Cigarette Experimentation in Mexican Origin Youth: Psychosocial and Genetic Determinants

Anna V. Wilkinson1,2, Melissa L. Bondy2,3, Xifeng Wu2, Jian Wang2, Qiong Dong2, Anthony M. D’Amelio Jr2, Alexander V. Prokhorov2, Xia Pu2, Robert K. Yu2, Carol J. Etzel2, Sanjay Shete2, and Margaret R. Spitz3

Abstract

Background: Established psychosocial risk factors increase the risk for experimentation among Mexican origin youth. Now, we comprehensively investigate the added contribution of select polymorphisms in candidate genetic pathways associated with sensation seeking, risk taking, and smoking phenotypes to predict experimentation.

Methods: Participants (N = 1,118 Mexican origin youth) recruited from a large population-based cohort study in Houston, TX, provided prospective data on cigarette experimentation over 3 years. Psychosocial data were elicited twice—baseline and final follow-up. Participants were genotyped for 672 functional and tagging variants in the dopamine, serotonin, and opioid pathways.

Results: After adjusting for gender and age, with a Bayesian False Discovery Probability set at 0.8 and prior probability of 0.05, six gene variants were significantly associated with risk of experimentation. After controlling for established risk factors, multivariable analyses revealed that participants with six or more risk alleles were 2.25 [95% confidence interval (CI), 1.62–3.13] times more likely to have experimented since baseline than participants with five or fewer. Among committed never-smokers (N = 872), three genes (OPRM1, SNAP25, HTR1B) were associated with experimentation as were all psychosocial factors. Among susceptible youth (N = 246), older age at baseline, living with a smoker, and three different genes (HTR2A, DRD2, SLC6A3) predicted experimentation.

Conclusions: Our findings, which have implications for development of culturally specific interventions, need to be validated in other ethnic groups.

Impact: These results suggest that variations in select genes interact with a cognitive predisposition toward smoking. In susceptible adolescents, the impact of the genetic variants appears to be larger than committed never-smokers. Cancer Epidemiol Biomarkers Prev; 21(1); 228–38. ©2011 AACR.

Introduction

Smoking remains the leading modifiable risk factor for lung cancer, increasing risk for the disease between 10- and 20-fold, depending on the duration and intensity (1). Moreover, compared with smoking initiation in late adolescence or early adulthood, early smoking initiation is associated with increased DNA damage in the lung (2) as well as higher levels of nicotine dependence and more years smoked (3), all factors that increase the risk of developing lung cancer (2, 4). The social, economic, and individual benefits to be gained by preventing youth from experimenting with cigarettes and becoming adult smokers are therefore profound.

Despite the large body of literature available on the prevalence of smoking behavior among United States (U.S.) adolescents, research examining the transition from never smoking to experimentation (defined as having tried cigarettes, but not smoking on a monthly basis) with cigarettes is limited, especially among Hispanic adolescents. We (5) and others (6, 7) have reported that cognitive susceptibility to smoking, a construct that combines peer social influence with behavioral intentions, is possibly the strongest single predictor of cigarette experimentation. We found that 45% of youth who self-reported as cognitively susceptible to smoking subsequently experimented with cigarettes, compared with 15% of their peers who self-reported as being committed never-smokers (5). Understanding factors associated with this transition is crucial to smoking prevention efforts because up to 64% of experimenters transition to daily smoking within 1 year of starting (8), most make the transition to regular smoking.
by 18 years of age (9) and almost 90% of adults who currently smoke initially experimented with smoking when they were younger than 18 years (10). Figure 1 presents transitions on the smoking continuum from committed never-smoker, to susceptible, to experimenting, and finally regular smoking.

Hispanics are the largest and most rapidly increasing ethnic group in the United States and those of Mexican origin comprise the U.S. majority of this subgroup (11). Nationally, Mexican origin youth report the highest rates of cognitive susceptibility to smoking—29% compared with 21% in non-Hispanic whites and 23% in non-Hispanic blacks (12) and Texas Hispanic adolescents exhibit the highest rates of experimentation with cigarettes among all ethnic groups—55% compared with 48% in non-Hispanic whites and 46% in non-Hispanic blacks report ever experimenting with cigarettes (13), underscoring their increased risk for adult smoking.

