Original investigation

An Adolescent Substance Prevention Model Blocks the Effect of *CHRNA5* Genotype on Smoking During High School

David J. Vandenberg PhD^{1,2,3}, Gabriel L. Schlomer PhD^{1,4},
H. Harrington Cleveland PhD⁴, Alisa E. Schink BS³, Kerry L. Hair MS^{1,4},
Mark E. Feinberg PhD⁵, Jenae M. Neiderhiser PhD⁶, Mark T. Greenberg
PhD^{4,5}, Richard L. Spoth PhD⁷, Cleve Redmond PhD⁷

¹Department of Biobehavioral Health, Penn State University, University Park, PA; ²Institute for the Neurosciences, Penn State University, University Park, PA; ³Molecular Cellular and Integrative Biosciences Program, Penn State University, University Park, PA; ⁴Department of Human Development and Family Studies, Penn State University, University Park, PA; ⁵Bennett Pierce Prevention Research Center, Penn State University, University Park, PA; ⁶Department of Psychology, Penn State University, University Park, PA; ⁷Partnerships in Prevention Science Institute, Iowa State University, Ames, IA

Corresponding Author: David J. Vandenberg, PhD, Department of Biobehavioral Health, Penn State University, 303 Chandlee Laboratory, University Park, PA 16802, USA. Telephone: 814-863-8430; Fax: 814-863-7525; E-mail: djv4@psu.edu

Abstract

Introduction: Prevention intervention programs reduce substance use, including smoking, but not all individuals respond. We tested whether response to a substance use prevention/intervention program varies based upon a set of five markers (rs16969968, rs1948, rs578776, rs588765, and rs684513) within the cluster of nicotinic acetylcholine receptor subunit genes (*CHRNA5/A3/B4*).

Methods: Participants ($N = 424$) were randomly assigned to either control condition, or a family-based intervention in grade 6 and a school-based drug preventive intervention in grade 7. Smoking in the past month was assessed in grades 9–12 using a four-point scale (0 = never smoked, 1 = smoked but not in last month, 2 = one or a few times, 3 = about once a week or more).

Results: There was a main effect of both the intervention ($b = -0.24$, $P < .05$) and genotype at rs16969968 ($b = 0.14$, $P < .05$) on high school smoking. Using dummy coding to allow for nonlinear effects, individuals with the A/A genotype smoked more often than those with G/G ($b = 0.33$, $P < .05$). A genotype $\times$ intervention effect was found with reduced smoking among those with A/A and G/A genotypes to levels similar to those with the G/G genotype (G/G vs. A/A: $b = -0.67$, $P < .05$; A/G vs. A/A: $b = -0.61$, $P < .05$; G/G vs. A/G *ns*). Results were nonsignificant for the other four markers.

Conclusions: Preventive interventions can reduce the genetic risk for smoking from rs16969968.

Introduction

Smoking-related behaviors are heritable, with approximately 40%–70% of the variance accounted for by genetic effects (ref.¹ for review). Similar heritability estimates are found for the early stages of smoking behaviors, such as smoking initiation,^{2,3} and first smoking

experiences.^{4–7} This evidence of heritability is consistent with results of specific genetic associations between smoking and markers in and around the cluster of three nicotinic receptor subunit genes (*CHRNA5*, *CHRNA3*, and *CHRNB4*) on chromosome 15q25.^{8,9} This cluster is in high linkage disequilibrium; nevertheless, there is

evidence for distinct effects of at least three loci in this cluster.^{10,11} Some studies find evidence for five loci.¹² The current study tested for an association between markers for these five loci and high school smoking in the context of substance misuse preventive intervention.

Of the five single nucleotide polymorphisms (SNPs) tested, rs16969968, which is located within the fifth exon of *CHRNA5*, has been the target of intense study. The minor allele (A) has been associated consistently with risk for several smoking-related traits, including heaviness of smoking (eg, cigarettes per day) and nicotine dependence.^{13–21} In addition to these findings focusing mostly on mature smokers, Sherva and colleagues have shown an association of the risk allele with a rush or buzz from the first cigarette in a sample of Caucasians.²² In an independent study, rs16969968 had an effect on smoking initiation adolescence,²³ but there are some potential differences in the age at which the effects were most notable.²⁴ These genetic findings for early smoking behaviors are important to understand because the risk for lifetime smoking is greatest for those who report positive experiences in early use of cigarettes^{25–29} and extend to a greater risk for becoming a heavy smoker later in life.³⁰

In comparison to the significant findings of the main effect of genetics, Gene $\times$ Environment (G $\times$ E) interactions involving smoking behaviors have received less attention. To date, much of the G $\times$ E work has focused on smoking cessation in adults,^{31,32} rather than early stages of use as in the present study. Chen et al.³¹ found that a haplotype consisting of SNPs rs16969968 and rs680244 (a nearby but genetically distinct site) interacted with drug treatment to predict cessation. Uhl et al.³² found that a polygenic risk score that included rs16969968 that moderated the effects of randomized drug therapy cessation treatment during adulthood was also related to substance use during adolescence. This work demonstrates the benefit of applying experimental designs to examine G $\times$ E processes and demonstrates that markers (and genes) that have documented main effects, such as rs16969968 (and *CHRNA5*), are also potential contributors to G $\times$ E interactions.

Similar to the value of randomized smoking cessation trials in providing evidence of G $\times$ E interactions on smoking cessation, preventive intervention trials provide approaches to examine G $\times$ E interactions on emerging risk behaviors. These studies, referred to as Gene by Intervention (G $\times$ I) studies, have numerous advantages over epidemiological studies. The random assignment of individuals or communities to intervention and control/comparison conditions³³ allow for: (1) greater control and understanding of environmental differences across participants, (2) increased statistical power as a result of the experimental design,³⁴ (3) reduced self-selection bias, diminishing active gene-by-environment correlation (rGE), and (4) the potential for insight into the environmental “pathways” that may connect genetic variation to phenotypes. Brody,³⁵ and Bakermans-Kranenburg and van IJzendoorn³⁶ elaborate on the advantages of randomized prevention trials for genetic research.

