gilles, Wu, Foster, Dillon, & Chanock, 1999). We used a 5 nuclease (TaqMan) real-time polymerase chain reaction (PCR) assay (ABI Prism 7700, Applied Biosystems, Foster City, CA) to measure *CYP2A6* copy number (Aarskog & Vedeler, 2000; Schaeffeler, Schwab, Eichelbaum, & Zanger, 2003). TaqMan assays in 25

μL total volume, performed in triplicate, used the albumin gene for reference, 20 ng of genomic DNA, 1X Universal Master mix (Applied Biosystems, Foster City, CA), 900 nM of each primer, 250 nM of probe, and PCR at 50°C for two minutes, 95°C for 10 minutes, and 40 cycles at 95°C for 15 seconds and 60°C for one minute. PCR produced 77 bp *CYP2A6* and 72 bp albumin products. The comparative standard consisted of a known wild-type *CYP2A6* sample run in triplicate at three different concentrations against albumin. The relative amounts of the *CYP2A6* and albumin PCR products determined the *CYP2A6* copy number estimate and associated *CYP2A6**4 genotype.

We used sampling weights to estimate allele and genotype frequencies, the conventional chi-square statistic to evaluate Hardy-Weinberg equilibrium at *CYP2A6**2 and *9, and Cochran-Mantel-Haenszel chi-square statistics to evaluate genotype associations with smoking-related variables. We used conditional logistic regression to estimate the relative odds (unadjusted and adjusted for other risk factors) of specific smoking-related outcomes according to genotype. Using models containing terms for genotype and secondary risk factors (e.g., average number of cigarettes smoked daily before study entry) and terms for the genotype by risk factor cross-classification, the statistical examination for effect modification used a log-likelihood ratio test applied to the cross-classification terms.

RESULTS

*CYP2A6**2 and *4 genotype results were obtained for 96.0% and 98.1% of subjects quit and not quit ($p = 0.08$), respectively, and *CYP2A6**9 results for 95.6% and 97.5% of subjects quit and not quit ($p = 0.14$), respectively. *CYP2A6**2, *4, and *9 allele frequencies were 2.6%, 1.8%, and 7.2%, respectively – estimates that were all in agreement with previously reported values (Lerman et al., 2010; Swan et al., 2009). Evaluations for Hardy-Weinberg equilibrium detected statistically significant excesses in the frequencies of *CYP2A6**2 homozygotes (0.6% observed, 0.1% expected) and *CYP2A6**9 homozygotes (1.2% observed, 0.5% expected).

A *CYP2A6**2, *4, or *9 genotype result was available for 878 subjects (48.0% men; 59.9% 50–59, 28.9% 60–69, and 11.2% 70–79 years of age). We observed an inverse dose–response relationship between average number of cigarettes smoked daily before study entry and the frequency of a genotype containing a *CYP2A6* allele associated with deficient or slow nicotine metabolism (Table 1). When compared to subjects who smoked ≥30 cigarettes a day, the odds of a genotype containing a *CYP2A6**2, *4, or *9 allele was 52% higher in subjects who smoked 20–29 cigarettes a day (95% confidence interval (CI): 0.94–2.45) and 86% higher in subjects who smoked <20 cigarettes a day (95% CI: 1.13–3.06). Overall, the difference between these odds ratios (ORs) was significant (p -trend 0.016) showing a trend that suggested that the less a person smoked at baseline, the more likely they were to have a variant allele.

Although not significant ($p > 0.25$), presence of a *CYP2A6**2, *4, or *9 allele appeared to increase the odds of a quit attempt (OR: 1.22; 95% CI: 0.85–1.75), the odds of a quit interval longer than 30 days (OR: 1.09; 95% CI: 0.76–1.55), and the odds of being quit for more than 30 days (OR: 1.05; 95% CI: 0.71–1.55). Adjustment for the average number of cigarettes smoked daily before study entry and adjustment for *DRD2* TaqIA genotype, a genetic factor previously associated with outcome in the same population (Styn et al., 2009), attenuated these associations. Neither average number of cigarettes smoked daily nor *DRD2* TaqIA genotype modified the association between *CYP2A6* genotype and any one-year smoking outcome ($p > 0.10$).