To enhance prevention efforts in this growing population, we evaluated the trajectory from never smoking to experimenting with cigarettes in a longitudinal cohort of Mexican origin adolescents between 11 and 13 years of age at enrollment who were followed for 3 years. Among this sample of more than a thousand youth, we have shown (5) that being a boy and older at enrollment, holding positive beliefs about the social benefits of smoking, living with a smoker, and having had at least one school detention—all significantly predicted becoming an experimenter over the course of the study. We also reported that baseline cognitive susceptibility to smoking status modified the relationship between experimentation at follow-up and the other established risk factors examined (5), suggesting that risk factors associated with experimentation vary by susceptibility status.

Contemporaneously, family, twin, candidate, and genome-wide association studies provide compelling evidence of a role for genetic variants governing smoking initiation, nicotine dependence, and cessation (14, 15) with estimated heritability rates ranging from 40% to 60% for initiation (16, 17) and around 50% for persistence (18). We now expand our analysis to assess the role of 58 genes and 672 single-nucleotide polymorphisms (SNP) covering genetic variants in candidate pathways that influence sensation-seeking behavior, risk-taking behavior, and smoking phenotypes, in the context of established demographic and psychosocial risk factors, among these same youth during the transition from never smoking to experimentation with cigarettes. We include SNPs associated with sensation-seeking and risk-taking behavior because both behaviors are associated with smoking initiation (19–21). Because our goal is to examine potential gene–environment interactions associated with the transition from never smoking to experimenting with cigarettes; in the current study, we focus on youth who had not experimented with cigarettes at baseline when they enrolled in the cohort.

Participants and Methods

Participant recruitment

Between May 2005 and December 2006, youth were recruited from a population-based cohort of Mexican-American households in the Houston metropolitan area. A detailed description of the cohort recruitment methodology has been published (22). A total of 3,000 households with potential age-eligible adolescents (11–13 years) were identified from the cohort database. Of the first 1,425 potential participants’ parents or legal guardians contacted...
Data collection

Data on smoking phenotype were collected via personal interview on 5 sequential encounters. Baseline and final interviews were completed in the home—the second home survey was completed 30 months on average (SD = 4.8 months) after baseline. The second, third, and fourth interviews were completed roughly 6, 12, and 18 months, respectively, following baseline, over the telephone. At baseline, after consenting into the study, each participant completed a 5-minute personal interview during which basic demographic [gender, age, nativity status (US or Mexico)] acculturation data (23) and parental educational attainment (available for 93.5% of the study participants) were collected. To assure confidentiality from parents overhearing the child’s responses, the remainder of the survey was completed on a personal digital assistant (PDA). All participants provided buccal samples at baseline. A detailed description of baseline data collection procedures has been published (24).

Measures

Our primary outcome variable was new experimentation with cigarettes, assessed using two questions derived from the Youth Risk Behavior Surveillance Survey (25), “Have you ever smoked a whole cigarette?” and “Have you ever tried a cigarette, even a puff?” Participants who answered “yes” to either question were defined as experimenters. All others were considered never experimenters. Participants who responded “no” at baseline, but “yes” to either question at any follow-up were designated as “new experimenters.”

We further examined the influence of several established psychosocial risk factors: risk-taking tendencies (21), outcome expectations related to smoking (26), household social influence (27–29), and cognitive susceptibility to smoking (5, 7). The measures used to assess these constructs are detailed in Table 1.

DNA collection

Saliva samples were obtained in Oragene vials (DNA Genotek). DNA extraction was conducted using a “Purifier” solution with alcohol precipitation per the manufacturer’s protocol. The median yield of DNA from 2 mL of saliva captured in 2 mL of Oragene DNA was 110 μg.