These G $\times$ I advantages have been leveraged in several adolescent risk behavior studies including substance misuse,^{37,38} externalizing behavior,^{39,40} aggression and impulsivity,⁴¹ and alcohol use.⁴² Until recently, little G $\times$ I work had been done on smoking. This comes in spite of the fact that there have been a number of successful preventive interventions for early smoking behaviors in school- and community-based programs⁴³; and that delaying the initiation and slowing the early stages of progression to regular smoking is enormously important from a public health perspective. However, a recent report demonstrated a role for genetic moderation of an intervention in elementary school that delayed initiation of first cigarette

use during adolescence.⁴⁴ Here we extend this type of analysis to examine the interaction between genetics and intervention on smoking in high school within the PROSPER project, a largely rural and Caucasian prevention trial population.⁴⁵

The PROSPER intervention program was delivered to approximately 11 000 students and reduced smoking risk at 10th grade.⁴⁶ We hypothesized that the intervention would reduce high school smoking in the smaller subsample with DNA data considered herein. Consistent with the genetic results that the minor allele (A) at rs16969968 is the risk allele for smoking-related phenotypes, we further hypothesized that there would be a main effect of genotype on smoking during high school. Finally, we hypothesized that genotype would interact with the intervention to reduce smoking based on the number of copies of the minor (risk) allele. Follow-up analyses were conducted with additional markers in the cluster that are not as consistently associated with smoking-related behaviors.

Methods

Participants and Study Procedures

Data were from the PROSPER Project, a longitudinal universal substance misuse prevention intervention study that includes 14 intervention and 14 control communities in Iowa and Pennsylvania. The schools were from districts with 1300–5200 students in total enrollment, and more than 15% of the families qualifying for free or reduced cost school lunch. The intervention communities formed collaborative teams of university, local school, and cooperative extension members to choose and deliver the intervention programs. All 14 intervention communities chose the “Strengthening Families Program: For Parents and Youth 10–14” as their family-focused program in sixth grade ($M_{\text{age}} = 11.77$ years, $SD = 0.34$). The participation rate in Strengthening Families Program: For Parents and Youth 10–14 was approximately 17%. For the in-school program, administered in seventh grade ($M_{\text{age}} = 12.26$ years, $SD = 0.34$), “Life Skills Training”⁴⁷ and “Project Alert”⁴⁸ were selected by four teams each, while the other six teams chose the “All Stars” curriculum.⁴⁹ These three programs utilize similar lessons that target social norms, personal goal setting, decision-making, and peer group affiliation. These school-based interventions were delivered during required classes as part of the curriculum in seventh grade, ensuring that nearly all students in intervention communities took part. Lesson activities included participation in question-answer sessions, role-play, and small-group activities focused on peer pressure, decision-making skills, and risks associated with substance misuse. Very high levels of implementation quality have been confirmed across intervention models and cohorts.⁵⁰

During their sixth grade year, students in participating communities were invited to complete in-school surveys with approximately 90% participation as indicated in the CONSORT flowchart (Figure 1). The second in-school data collection was 6 months later (Wave 2) and data were collected annually thereafter. A random sub-sample of 2267 PROSPER families were invited to participate in an additional in-home assessment, which included in-depth interviews with the child and one or both parents if available. Of the families invited, 979 (43%) participated. These in-home participants were similar to those of the full sample for measures of general risk (Table 2).⁵¹ However, in-home participants did report slightly lower perceived benefits from using substances ($M = 4.71$ vs. $M = 4.77$, $F(1,27) = 12.36$, $P < .01$) than the full sample of youth in the PROSPER project responding to school-based assessments. Effect

Figure 1. CONSORT flow chart of genotyped sample from the PROSPER study. Recruitment numbers of the PROSPER study and in-home sub-study from which participants were recruited for genetic study (gPROSPER). **Demographic details for participants at the assignment stage, in-home stage, and provided DNA stage are available in Table 2.

sizes for these differences were small ($r_s < 0.03$). DNA samples were collected from a subset (594) of adolescents either in ninth grade (Wave 5) or when recontacted after high school. The current analysis is based on $N = 424$ in-home adolescent participants who had both DNA data for the rs16969968 SNP and smoking data during Waves 5–8. Participants in this sample were primarily Caucasian (90.8%) with smaller groups reporting Hispanic (5.2%), black (0.7%), Asian (0.7%), and other (2.6%) ethnicities.

Measures

Genotyping

Data for rs16969968 were generated by Affymetrix Inc (Santa Clara, CA) on an Exome 319 Array. Four of the markers studied (rs1948, rs578776, rs588765, and rs684513) were not present on the Exome Array, but were genotyped later using the OpenArray system from Life Technologies at the Penn State Genomics Core Facility. Genotype frequencies are presented in Table 1, and all markers were in Hardy-Weinberg Equilibrium ($P_s > .4$, using SAS Proc Allele). No differences were found between these cases and the analysis sample on any study variables or demographics (Table 2).

Table 1. Genotype Frequencies at the Five SNPs Tested in the *CHRNA5/A3/B4* Cluster

SNP	Genotype, N(%)		
rs16969968	G/G, 193(45.5)	G/A, 180 (42.5)	A/A, 51(12.0)
rs1948	G/G, 204(45.9)	G/A, 188(42.3)	A/A, 52(11.7)
rs578776	C/C, 224(50.7)	C/T, 178(40.3)	T/T, 40(9.0)
rs588765	C/C, 162(36.5)	C/T, 203(45.7)	T/T, 79(17.4)
rs684513	C/C, 267(61.7)	C/G, 144(33.3)	G/G, 22(5.1)

Condition

Intervention condition was coded 0 = control ($n = 175$, 41.3%) 1 = intervention (249, 58.7%).