A systematic evaluation of change in smoking behavior in relation to *CYP2A6**2, *4, and *9 variants considered separately under additive, dominant, and recessive genetic models identified only one statistically significant association. Specifically, not smoking for more than 30 days at first follow-up was more frequent in subjects with two *CYP2A6**2 alleles than subjects with one or none (OR: 8.16; 95% CI: 1.51–44.1; $p = 0.015$). Five of seven subjects homozygous for *CYP2A6**2 were not smoking for more than 30 days at first follow-up. To identify persons with presumably very low CYP2A6 activity, we also defined a genotype grouping consisting of persons with homozygous *CYP2A6**2, homozygous *CYP2A6**9, both *CYP2A6**2 and *CYP2A6**9, or one *CYP2A6* copy and *CYP2A6**2 or *CYP2A6**9. Though not statistically significant, not smoking for more than 30 days at first follow-up was more frequent in these subjects ($n = 22$, weighted frequency 2.3%) than in subjects with two or three CYP2A6 copies and no variant *CYP2A6* alleles (OR: 2.20; 95% CI: 0.90–5.37; $p = 0.073$).

The 13 subjects who had three *CYP2A6* copies (1.3% genotype frequency) initiated smoking at an older age (median: 20 years; interquartile range (IQR): 16–23 years) than the 865 subjects with one or two *CYP2A6* copies (median: 17 years; IQR: 15–20 years; $p = 0.045$). *CYP2A6* duplication was less frequent in heavier (≥ 20 cigarettes/day) than lighter (< 20 cigarettes/day) smokers (OR: 0.20; 95% CI: 0.06–0.68; $p = 0.010$). Prospectively, the presence of an extra *CYP2A6* copy appeared to increase the odds of a subsequent quit interval longer than 30 days (OR: 3.32, 95% CI: 1.07–10.3, $p = 0.037$; adjusted for number of cigarettes smoked daily before entry, OR: 2.89, 95% CI: 0.93–9.05, $p = 0.068$) and the odds of being quit for more than 30 days one year later (OR: 3.49, 95% CI: 1.17–10.4, $p = 0.026$; adjusted for number cigarettes smoked daily before entry, OR: 2.93, 95% CI: 0.97–8.84, $p = 0.056$).

DISCUSSION

Smokers who inherit *CYP2A6* alleles associated with slower nicotine metabolism presumably need to smoke fewer cigarettes to achieve the same pharmacological effect experienced by smokers who inherit *CYP2A6* alleles associated with faster nicotine metabolism. Accordingly, confirming reports of association between cigarette dose and *CYP2A6* variation (Wassenaar et al., 2011), we observed an inverse dose–response relationship between average number of cigarettes smoked daily and genotypes containing a metabolically deficient *CYP2A6**2, *4, or *9 allele.

In our study population of volunteers for CT lung cancer screening, but otherwise unselected for motivation to quit or exposure to nicotine replacement therapy, we did not observe statistically significant association between genotypes containing a metabolically deficient *CYP2A6* allele and more favorable smoking outcomes over time. On the other hand, though possibly a chance finding, smokers with an extra *CYP2A6* copy smoked fewer cigarettes a day and experienced better smoking outcomes over time. None of our 13 subjects with an extra *CYP2A6* copy reported smoking more than 20–29 cigarettes a day, on average, before study entry. Any relationship established between *CYP2A6* duplication and smoking behavior must be reconciled with the possible enhancing effect of this genetic change on nicotine metabolism.

Smoking depends on many genetic and situational factors. Though we genotyped subjects for three common *CYP2A6* variants, we did not genotype subjects for a fourth important variant – *CYP2A6**12, 2.5% allele frequency (Swan et al., 2009). We estimate that 4.4% of subjects in our reference category (no *CYP2A6**2, *4, or *9) have *CYP2A6**12, possibly diluting associations between CYP2A6-deficient genotype and smoking outcome. Also, a

meaningful effect of *CYP2A6* on change in smoking may appear only in the presence of other genetic factors or in response to specific interventions.