Table 1. Description of the psychosocial measures used to assess each construct

<table>
<thead>
<tr>
<th>Construct</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Linguistic acculturation</td>
<td>Assessed at baseline using 4 items from the language use subscale of the brief acculturation scale for Hispanics (23), which ascertains language used when reading, speaking at home, with friends, and thinking. This scale had good internal reliability (α = 0.76).</td>
</tr>
<tr>
<td>Risk taking</td>
<td>Assessed using two items “I look for dangerous things to do, just for excitement” and “If I got a chance to skydive from an airplane, I’d do it.” Responses were made on a 4-point scale ranging from “just like me” to “not at all like me” and were averaged. For descriptive purposes, the variable was categorized into “high” and “low” on the basis of the median split, and in the multivariable analysis, it was entered as continuous. Higher scores indicate greater risk-taking tendencies.</td>
</tr>
<tr>
<td>Outcome expectations</td>
<td>Assessed at baseline using a 7-item scale developed by Dalton and colleagues (26). Examples include “I think smoking would help me to feel more comfortable at parties” and “I think smoking would make me look more mature.” Responses are made on a 4-point scale ranging from “strongly disagree” to “strongly agree.” We have found the scale to have good internal consistency (Cronbach’s α = 0.86). For descriptive purposes, the variable was categorized into “none” and “some”; however, in the multivariable analysis, it was entered as continuous. Higher scores indicate more positive perceived consequences ascribed to smoking behavior.</td>
</tr>
<tr>
<td>Household social influence</td>
<td>For each potential household member [i.e., father, mother, sister(s), brother(s), and other(s)], the participant was asked “Does your father smoke?” and “Do you live with your father?” To ensure the variable reflected social influence from all household members with whom the participant currently lived, we summed the number of smoking household members residing with participant. In the multivariable analyses, this variable was coded as “none” and “at least one,” with “none” serving as the reference category.</td>
</tr>
<tr>
<td>Cognitive susceptibility</td>
<td>Combines behavioral intentions and peer influence and was assessed among never-smokers only (7). To be coded as “non-susceptible,” participants responded “no” to “Do you think you will try a cigarette soon?” and “definitely not” to “If one of your best friends were to offer you a cigarette would you smoke it?” and “Do you think you will be smoking cigarettes 1 year from now?” All other participants were coded as susceptible.</td>
</tr>
</tbody>
</table>
SNP selection
Candidate genes were identified from published reviews (30) and PubMed searches using the following key words: sensation seeking, risk taking, gambling, smoking onset, and initiation. This list was cross-referenced with the Gene Ontology Database (31) and Kegg Pathway to confirm pathway information. Tagging SNPs were selected from the International HapMap Project (Release 21 with NCBI build 36; ref. 32). The following selection criteria were used: located in the respective gene or within 10 kb upstream or downstream of the gene ends to cover the regulatory regions; minor allele frequency (MAF) >5% in various ethnic groups; and not already represented by a current tag SNP at a linkage disequilibrium of $r^2 > 0.80$. We also included SNPs in coding (synonymous SNPs, nonsynonymous SNPs) and regulatory regions [promoter, splicing site, 5' untranslated region (UTR), and 3'-UTR]. In addition, functional SNPs and SNPs previously reported to be associated with smoking phenotypes were included. The gene, position, allelic change, and chromosome are summarized in Supplementary Table S1.

Genotyping
A total of 1,274 samples were sent for genotyping. We designed an Illumina GoldenGate assay for candidate SNPs (Illumina, Inc.). Ninety-three percent of the SNPs had Illumina SNP scores of >0.6. DNA samples (250 ng) were genotyped following the standard 3-day Illumina protocol. Data from the array were autocalled by the BeadArray Reader (Illumina, Inc.). Cluster definitions for each SNP were determined using Illumina BeadStudio Genotyping Module v 2.3.41. Genotype calls were made when a genotype yielded a quality score (Gencall value) of 95% or higher. Among these markers, 1.2% of calls were missing (8 of 672). Seventy blind duplicate pairs were included, and the concordance of SNP genotype calls was greater than 99%.

Statistical analyses
The $\chi^2$ tests were used to examine associations between experimenter status and both categorical demographic and smoking-related covariates. Student’s $t$ tests and one-way ANOVA were used to examine mean differences on the continuous covariates. Linkage disequilibrium between SNPs was assessed by calculating pairwise Lewontin $D'$ and $r^2$ using Haplovie version 3.32.