High School Smoking

The outcome was constructed from reports of past month smoking assessed across the high school years (grades 9–12). We did not analyze eighth grade smoking due to the low frequencies of any reports of smoking, and because of the discrete shift from middle school to high school at ninth grade. As part of the in-school assessment, adolescents

Table 2. Descriptive Data for the Analytic Sample, the In-Home Sub-Study, and Full Sample

Sample characteristic	Analytic sample, N = 424	In-home sample, N = 977	Full sample, N = 10 849
Sex (% female)	53.5	52.4	50.4
Ethnicity (% European)	90.8	87.5	84.6
Both biological parents (% yes) ^a	60.3	51.1	61.9
Ever smoked (Wave 1; % yes)	3.6	4.4	6.9
rs16969968 (% "A" allele)	54.5	—	—
Condition (% intervention)	58.7	52.2	49.1
High school smoking	0.60 (<i>SD</i> = 0.84)	0.59 (<i>SD</i> = 0.84)	0.77 (<i>SD</i> = 0.93)
Age (Wave 5)	15.23 (<i>SD</i> = 0.34)	14.90 (<i>SD</i> = 0.47)	15.27 (<i>SD</i> = 0.44)
Maternal education ^b	13.69 (<i>SD</i> = 2.24)	13.26 (<i>SD</i> = 2.41)	13.26 (<i>SD</i> = 2.41)

^aFrequencies of biological parent presence and maternal education were taken from Wave 1.

^bMaternal education was collected as part of the in-home data collection only.

Table 3. Characteristics of Smoking Measures During High School

	Age, mean (<i>SD</i>)	Ever smoked a cigarette	Smoked at all in the past month	Past month smoking, mean (<i>SD</i>)
Wave 5	15.23 (0.34)	20.6%	10.6%	1.14 (0.42)
Wave 6	16.22 (0.34)	31.9%	13.7%	1.22 (0.59)
Wave 7	17.22 (0.34)	41.6%	19.9%	1.30 (0.64)
Wave 8	18.16 (0.33)	49.9%	24.4%	1.37 (0.70)

Percentages reflect affirmative responses. Past month smoking does not distinguish between those who have never smoked and those who have smoked but not in the past month.

were asked at each wave if they had ever smoked a cigarette and if so, how often they smoked in the past month. Response categories for the past month smoking item ranged from 1 = not at all to 5 = more than once a week. Although this past month smoking variable provides important information on the amount of smoking, it captures smoking behavior during only one of a possible 12 months, and relying solely on this item would risk underestimating smoking. To address this risk, we used information from an "ever smoking" item. The combination of these items allowed us to construct an outcome variable capturing the smoking behavior of participants who had smoked during high school but not within the month prior to the annual assessments. The resulting variable was 0 for never smoking, 1 for having smoked but not in last month, 2 for once to a few times in the past month, and 3 for about once a week or more. The across-year scores were strongly correlated with each other (mean $r = 0.65$) and showed good test-retest reliability ($\alpha = 0.88$; $M = 0.60$, $SD = 0.84$). These scores were averaged across high school years (Waves 5, 6, 7, and 8, approximate ages of 15–18 years) to create an overall high school smoking score. There were 139 (25%) cases missing smoking data due to attrition, but there were no resulting differences in condition, sex, ethnicity, or rs16969968 allele frequencies. Wave 1 smoking was very rare in both samples: Less than 4% and less than 7% in analytic and full samples, respectively (Table 3).

Analysis

The adolescents in this study were nested within communities, and thus the assumption of statistical independence may have been violated (eg, due to shared school/community environmental influences as well as peer influence effects from interactions among students attending school together⁵²). While little clustering within communities was detected by intra-class correlation ($ICC = 0.03$), the data were analyzed using the REPEATED statement in SAS PROC MIXED⁵³ to adjust parameters for nonindependence.

We used two approaches for genetic analysis. First, additive coding was used, which facilitates comparison with other studies. For rs16969968 the risk allele is A ($G/G = 0$, $G/A = 1$, $A/A = 2$). Second, dummy coding was used to permit nonlinear associations between numbers of rs16969968 "A" alleles and high school smoking.⁵⁴ The two dummy codes were created using G/G as the reference group so that the associations reflect G/G versus A/G and G/G versus A/A . To compare G/A with A/A , an additional dummy code was created for analysis with A/G as the reference group.

Results

Main Effects and Interactions of Genotype and Condition

In our first model we tested the main effects of condition and rs16969968 additively coded. Results showed reduced smoking during high school among intervention adolescents ($b = -0.24$, $P < .05$). A main effect of rs16969968 ($b = 0.14$, $P < .05$) indicated that high school smoking prevalence increases with the number of A alleles among adolescents. The interaction between the additive effect of rs16969968 and intervention was not significant ($b = -0.020$, $P = .12$). In the second model with dummy coded rs16969968 (using G/G as the reference group) the intervention was again associated with reduced high school smoking ($b = -0.24$, $P < .05$), and there was a significant effect of genotype between G/G and A/A genotypes ($b = 0.33$, $P < .05$) such that smoking was more prevalent among adolescents with A/A genotype compared to G/G (Table 4). A nearly significant difference was found between A/G and A/A ($b = 0.24$, $P < .07$), while no difference was found between G/G and G/A genotypes ($b = 0.10$, *ns*). There were no significant effects for rs1948, rs578776, rs588765, or rs684513 in either model.

Of the five markers tested, only rs16969968 showed a significant interaction between genotype and intervention (comparing G/G vs.

A/A genotype [$b = -0.67, P < .05$]; however there was no interaction for G/G versus A/G genotype ($b = -0.06, ns$). An additional analysis using A/G as the reference group showed an additional significant interaction between intervention condition and A/G versus A/A ($b = -0.61, P < .05$) (Figure 2).