In the past decade, there has been increasing interest in uncovering genetic influences on smoking cessation that could lead to tailored treatment strategies (Cox et al., 2011; Lerman et al., 2010). Indeed, at least one company has already attempted to profit from the limited data available pertaining to *CYP2A6* and smoking behaviors, although not without harsh criticism from the scientific community (GeneWatch UK, 2004). The potential of genetically tailored treatment strategies can only be realized if we carefully examine the contribution of candidate genes to clearly defined phenotypes. Through this careful examination, we can narrow our focus to specific genes or combinations of genes that have the most influence on smoking cessation and prevention strategies. Thus, eliminating non-influential genes is as important as identifying influential ones.

In summary, smokers inheriting a functionally deficient *CYP2A6* allele smoked fewer cigarettes per day. Although functional genetic variation in *CYP2A6* may influence daily smoking frequency, *CYP2A6* genetic variation may not determine quit smoking outcomes in adult smokers in the absence of nicotine replacement treatment.

Acknowledgments

This study received financial support from the University of Pittsburgh Cancer Institute Specialized Program of Research Excellence in Lung Cancer grant NCI P50 CA90440.

Biographies

Stephanie Land, PhD, is a program director in the Behavioral Research Program's (BRP) Tobacco Control Research Branch at the National Cancer Institute (NCI). She is an associate editor for the *Journal of Clinical Oncology*, the NCI Special Studies Institutional Review Board, and a member of the American Association for Cancer Research Task Force on Tobacco and Cancer. Prior to joining the NCI, Dr. Land was the co-founder and director of the Reduce Smoking and Exposure to Tobacco (ReSET) Center, an associate professor of biostatistics at the University of Pittsburgh, and a member of the American Schools of Public Health Tobacco Use Council. She was the PI for an NCI-funded project regarding smoking and other lifestyle behaviors as predictors of patient adherence to chemoprevention in the setting of breast cancer, and PI for an intramurally funded study of tobacco use and social network formation during the first year of college.

Tomoko Nukui, PhD, is currently associated with the Center for Clinical Pharmacology, University of Pittsburgh School of Medicine. Dr. Nukui's main research focus is identifying unique genetic and genomic variations/patterns as biomarkers for disease prognosis and treatment decisions in different types of cancer. In addition, she is also involved in

evaluating unique patterns of genetic variation that modulate risk of metabolic syndrome in a high-risk community.

Kenneth Perkins, PhD, is professor of psychiatry, psychology, and epidemiology at the University of Pittsburgh. Dr. Perkins is a past-president of the Society for Research on Nicotine and Tobacco (SRNT) and is a fellow of the American Psychological Association and of the Society of Behavioral Medicine. For over 25 years, his laboratory has examined the acute effects of nicotine and cigarette smoking in adults that may explain the persistence of tobacco dependence. Ongoing interests in all studies include sex differences and other individual differences in the pharmacological and non-pharmacological factors promoting smoking, and environmental factors that moderate responses to nicotine or cigarette smoking.

Marjorie Romkes, PhD, is an associate professor of medicine at the University of Pittsburgh. Dr. Romkes' current research efforts are focused on pharmacogenomics and genetic epidemiology. Dr. Romkes and her research group are studying multiple genes involved in drug metabolism and response to assess the genetic contribution to inter-individual differences in drug efficacy and response outcomes in tobacco-related and other cancers.

Mindi Styn, PhD, is a research assistant professor of epidemiology and nursing at the University of Pittsburgh. Dr. Styn's research interests focus on genetic and physiological influences on behavior change specific to smoking cessation and weight reduction.

Joel L. Weissfeld, MD, MPH, is an associate professor of epidemiology at the University of Pittsburgh. Dr. Weissfeld has filled several leadership roles for the UPCI Cancer Epidemiology Prevention and Control Program since 1990. His research interests focus on cancer prevention and early detection.

GLOSSARY

Nicotine Metabolism	The process by which the body breaks down nicotine. The rate, or speed, of nicotine metabolism influences nicotine levels in the body.
Allele	One of the possible forms of a gene. Individuals receive one allele from each parent.
Allele Frequency	The percentage of time a given allele is observed in a population or sample. In humans, the number of alleles in a sample is equal to twice the number of people in the sample.
Genotype	An individual's combination of alleles.