Two multivariable models were developed (one with and one without genotype data). Psychosocial risk factors with a significant bivariate association ($P < 0.05$) with new experimentation were entered into unconditional logistic regression analyses. We used Bayesian False Discovery Probability tests (BFDP; ref. 33) to evaluate the chance of false-positive associations for the variants studied. We set four levels of prior probability (0.01, 0.03, 0.05, and 0.07), prior ORs at 1.5, and the selected level of noteworthyness for BFDP at 0.8, the recommended threshold by Wakefield (33).

Because a priori we do not know the mode of inheritance, we tested each significant SNP using dominant, recessive, and additive models and selected the most parsimonious. SNPs retained in the multivariable model were summed to create a genetic risk score. SNPs that exhibited a protective effect were reverse coded before creating the genetic risk score. Thus, higher scores on the genetic risk score reflected increased risk.

Next principal components analysis was conducted to test for the possible underlying ethnic stratification, with the use of EIGENSTRAT software (34). We first applied the principal components analysis to the genotype data to infer continuous axes of genetic variation, which are defined as eigenvectors. We then used the top axes of variation as the covariates in the unconditional logistic regressions to develop the multivariable models.

Finally, having determined that cognitive susceptibility modifies the influence of the psychosocial variables on experimentation (5) and because including the genotype data did not significantly attenuate the ORs derived from the first model, we applied a standard methodology (35, 36) to determine whether cognitive susceptibility to smoking moderated (modified) the influence of the genotype data on experimentation. The interaction term (cognitive susceptibility by genetic risk score) and the main effects were simultaneously entered into unconditional logistic regression models. The interaction reflects the interaction between putative risk alleles and being cognitively susceptible to smoking.

Results
DNA from 1,274 youth enrolled was available for analysis. The 120 youth who reported experimenting at baseline were excluded as were an additional 36 for whom complete follow-up information was missing. The final sample size was 1,118, of which 584 (52.2%) were girls.

Table 2 summarizes demographic characteristics and psychosocial risk factors. Of the 211 (18.9%) participants who began experimenting over the course of the study, 62.6% were boys compared with 44.3% for never experimenters ($P < 0.001$). Experimenters were significantly more likely to be 13 at baseline (42.7% vs. 21.3%; $P < 0.001$) and live with at least one smoker (55.4% vs. 35.3%; $P < 0.001$). A higher proportion of experimenters held positive outcome expectations about smoking (56.9% vs. 34.6%; $P < 0.001$), reported risk-taking tendencies (70.1% vs. 50.5%; $P < 0.001$), and being cognitively susceptible to smoking (43.6% vs. 17.0%) than never experimenters.

Genotyping was completed on a total of 672 SNPs; 8 SNPs had missing genotype data for all participants, 12 SNPs failed the Hardy–Weinberg equilibrium test ($P < 0.00001$) for the entire data set, and 86 SNPs failed the frequency test (MAF < 0.05). Thus, 574 SNPs were included in the analysis. There were 38 SNPs with $P < 0.05$ based on the best model fit (additive, dominant, or recessive model). After controlling for false discovery, we...
identified 12 SNPs with a statistically significant BFDP at 0.8 and prior probability of 0.05. Finally, using unconditional logistic regression analysis with backward selection and adjusting for age and sex, 6 of the 12 SNPs were selected at $P < 0.05$. Table 3 presents the distribution of the 6 SNPs retained for the multivariable analyses—3 in the dopamine pathway (rs12422191 on DRD2, rs10052016 on SLC6A3, and rs8119844 on SNAP25), 2 in the serotonin pathway (rs6297 on HTR1B and rs9567732 on HTR2A), and an opioid receptor variant (rs9322451 on OPMR1).

To conduct the principal components analysis, we used $N = 1,118$ participants and 536 SNPs, which were shown to be unassociated with the experimentation outcome at a significance level 0.05 based on the best model fit (additive, dominant, or recessive model). We did not observe significant ethnic stratification in our data from the principal components analysis. Because only the top 2 eigenvalues (derived from the top 2 principal components) were significantly larger than the subsequent eigenvalues, we used these 2 largest principal components in our analyses (Tables 4 and 5; refs. 37, 38). We also considered controlling for the top 3 and top 10 largest principal components but found no significant differences in the resulting multivariable models.