Tests for Potential Confounds

An alternative explanation for these findings may be that differences are an artifact of allele frequencies that differ due to genetic ancestry. To help rule out this confound, genetic ancestry was assessed by principal coordinate analysis⁵⁵ on over 47 000 polymorphic SNPs collected from these samples (see ref.⁴² for additional details). Briefly, the first coordinate (PC1) reflected variation in non-European genetic ancestry based on self-report of race. Although we specified

both a main effect of PC1 and interactions with the dummy variables, including PC1 did little to affect the interaction terms reported above (both bs -0.68 or less, $P < .05$). In addition, PC1 was used to create a dichotomous variable to distinguish those with and without non-European genetic ancestry.⁴² The analyses were conducted again, this time dropping those participants with non-European ancestry ($n = 43$); the results were the same (both bs -0.64 or less, $P < .05$). Similar results were obtained when dropping self-reported non-Europeans ($n = 39$).

Discussion

Consistent with our hypotheses, there was a main effect both for condition (intervention) and genotype (but only for rs16969968) on

Table 4. Regression Analyses for rs16969968 (Other SNPs Nonsignificant)

Variables		Parameter estimates (standard errors)	
		Model 1	Model 2
Main effects	Condition	-0.24(0.09)*	0.16(0.13)
	G/G vs. G/A	0.10(0.09)	0.13(0.13)
	G/G vs. A/A	0.33(0.13)*	0.83(0.26)*
	A/G vs. A/A	0.24(0.13)**	0.70(0.26)*
Interactions	Cond*G/G vs. G/A		-0.06(0.17)
	Cond*G/G vs. A/A		-0.67(0.30)*
	Cond*G/A vs. A/A		-0.61(0.30)*

Cond = condition (0 = control, 1 = intervention). Each model was tested twice, once using only rs169_dum1 and rs169_dum2 (G/G as the reference group) and a second time using only rs169_dum2 and rs169_dum3 (A/G as the reference group).

* $P < .05$; ** $P < .10$.

Figure 2. Effect of intervention status on smoking in the past month based on CHRNA5 Genotype. Level of high school smoking (0 = never to 3 = about once a week or more) is plotted for control and intervention participants based on genotype at rs16969968.

smoking in high school. Analysis of the interaction of condition and genotype revealed that the increase in smoking based on the number of risk alleles was not present in the intervention group. This G×I effect presents opportunities to refine our understanding of interventions on smoking-related behaviors and the biological underpinnings of the behaviors.

Variance in our high school smoking outcome is contributed to both by initiation (0 = never smoked vs. 1 = smoked but not in last month) and level of smoking during the month preceding annual in-school assessments (2 = one or a few times vs. 3 = about once a week or more). Of course, level of smoking depends upon having initiated. Our results suggest that the effect of the intervention might lie in reducing the likelihood that adolescents initiate smoking, thereby eliminating the potential effect of the risk allele on progression from smoking initiation to smoking regularly. This result is consistent with: (1) the intervention design that targets peer refusal skills and decision-making and (2) a genotype effect based on nicotine's psychoactive effects as shown in studies on nicotine dependence¹⁶ and transition from daily to heavy smoking.¹⁵ Too little is known about neural pathways and biochemical mechanisms that underlie peer refusal and decision-making to describe specific mechanisms. However, it is promising that an intervention designed to target decision making was able to mitigate the effects of the risk allele at this gene. Perhaps the biochemical pathway for decision-making is related in some way to function of *CHRNA5*. Thus, in addition to our general G×I finding needing replication from independent samples, any role for the genotype at rs16969968 in the initial choice to smoke needs to be explored in separate studies with methods that are more sensitive to decision making.

Aspects of the results of a genotype-by-smoking cessation drug trial by Chen et al.³¹ that studied rs16969968 are relevant to the current study. In the cessation trial, genotype predicted abstinence only in participants receiving placebo (the risk allele was associated with cessation failure), but there was no effect of genotype in those receiving drug therapy. These findings parallel our result that the risk allele is associated with high school smoking in control participants, while there was no association in participants receiving the intervention.

A large body of neuropharmacological data exists on nicotinic receptors with potential roles in many behaviors (ref.⁵⁶ for review). The finding of a genetic association of rs16969968 to smoking traits is of particular interest because the SNP is a nonsynonymous change resulting in two different amino acids at position 398 of the $\alpha 5$ receptor subunit (A encodes asparagine [N] and G encodes aspartate [D]). These two alleles in the protein are associated with distinct functions with respect to gene expression and protein activity. Tests of mRNA indicate that the risk allele of rs16969968 is expressed at a lower level than the nonrisk allele in human post-mortem frontal cortex,⁵⁷ which was confirmed in an African population for which the extent of linkage disequilibrium is more limited, reducing the number of other polymorphic sites that could confound the analysis.⁵⁸ At the protein level, nicotinic receptors that contain the $\alpha 5$ subunit protein with the risk allele are less sensitive to a nicotine agonist,¹³ and have both lower Ca^{2+} permeability and greater short-term desensitization.⁵⁹ Thus, the genetic differences extend to protein activity differences that are relevant to nicotine's actions in the brain. That this relationship is causal relationship is supported in transgenic mouse studies in which knocking out the gene increases nicotine consumption.⁶⁰ The mouse model has a more severe consequence by removing all $\alpha 5$ activity, but it is similar in direction of effect to the human risk-allele.