REFERENCES

- Aarskog NK, Vedeler CA. Real-time quantitative polymerase chain reaction: a new method that detects both the peripheral myelin protein 22 duplication in Charcot-Marie-Tooth type 1A disease and the peripheral myelin protein 22 deletion in hereditary neuropathy with liability to pressure palsies. *Human Genetics*. 2000; 107(5):494–498. [PubMed: 11140948]
- Benowitz NL, Swan GE, Jacob P III, Lessov-Schlaggar CN, Tyndale RF. CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. *Clinical Pharmacology & Therapeutics*. 2006; 80(5):457–467. [PubMed: 17112802]
- Cox LS, Faseru B, Mayo MS, Krebill R, Snow TS, Bronars CA, et al. Design, baseline characteristics, and retention of African American light smokers into a randomized trial involving biological data. *Trials*. 2011; 12:22. [PubMed: 21266057]
- GeneWatch UK. Three reasons not to buy the NicoTest™ genetic test. 2004 Retrieved November 9, 2012, from http://www.genewatch.org/uploads/f03c6d66a9b354535738483c1c3d49e4/Nicotest_brief_final.pdf.
- Gilles PN, Wu DJ, Foster CB, Dillon PJ, Chanock SJ. Single nucleotide polymorphic discrimination by an electronic dot blot assay on semiconductor microchips. *Nature Biotechnology*. 1999; 17(4): 365–370.
- Lerman C, Jepson C, Wileyto EP, Patterson F, Schnoll R, Mroziewicz M, et al. Genetic variation in nicotine metabolism predicts the efficacy of extended-duration transdermal nicotine therapy. *Clinical Pharmacology & Therapeutics*. 2010; 87(5):553–557. [PubMed: 20336063]
- Malarcher A, Dube S, Shaw L, Babb S, Kaufmann R. Quitting smoking among adults: United States, 2001–2010. *Morbidity and Mortality Weekly Report*. 2011; 60(44):1513–1519. [PubMed: 22071589]
- Schaeffeler E, Schwab M, Eichelbaum M, Zanger UM. CYP2D6 genotyping strategy based on gene copy number determination by TaqMan real-time PCR. *Human Mutation*. 2003; 22(6):476–485. [PubMed: 14635107]
- Strasser A, Benowitz N, Pinto A, Tang K, Hecht S, Carmella S, et al. Nicotine metabolite ratio predicts smoking topography and carcinogen biomarker level. *Cancer Epidemiology, Biomarkers & Prevention*. 2011; 20(2):234–238.
- Styn MA, Nukui T, Romkes M, Perkins K, Land SR, Weissfeld JL. The impact of genetic variation in DRD2 and SLC6A3 on smoking cessation in a cohort of participants 1 year after enrollment in a lung cancer screening study. *American Journal of Medical Genetics*. 2009; 150B(2):254–261. [PubMed: 18563706]
- Swan GE, Lessov-Schlaggar CN, Bergen AW, He Y, Tyndale RF, Benowitz NL. Genetic and environmental influences on the ratio of 3-hydroxycotinine to cotinine in plasma and urine. *Pharmacogenetics and Genomics*. 2009; 19(5):388–398. [PubMed: 19300303]
- Wassenaar C, Dong Q, Wei Q, Amos C, Spitz M, Tyndale R. Relationship between CYP2A6 and CHRNA5-CHRNA3-CHRNA4 variation and smoking behaviors and lung cancer risk. *Journal of the National Cancer Institute*. 2011; 103(17):1342–1346. [PubMed: 21747048]

Conditional logistic regression results (controlling for sex, age, and month of entry; *N* = 878); odds of genotype according to average number of cigarettes smoked daily before entry

TABLE 1

Genotype	<i>N</i>	20–29 vs. 30 cigarettes/day		<20 vs. 30 cigarettes/day		<i>p</i> -trend across dosage groups
		OR	95% CI	OR	95% CI	
Any <i>CYP2A6</i> *2	41	1.15	0.48–2.72	1.75	0.73–4.19	0.149
Any <i>CYP2A6</i> *4	16	0.89	0.12–6.52	3.43	0.75–15.8	0.952
Any <i>CYP2A6</i> *9	113	1.33	0.75–2.38	1.87	1.04–3.38	0.040
Any <i>CYP2A6</i> *2, *4, or *9	166	1.52	0.94–2.45	1.86	1.13–3.06	0.016

Note. Five subjects with missing genotype excluded from *CYP2A6**9 analysis; OR, odds ratio; CI, confidence interval.