Including the genes in the model (Table 4) did not significantly attenuate the impact of the psychosocial

### Table 2. Distribution of demographic characteristics and smoking-related covariates by new experimenter status ($N = 1,118$)

<table>
<thead>
<tr>
<th>Experimenter status</th>
<th>New experimenter</th>
<th>Never experimenter</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$N$ (%)</td>
<td>$N$ (%)</td>
<td>$N$ (%)</td>
</tr>
<tr>
<td>Overall</td>
<td>1,118 (100.0)</td>
<td>211 (18.9)</td>
<td>907 (81.1)</td>
</tr>
<tr>
<td><strong>Demographic characteristics</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>584 (52.2)</td>
<td>79 (37.4)</td>
<td>505 (55.7)</td>
</tr>
<tr>
<td>Males</td>
<td>534 (47.8)</td>
<td>132 (62.6)</td>
<td>402 (44.3)</td>
</tr>
<tr>
<td>Age, y</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 or 12</td>
<td>835 (74.7)</td>
<td>121 (57.3)</td>
<td>714 (78.7)</td>
</tr>
<tr>
<td>13</td>
<td>283 (25.3)</td>
<td>90 (42.7)</td>
<td>193 (21.3)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>11.89 (0.84)</td>
<td>12.18 (0.87)</td>
<td>11.75 (0.80)</td>
</tr>
<tr>
<td>Country of birth</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mexico</td>
<td>297 (26.6)</td>
<td>48 (22.7)</td>
<td>249 (27.5)</td>
</tr>
<tr>
<td>United States</td>
<td>821 (73.4)</td>
<td>163 (77.3)</td>
<td>658 (72.5)</td>
</tr>
<tr>
<td><strong>Linguistic acculturation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low</td>
<td>256 (22.9)</td>
<td>41 (19.4)</td>
<td>215 (23.8)</td>
</tr>
<tr>
<td>High</td>
<td>861 (77.1)</td>
<td>170 (80.6)</td>
<td>691 (76.2)</td>
</tr>
<tr>
<td>Parental education ($N = 1,051$)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;High school</td>
<td>619 (65.6)</td>
<td>64 (32.3)</td>
<td>501 (35.3)</td>
</tr>
<tr>
<td>$\geq$High school graduate</td>
<td>324 (34.4)</td>
<td>134 (67.7)</td>
<td>552 (64.7)</td>
</tr>
<tr>
<td><strong>Smoking-related covariates</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of household members who smoke</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>681 (60.9)</td>
<td>94 (44.6)</td>
<td>587 (64.7)</td>
</tr>
<tr>
<td>1</td>
<td>341 (30.5)</td>
<td>84 (39.8)</td>
<td>257 (28.4)</td>
</tr>
<tr>
<td>$\geq$2</td>
<td>96 (8.6)</td>
<td>33 (15.6)</td>
<td>63 (6.9)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>0.55 (0.76)</td>
<td>0.75 (0.84)</td>
<td>0.43 (0.66)</td>
</tr>
<tr>
<td>Risk taking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Averse</td>
<td>512 (45.8)</td>
<td>63 (29.9)</td>
<td>449 (49.5)</td>
</tr>
<tr>
<td>Some</td>
<td>606 (54.2)</td>
<td>148 (70.1)</td>
<td>458 (50.5)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1.68 (0.81)</td>
<td>1.95 (0.90)</td>
<td>1.58 (0.76)</td>
</tr>
<tr>
<td>Outcome expectations</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>684 (61.2)</td>
<td>91 (43.1)</td>
<td>593 (65.4)</td>
</tr>
<tr>
<td>Some</td>
<td>434 (38.8)</td>
<td>120 (56.9)</td>
<td>314 (34.6)</td>
</tr>
<tr>
<td>Mean (SD)</td>
<td>1.26 (0.40)</td>
<td>1.35 (0.42)</td>
<td>1.19 (0.35)</td>
</tr>
<tr>
<td>Susceptible at baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>872 (78.0)</td>
<td>119 (56.4)</td>
<td>753 (83.0)</td>
</tr>
<tr>
<td>Yes</td>
<td>246 (22.0)</td>
<td>92 (43.6)</td>
<td>154 (17.0)</td>
</tr>
</tbody>
</table>