It is not uncommon for a single marker within a locus that contains several other polymorphic sites to be the only one associated with the trait tested. The SNP rs16969968 has been associated consistently with smoking phenotypes in other studies (described above) with *P* values remaining significant in genome-wide association studies (GWAS) ($<5 \times 10^{-8}$). The SNPs used in multiple marker analyses (polygenic risk scores or gene scores) are frequently drawn from those SNPs deemed significant or nearly significant in GWAS style analyses with severe accounting for multiple testing. Pooling these SNPs together in a gene score may capture more genetic variance than is typically explained by a single marker,^{32,61–63} but a single SNP can still account for the bulk of the variation in some risk scores.⁶³

Genetic variation in other genes is likely involved in high school smoking in addition to the variation from the *CNRNA5/A3/B4* cluster. Research points to other nicotinic receptor subunit genes, such as *CHRNA2*, which has been associated with subjective responses to nicotine,⁶⁴ as have the *CHRNA6* and *CHRNA3* genes.⁶⁵ Using markers that have a reliable and well-characterized role in smoking behaviors provides potential insight into how environments might be contributing to smoking behavior. That said, future work would be needed to understand how to incorporate other nicotinic receptor subunit genes, and other genes whose proteins do not have such direct associations with nicotine. Approaches that incorporate neurobiological pathway data as a means to address main effects of genes on smoking behaviors (ref.⁶⁶ for review), or genetic risk scores^{67,68} show promise, but more work is needed on aspects such as proper weighting of risk alleles in a genetic risk score to gain the most information from these techniques.

This study represents an early step in understanding the complicated pathways through which phenotypes used in genetic studies relate to a behavioral intervention (eg, peer, familial, and social factors). Testing for G×I effects in prevention trials provides a means for evaluating these pathways and also broadens the conceptual basis for development and refinement of intervention models.³⁵ Given that the PROSPER interventions target behavioral change, rather than physiological responses to nicotine, their effects are likely focused on the decision to initiate smoking and perhaps less so on smoking escalation.

Results from this type of G×I research may lead to small, efficient trials (microtrials) that target individuals with specific genotypes or test more narrowly defined interventions and outcomes, or both.³⁵ A second stage of full-scale studies might then test multiple intervention variables and outcome measures simultaneously to examine synergies and interferences between the parts of the intervention.⁶⁹ For a complex behavior such as response to a behavioral intervention, we anticipate that the action of many loci would be involved—leading to genetic risk being distributed along a broad continuum. Thus, targeted studies will help shape universal interventions that significantly reduce risky behaviors in the large portion of the population at moderate risk.

It is important that interventions to prevent smoking be presented at an early age. The adolescent brain responds to the presence of nicotine resulting in symptoms of nicotine dependence within the first few cigarettes.^{70,71} In addition, the likelihood to become a lifelong smoker is greatest in those who start smoking during adolescence.^{72,73} Work from other studies suggests that adolescents possessing the risk allele at rs16969968, might have heightened sensitivity to nicotine,³⁰ and convert to daily smoking as teenagers.⁷⁴ Our finding that this specific genetic risk for adolescent smoking was not present in those exposed to a universal prevention model

designed to reduce substance misuse is valuable given the significant public health implications of reducing smoking. It remains to be seen if this G×I effect extends to other drugs as well. The rs16969968 SNP is also associated with alcohol use in adolescents,⁷⁴ and the *CHRNA5/A3/B4* cluster surrounding the SNP has been associated with adolescent use of other drugs,⁷⁵ suggesting that the consequences of genotype status in this region are not limited to nicotine.

Our results, along with these additional studies, reinforce the call by Belsky, Moffitt, and Caspi⁷⁶ for genetically-informed intervention trials to reduce life-long health risks like smoking. Genetic findings such as ours contribute significantly to our understanding of variability in response to prevention; that is, who derives the greatest benefits from prevention and who derives little or no benefit. Targeting individuals for preventive interventions based on genetic factors is controversial; for example, some fear that targeting could lead to stigma based on genetic susceptibility. However, in an age wherein personalized medicine based on genes is gaining traction in the medical and pharmaceutical domains, the potential benefits of using similar approaches for behavioral prevention should be considered and balanced against the potential downsides. As personalized medicine advances, and with it the understanding of inherited risk among the lay population, the potential for stigma may decrease over time. Moreover, understanding the genetic basis of variability in response to prevention should also motivate additional work on building effective prevention strategies that either are more effective with the entire population or are able to benefit the portions of the population who are relatively unaffected by current preventive strategies.

Funding

This work was supported by the National Institute on Drug Abuse (grant numbers DA030389 and DA013709).

Declaration of Interests

None declared.

Acknowledgments

The authors would like to thank Dr Deborah Grove and Ms Ashley Price of the Penn State Genomics Core Facility for DNA purification and genotyping. Shirley Huck, Cathy Owen, Debra Bahr, and Anthony Connor of the Iowa State University Survey and Behavioral Research Services, and Larissa Witmer, Rob Schofield and Dean Stankowski of the Penn State University Survey Research Center assisted with participant recruitment. Lee Carpenter and Amanda Griffin of Penn State provided support and critical comments on the paper.

References

- Hall W, Madden P, Lynskey M. The genetics of tobacco use: methods, findings and policy implications. *Tob Control*. 2002;11(2):119–124.
- Sullivan PF, Kendler KS. The genetic epidemiology of smoking. *Nicotine Tob Res*. 1999;1(suppl 2):S51–57; discussion S69–70.
- Rose RJ, Broms U, Korhonen T, Dick DM, Kaprio J. Genetics of Smoking Behavior. In: Kim YK, ed. *Handbook of Behavior Genetics*. 1st ed. New York, NY: Springer; 2009:411–432. doi:10.1007/978-0-387-76727-728.
- Li MD, Cheng R, Ma JZ, Swan GE. A meta-analysis of estimated genetic and environmental effects on smoking behavior in male and female adult twins. *Addiction*. 2003;98(1):23–31.
- Haberstick BC, Ehringer MA, Lessem JM, Hopfer CJ, Hewitt JK. Diziness and the genetic influences on subjective