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factors as seen by overlapping confidence intervals (CI). Being cognitively susceptible to smoking was the strongest predictor of new experimentation (OR = 2.55; 95% CI, 1.74–3.73), followed by being 13 at baseline (OR = 2.27; 95% CI, 1.59–3.23), male (OR = 1.96; 95% CI, 1.40–2.75), holding positive outcome expectations about smoking (OR = 1.65; 95% CI, 1.07–2.53), living with somebody who smokes (OR = 1.53; 95% CI, 1.23–1.90), and exhibiting risk-taking tendencies (OR = 1.30; 95% CI, 1.07–1.59). All 6 SNPs were significantly associated with new experimentation. Four SNPs, rs9322451 on OPRM1, rs6297 on HTR1B, rs10052016 on SLC6A3, and rs9567732 on SNAP25, were protective, whereas 2 others, specifically rs6297 on HTR1B (OR = 2.10; 95% CI, 1.20–3.66), and rs8119844 on the SNAP25 gene (OR = 0.21; 95% CI, 0.08–0.53). However, among susceptible youth, only 2 of the variables maintained significance (being 13 years at baseline and living with a smoker), as did 3 different SNPs, specifically rs10052016 on SLC6A3 (OR = 0.37; 95% CI, 0.20–0.68), rs9567732 on HTR2A (OR = 0.46; 95% CI, 0.25–0.86), and rs12422191 on DRD2 (OR = 4.88; 95% CI, 2.89–12.56).

In each model adding the genetic information to the psychosocial factors increased the area under the receiver operating characteristic (ROC) curve (Table 6), this increase was greatest in the model based on susceptible youth only. Among susceptible youth, adding the genetic information resulted in a 7% area under the curve increase from 69% (95% CI, 62–76) to 76% (95% CI, 70–82), compared with a 4% area under the curve increase from 70% (95% CI, 65–75) to 74% (95% CI, 69–79) among the committed never-smokers (or nonsusceptible youth).

Discussion

In this longitudinal study, we evaluated the added predictive value of select variants of candidate smoking-related phenotype genes in the dopamine, serotonin, and opioid pathways on smoking experimentation as well as the impact of a gene–environment interaction. Our results confirm the relationship between experimenting with cigarettes and established psychosocial and
demographic risk factors and identify 6 SNPs significantly associated with new experimentation: 3 in the dopamine pathway, 2 in the serotonin pathway, and an opioid receptor. Adding these SNPs to the multivariable models significantly improved the risk prediction of each model examined. Participants who carried 6 risk alleles were two times more likely to have experimented since baseline than participants with 5 or fewer risk alleles. Our overall experimentation rate of 26.3%, and 44.3% among those who were 13 at baseline, appears comparable with rates examined.

Table 4. Logistic regression for new experimentation (N = 1,118)

<table>
<thead>
<tr>
<th>Social and psychologic factors</th>
<th>Psychosocial factors only</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>Psychosocial and genetic factors</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitively susceptible</td>
<td></td>
<td>2.34 (1.62–3.37)</td>
<td>&lt;0.001</td>
<td>2.55 (1.74–3.73)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Age 13 at baseline</td>
<td></td>
<td>2.13 (1.51–2.99)</td>
<td>&lt;0.001</td>
<td>2.27 (1.59–3.23)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td></td>
<td>1.87 (1.35–2.59)</td>
<td>&lt;0.001</td>
<td>1.96 (1.40–2.75)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Outcome expectations</td>
<td></td>
<td>1.69 (1.11–2.57)</td>
<td>0.014</td>
<td>1.65 (1.07–2.53)</td>
<td>0.023</td>
<td></td>
</tr>
<tr>
<td>Household member smokes</td>
<td></td>
<td>1.51 (1.22–1.86)</td>
<td>&lt;0.001</td>
<td>1.53 (1.23–1.90)</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>Risk-taking tendencies</td>
<td></td>
<td>1.35 (1.12–1.64)</td>
<td>0.002</td>
<td>1.30 (1.07–1.59)</td>
<td>0.009</td>
<td></td>
</tr>
</tbody>
</table>

Gene (SNP)

SNAP25 (rs8119844) — (—) — 0.26 (0.11–0.61) | 0.002
OPRM1 (rs9322451) — (—) — 0.53 (0.35–0.81) | 0.003
HTR1B (rs6297) — (—) — 1.89 (1.17–3.03) | 0.009
SLC6A3 (rs10052016) — (—) — 0.64 (0.45–0.90) | 0.009
HTR2A (rs9567732) — (—) — 0.70 (0.50–0.98) | 0.038
DRD2 (rs12422191) — (—) — 2.10 (1.31–3.36) | 0.002

AIC criteria 958.41 930.10

NOTE: New experimentation includes participants who reported that they had not experimented at baseline and reported that they had experimented on one or more of the 4 subsequent contacts. Abbreviation: AIC, Akaike information criterion.