- experiences to initial cigarette use. *Addiction*. 2011;106(2):391–399. doi:10.1111/j.1360-0443.2010.03133.x.
- Lessov-Schlaggar CN, Kristjansson SD, Bucholz KK, Heath AC, Madden PA. Genetic influences on developmental smoking trajectories. *Addiction*. 2012;107(9):1696–1704. doi:10.1111/j.1360-0443.2012.03871.x.
- Agrawal A, Madden PA, Bucholz KK, Heath AC, Lynskey MT. Initial reactions to tobacco and cannabis smoking: a twin study. *Addiction*. 2014;109(4):663–671. doi:10.1111/add.12449.
- Berrettini WH, Doyle GA. The *CHRNA5-A3-B4* gene cluster in nicotine addiction. *Mol Psychiatry*. 2012;17(9):856–866. doi:10.1038/mp.2011.122.
- Vandenbergh DJ, Schlomer GL. Finding genomic function for genetic associations in nicotine addiction research: the ENCODE project's role in future pharmacogenomic analysis. *Pharmacol Biochem Behav*. 2014;123:34–44. doi:10.1016/j.pbb.2014.01.009.
- Bierut LJ, Stitzel JA, Wang JC, et al. Variants in nicotinic receptors and risk for nicotine dependence. *Am J Psychiatry*. 2008;165(9):1163–1171. doi:10.1176/appi.ajp.2008.07111711.
- Saccone NL, Culverhouse RC, Schwantes-An TH, et al. Multiple independent loci at chromosome 15q25.1 affect smoking quantity: a meta-analysis and comparison with lung cancer and COPD. *PLoS Genet*. 2010;6(8):pii: e1001053. doi:10.1371/journal.pgen.1001053.
- Stephens SH, Hartz SM, Hoft NR, et al. Distinct loci in the *CHRNA5/CHRNA3/CHRNA4* gene cluster are associated with onset of regular smoking. *Genet Epidemiol*. 2013;37(8):846–859. doi:10.1002/gepi.21760.
- Saccone SF, Hinrichs AL, Saccone NL, et al. Cholinergic nicotinic receptor genes implicated in a nicotine dependence association study targeting 348 candidate genes with 3713 SNPs. *Hum Mol Genet*. 2007;16(1):36–49.
- Thorgeirsson TE, Geller F, Sulem P, et al. A variant associated with nicotine dependence, lung cancer and peripheral arterial disease. *Nature*. 2008;452(7187):638–642. doi:10.1038/nature06846.
- Berrettini W, Yuan X, Tozzi F, et al. Alpha-5/alpha-3 nicotinic receptor subunit alleles increase risk for heavy smoking. *Mol Psychiatry*. 2008;13(4):368–373. doi:10.1038/sj.mp.4002154.
- Saccone NL, Saccone SF, Hinrichs AL, et al. Multiple distinct risk loci for nicotine dependence identified by dense coverage of the complete family of nicotinic receptor subunit (*CHRN*) genes. *Am J Med Genet B Neuropsychiatr Genet*. 2009;150B(4):453–466. doi:10.1002/ajmg.b.30828.
- Liu JZ, Tozzi F, Waterworth DM, et al. Meta-analysis and imputation refines the association of 15q25 with smoking quantity. *Nat Genet*. 2010;42(5):436–440. doi:10.1038/ng.572.
- Tobacco and Genetics Consortium (Furberg). Genome-wide meta-analyses identify multiple loci associated with smoking behavior. *Nat Genet*. 2010;42(5):441–447. doi:10.1038/ng.571.
- Thorgeirsson TE, Gudbjartsson DF, Surakka I, et al. Sequence variants at *CHRNA3-CHRNA6* and *CYP2A6* affect smoking behavior. *Nat Genet*. 2010;42(5):448–453. doi:10.1038/ng.573.
- Chen LS, Baker TB, Gruzza R, et al. Dissection of the phenotypic and genotypic associations with nicotinic dependence. *Nicotine Tob Res*. 2012;14(4):425–433. doi:10.1093/ntr/ntr231.
- Chen LS, Saccone NL, Culverhouse RC, et al. Smoking and genetic risk variation across populations of European, Asian, and African American ancestry—a meta-analysis of chromosome 15q25. *Genet Epidemiol*. 2012;36(4):340–351. doi:10.1002/gepi.21627. Erratum in: *Genet Epidemiol*. 2012 Jul;36(5):525–6.
- Sherva R, Wilhelmsen K, Pomerleau CS, et al. Association of a single nucleotide polymorphism in neuronal acetylcholine receptor subunit alpha 5 (*CHRNA5*) with smoking status and with 'pleasurable buzz' during early experimentation with smoking. *Addiction*. 2008;103(9):1544–1552. doi:10.1111/j.1360-0443.2008.02279.x.
- Weiss RB, Baker TB, Cannon DS, et al. A candidate gene approach identifies the *CHRNA5-A3-B4* region as a risk factor for age-dependent nicotine addiction. *PLoS Genet*. 2008;4(7):e1000125. doi:10.1371/journal.pgen.1000125.