Table 5. Logistic regression for new experimentation stratified by cognitive susceptibility

<table>
<thead>
<tr>
<th>Social and psychologic factors</th>
<th>Committed never-smoker</th>
<th>OR (95% CI)</th>
<th>P</th>
<th>Susceptible</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age 13 at baseline</td>
<td>2.04 (1.31–3.20)</td>
<td>0.002</td>
<td>3.00 (1.61–5.62)</td>
<td>0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>2.08 (1.37–3.17)</td>
<td>0.001</td>
<td>1.64 (0.89–3.02)</td>
<td>0.116</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outcome expectations</td>
<td>2.87 (1.63–5.06)</td>
<td>&lt;0.001</td>
<td>0.93 (0.48–1.80)</td>
<td>0.825</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household member smokes</td>
<td>1.52 (1.15–2.01)</td>
<td>0.003</td>
<td>1.63 (1.12–2.38)</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Risk-taking tendencies</td>
<td>1.46 (1.14–1.88)</td>
<td>0.003</td>
<td>1.17 (0.83–1.64)</td>
<td>0.379</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Gene (SNP)

SNAP25 (rs8119844) 0.21 (0.08–0.53) | 0.001 | 0.42 (0.04–4.10) | 0.454
OPRM1 (rs9322451) 0.54 (0.32–0.92) | 0.025 | 0.54 (0.26–1.11) | 0.095
HTR1B (rs6297) 2.10 (1.20–3.66) | 0.009 | 1.44 (0.58–3.58) | 0.433
SLC6A3 (rs10052016) 0.85 (0.56–1.28) | 0.429 | 0.37 (0.20–0.68) | 0.002
HTR2A (rs9567732) 0.80 (0.53–1.21) | 0.287 | 0.46 (0.25–0.86) | 0.015
DRD2 (rs12422191) 1.49 (0.81–2.74) | 0.199 | 4.88 (1.89–12.56) | 0.001

AIC criteria 640.45 291.66

NOTE: New experimentation includes participants who reported that they had not experimented at baseline and reported that they had experimented on one or more of the 4 subsequent contacts. Abbreviation: AIC, Akaike information criterion.
In addition, 3 different SNPs (HTR2A increasing age and living with a smoker had an impact. Psychosocial factors are less relevant because only among susceptible youth, it appears that the experimentation as were all the psychosocial factors. SNPs (analyses. Among committed never-smokers, 3 of the occurrences (45), predicted experimentation (20, 46, 47). Risk-taking tendencies, which tend to peak in adolescence (27–29). Consistent with previous work, is a strong, consistent predictor of smoking initiation (5, 44). Our data confirmed a roughly 70% increased risk of experimenting associated with positive outcome expectations. Among committed never-smokers only, the risk increased to almost 3-fold and holding positive outcome expectations exerted the strongest independent influence on experimenting among all psychosocial risk factors examined. Having a parent who smokes is a strong, consistent predictor of smoking initiation among youth (27–29). Consistent with previous work, risk-taking tendencies, which tend to peak in adolescents (45), predicted experimentation (20, 46, 47).