24. Grucza RA, Johnson EO, Krueger RF, et al. Incorporating age at onset of smoking into genetic models for nicotine dependence: evidence for interaction with multiple genes. *Addict Biol.* 2010;15(3):346–357. doi:10.1111/j.1369-1600.2010.00220.x.
25. Pomerleau OF, Pomerleau CS, Namenek RJ. Early experiences with tobacco among women smokers, ex-smokers, and never-smokers. *Addiction.* 1998;93(4):595–599.
26. Chen X, Stacy A, Zheng H, et al. Sensations from initial exposure to nicotine predicting adolescent smoking in China: a potential measure of vulnerability to nicotine. *Nicotine Tob Res.* 2003;5(4):455–463.
27. DiFranza JR, Savageau JA, Fletcher K, et al. Recollections and repercussions of the first inhaled cigarette. *Addict Behav.* 2004;29(2):261–272.
28. Pomerleau OF, Pomerleau CS, Mehringer AM, Snedecor SM, Cameron OG. Validation of retrospective reports of early experiences with smoking. *Addict Behav.* 2005;30(3):607–611.
29. Pomerleau CS, Pomerleau OF, Namenek RJ, Marks JL. Initial exposure to nicotine in college-age women smokers and never-smokers: a replication and extension. *J Addict Dis.* 1999;18(3):13–19.
30. Hartz SM, Short SE, Saccone NL, et al. Increased genetic vulnerability to smoking at CHRNA5 in early-onset smokers. *Arch Gen Psychiatry.* 2012;69(8):854–860. doi:10.1001/archgenpsychiatry.2012.124.
31. Chen LS, Baker TB, Piper ME, et al. Interplay of genetic risk factors (CHRNA5-CHRNA3-CHRNA4) and cessation treatments in smoking cessation success. *Am J Psychiatry.* 2012;169(7):735–742. doi:10.1176/appi.ajp.2012.11101545.
32. Uhl GR, Walther D, Musci R, et al. Smoking quit success genotype score predicts quit success and distinct patterns of developmental involvement with common addictive substances. *Mol Psychiatry.* 2014;19(1):50–54. doi:10.1038/mp.2012.155.
33. McClelland GH, Judd CM. Statistical difficulties of detecting interactions and moderator effects. *Psychol Bull.* 1993;114(2):376–390.
34. van Ijzendoorn MH, Bakermans-Kranenburg MJ, Belsky J, et al. Gene-by-environment experiments: a new approach to finding the missing heritability. *Nat Rev Genet.* 2011;12(12):881 doi:10.1038/nrg2764-c1.
35. Brody GH, Beach SR, Hill KG, Howe GW, Prado G, Fullerton SM. Using genetically informed, randomized prevention trials to test etiological hypotheses about child and adolescent drug use and psychopathology. *Am J Public Health.* 2013;103(suppl 1):S19–24. doi:10.2105/AJPH.2012.301080.
36. Bakermans-Kranenburg MJ, van Ijzendoorn MH. The hidden efficacy of interventions: genexenvironment experiments from a differential susceptibility perspective. *Annu Rev Psychol.* 2015;66:381–409.
37. Brody GH, Chen YF, Beach SR, Philibert RA, Kogan SM. Participation in a family-centered prevention program decreases genetic risk for adolescents' risky behaviors. *Pediatrics.* 2009;124(3):911–917. doi:10.1542/peds.2008-3464.
38. Brody GH, Yu T, Beach SR. A differential susceptibility analysis reveals the “who and how” about adolescents' responses to preventive interventions: tests of first- and second-generation Gene x Intervention hypotheses. *Dev Psychopathol.* 2015;27(1):37–49. doi:10.1017/S095457941400128X.
39. Albert D, Belsky DW, Crowley DM, et al. Developmental mediation of genetic variation in response to the Fast Track prevention program. *Dev Psychopathol.* 2015;27(1):81–95.
40. Albert D, Belsky DW, Crowley DM, et al. Developmental mediation of genetic variation in response to the Fast Track prevention program. *Dev Psychopathol.* 2015;27(1):81–95. doi:10.1017/S095457941400131X.
41. Musci RJ, Bradshaw CP, Maher B, Uhl GR, Kellam SG, Ialongo NS. Reducing aggression and impulsivity through school-based prevention programs: a gene by intervention interaction. *Prev Sci.* 2014;15(6):831–840. doi:10.1007/s11121-013-0441-3.
42. Cleveland HH, Schlomer GL, Vandenbergh DJ, et al. The conditioning of intervention effects on early adolescent alcohol use by maternal involvement and dopamine receptor D4 (DRD4) and serotonin transporter linked polymorphic region (5-HTTLPR) genetic variants. *Dev Psychopathol.* 2015;27(1):51–67. doi:10.1017/S0954579414001291.
43. Patnode CD, O'Connor E, Whitlock EP, Perdue LA, Soh C. Primary care-relevant interventions for tobacco use prevention and cessation in children and adolescents: a systematic evidence review for the U.S. Preventive Services Task Force. *Ann Intern Med.* 2013;158(4):253–260. doi:10.7326/0003-4819-158-4-201302190-00580.
44. Musci RJ, Bradshaw CP, Maher B, Uhl GR, Kellam SG, Ialongo NS. Reducing aggression and impulsivity through school-based prevention programs: a gene by intervention interaction. *Prev Sci.* 2014;15(6):831–840. doi:10.1007/s11121-013-0441-3.
45. Spoth R, Greenberg M, Bierman K, Redmond C. PROSPER community-university partnership model for public education systems: capacity-building for evidence-based, competence-building prevention. *Prev Sci.* 2004;5(1):31–39.
46. Spoth RL, Redmond C, Shin C. Randomized trial of brief family interventions for general populations: adolescent substance use outcomes 4 years following baseline. *J Consult Clin Psychol.* 2001;69(4):627–642.
47. Botvin GJ, Kantor LW. Preventing alcohol and tobacco use through life skills training. *Alcohol Res Health.* 2000;24(4):250–257.
48. Ellickson PL, McCaffrey DE, Ghosh-Dastidar B, Longshore DL. New inroads in preventing adolescent drug use: results from a large-scale trial of project ALERT in middle schools. *Am J Public Health.* 2003;93(11):1830–1836.
49. McNeal RB Jr, Hansen WB, Harrington NG, Giles SM. How all stars works: an examination of program effects on mediating variables. *Health Educ Behav.* 2004;31(2):165–178.
50. Spoth R, Gyll M, Lillehoj CJ, Redmond C, Greenberg M. Prosper Study Of Evidence-Based Intervention Implementation Quality By Community-University Partnerships. *J Community Psychol.* 2007;35(8):981–999.
51. Lippold MA, Greenberg MT, Collins LM. Youths' substance use and changes in parental knowledge-related behaviors during middle school: a person-oriented approach. *J Youth Adolesc.* 2014;43(5):729–744. doi:10.1007/s10964-013-0010-x.
52. Kenny DA, Kashy DA, Cook WL. *Dyadic Data Analysis.* New York, NY: Guilford Press; 2006.
53. SAS Institute Inc. *SAS/STAT® 9.3 User's Guide.* Cary, NC: SAS Institute Inc; 2011.
54. Aliev F, Latendresse SJ, Bacanu SA, Neale MC, Dick DM. Testing for measured gene-environment interaction: problems with the use of cross-product terms and a regression model reparameterization solution. *Behav Genet.* 2014;44(2):165–181. doi:10.1007/s10519-014-9642-1.
55. Halder I, Shriver M, Thomas M, Fernandez JR, Frudakis T. A panel of ancestry informative markers for estimating individual biogeographical ancestry and admixture from four continents: utility and applications. *Hum Mutat.* 2008;29(5):648–658. doi:10.1002/humu.20695.
56. Rogers SW, Gould TJ, Baker TB. Mouse Models and the Genetics of Nicotine Dependence. In: Lerman C, Perkin KA, Gould TJ, eds. *Phenotypes and Endophenotypes Foundations for Genetic Studies of Nicotine Use and Dependence.* Bethesda, MD: National Cancer Institute; 2012:133–186.
57. Wang JC, Cruchaga C, Saccone NL, et al. Risk for nicotine dependence and lung cancer is conferred by mRNA expression levels and amino acid change in CHRNA5. *Hum Mol Genet.* 2009;18(16):3125–3135. doi:10.1093/hmg/ddp231.
58. Wang JC, Spiegel N, Bertelsen S, et al. Cis-regulatory variants affect CHRNA5 mRNA expression in populations of African and European ancestry. *PLoS One.* 2013;8(11):e80204. doi:10.1371/journal.pone.0080204.
59. Kuryatov A, Berrettini W, Lindstrom J. Acetylcholine receptor (AChR) $\alpha 5$ subunit variant associated with risk for nicotine dependence and lung cancer reduces ($\alpha 4\beta 2$) $\alpha 5$ AChR function. *Mol Pharmacol.* 2011;79(1):119–125. doi:10.1124/mol.110.066357.
60. Fowler CD, Arends MA, Kenny PJ. Subtypes of nicotinic acetylcholine receptors in nicotine reward, dependence, and withdrawal: evidence from genetically modified mice. *Behav Pharmacol.* 2008;19(5–6):461–484. doi:10.1097/FBP.0b013e32830c360e.
61. Belsky DW, Moffitt TE, Baker TB, et al. Polygenic risk and the developmental progression to heavy, persistent

- smoking and nicotine dependence: evidence from a 4-decade longitudinal study. *JAMA Psychiatry*. 2013;70(5):534–542. doi:10.1001/jamapsychiatry.2013.736.
62. Vrieze SI, McGue M, Iacono WG. The interplay of genes and adolescent development in substance use disorders: leveraging findings from GWAS meta-analyses to test developmental hypotheses about nicotine consumption. *Hum Genet*. 2012;131(6):791–801. doi:10.1007/s00439-012-1167-1.
 63. Meyers JL, Cerdá M, Galea S, et al. Interaction between polygenic risk for cigarette use and environmental exposures in the Detroit Neighborhood Health Study. *Transl Psychiatry*. 2013;3:e290. doi:10.1038/tp.2013.63.
 64. Ehringer MA, Clegg HV, Collins AC, et al. Association of the neuronal nicotinic receptor beta2 subunit gene (CHRNA2) with subjective responses to alcohol and nicotine. *Am J Med Genet B Neuropsychiatr Genet*. 2007;144B(5):596–604.
 65. Zeiger JS, Haberstick BC, Schlapfer I, et al. The neuronal nicotinic receptor subunit genes (CHRNA6 and CHRNA3) are associated with subjective responses to tobacco. *Hum Mol Genet*. 2008;17(5):724–734.
 66. Ramanan VK, Shen L, Moore JH, Saykin AJ. Pathway analysis of genomic data: concepts, methods, and prospects for future development. *Trends Genet*. 2012;28(7):323–332. doi:10.1016/j.tig.2012.03.004.
 67. Wray NR, Goddard ME, Visscher PM. Prediction of individual genetic risk of complex disease. *Curr Opin Genet Dev*. 2008;18(3):257–263. doi:10.1016/j.gde.2008.07.006.
 68. Plomin R, Haworth CM, Davis OS. Common disorders are quantitative traits. *Nat Rev Genet*. 2009;10(12):872–878.
 69. Collins LM, Baker TB, Mermelstein RJ, et al. The multiphase optimization strategy for engineering effective tobacco use interventions. *Ann Behav Med*. 2011;41(2):208–226.
 70. DiFranza J, Savageau J, Rigotti N, et al. Development of symptoms of tobacco dependence in youths: 30 month follow up data from the DANDY study. *Tob Control*. 2002;11(3):228–235. doi:10.1136/tc.11.3.228.
 71. DiFranza JR. Hooked from the first cigarette. *J Fam Pract*. 2007;56(12):1017–1022.
 72. Breslau N, Peterson EL. Smoking cessation in young adults: age at initiation of cigarette smoking and other suspected influences. *Am J Public Health*. 1996;86(2):214–220.
 73. Chassin L, Presson CC, Pitts SC, Sherman SJ. The natural history of cigarette smoking from adolescence to adulthood in a midwestern community sample: multiple trajectories and their psychosocial correlates. *Health Psychol*. 2000;19(3):223–231.
 74. Schlapfer IR, Hoft NR, Collins AC. The CHRNA5/A3/B4 gene cluster variability as an important determinant of early alcohol and tobacco initiation in young adults. *Biol Psychiatry*. 2008;63(11):1039–1046. doi:10.1016/j.biopsych.2007.10.024.
 75. Lubke GH, Stephens SH, Lessem JM, Hewitt JK, Ehringer MA. The CHRNA5/A3/B4 gene cluster and tobacco, alcohol, cannabis, inhalants and other substance use initiation: replication and new findings using mixture analyses. *Behav Genet*. 2012;42(4):636–646. doi:10.1007/s10519-012-9529-y.
 76. Belsky DW, Moffitt TE, Caspi A. Genetics in population health science: strategies and opportunities. *Am J Public Health*. 2013;103(suppl 1):S73–83. doi:10.2105/AJPH.2012.301139.