Next, we focus on the results from the stratified analyses. Among committed never-smokers, 3 of the SNPs (OPRM1, SNAP25, HTR1B) were associated with experimentation as were all the psychosocial factors. However, among susceptible youth, it appears that the psychosocial factors are less relevant because only increasing age and living with a smoker had an impact. In addition, 3 different SNPs (HTR2A, DRD2, SLC6A3) were associated with experimentation in this subgroup. Adding the genetic data improved the predictiveness of both models as assessed by the area under the curve; this improvement was more marked among susceptible participants. It must be noted that these genetic variants are not predicting a response to nicotine, rather sensation seeking, risk taking, or curiosity, as this analysis only included participants who had not tried cigarettes at baseline. In summary, psychosocial risk factors appear to be more important predictors of experimentation among the committed never-smokers (nonsusceptible youth) relative to the susceptible youth, based on previous work (5) and the current analysis. The genetic risk factors appear to be more important predictors of experimenting among the susceptible youth than the committed never-smokers, as fewer psychosocial risk factors maintained significance among the susceptible youth compared with committed never-smokers.

The dopaminergic system is important in mediating the pleasurable feelings of reward and novelty seeking behavior. Individuals with a high level of such behaviors are likely to be more susceptible to cues for potential reward, such as initiating smoking. We found that a SNP on DRD2 (rs12422191) was significantly associated with new experimentation; in the analysis stratified on susceptibility status, DRD2 maintained significance among participants identified as susceptible to smoking only, among whom this variant exhibited the strongest association with smoking behavior (OR = 4.88) among all genes and psychosocial factors examined.

There are conflicting reports about the association between DRD2 and smoking behavior among both adolescents and adults (48–51). Among adults, we found rs1800497 on DRD2 (which is in perfect linkage disequilibrium with rs12422191 for both CEU and Mexican populations) was associated with continuation of smoking. Carriers of the rare allele are reported to exhibit an earlier age at onset of smoking (50) and in an adolescent Caucasian population to be associated with progression to smoking (52). Neuroimaging findings support a functional role of the gene in regulation of DRD2 availability in the frontal cortex (53), and Noble (54) has shown that carriers of at least one variant allele have 40% fewer receptors relative to wild-type carriers.

The dopamine transporter gene (SLC6A3) regulates dopamine reuptake into presynaptic terminals and has also been studied extensively in relation to smoking behavior and cessation (55). We found a rare allele of SLC6A3 (rs10052016) to be protective against experimentation in susceptible participants. The 9 repeat allele of SLC6A3 variable number of tandem repeats sequence in the 3'-UTR has previously shown to have a protective in several diverse populations, including young Israeli women (56) and adolescents (57).

The opioid receptors and their endogenous ligands have long been implicated in addiction, sensation-seeking behaviors, and smoking initiation (58). The OPMRI

| Table 6. Area under the curve and 95% CIs for each model’s ROC curve |
|---------------------------|---------------------------|
|                          | Psychosocial factors only | Psychosocial and genetic factors |
|                          | Area under the curve | 95% CI      | Area under the curve | 95% CI      |
| All                      | 0.74            | 0.70–0.78   | 0.77            | 0.73–0.81   |
| Committed never-smoker   | 0.70            | 0.65–0.75   | 0.74            | 0.69–0.79   |
| Susceptible              | 0.69            | 0.62–0.76   | 0.76            | 0.70–0.82   |

reported elsewhere. In Brazil, 30% of 7th to 10th grade (39) and up to 50% of 15- to 16-year-old European students (40) report experimenting.

Overall cognitive susceptibility to smoking, a psychosocial risk factor that assesses both peer influence and behavioral intentions (7) and an early phase in the transition from never to ever smoking (5), was the strongest psychosocial risk factor (>2-fold) for experimentation. Risk factors associated with being susceptible to smoking included having friends who smoke, living with a smoker, having lower subjective social status and more detentions at school, and reporting more temptations to try smoking (24). We, and others, have shown that smoking initiation is strongly influenced by gender and age (5, 41, 42). Higher perceived outcome expectations, which assess the perceived social benefits from smoking, are associated with both intentions to smoke (43) and experimenting with cigarettes (5, 44). Our data confirmed a roughly 70% increased risk of experimentation associated with positive outcome expectations. Among committed never-smokers only, the risk increased to almost 3-fold and holding positive outcome expectations exerted the strongest independent influence on experimenting among all psychosocial risk factors examined. Having a parent who smokes is a strong, consistent predictor of smoking initiation among youth (27–29). Consistent with previous work, risk-taking tendencies, which tend to peak in adolescents (45), predicted experimentation (20, 46, 47).

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variant had a protective effect among the full sample and among committed never-smokers but was not significantly associated with new experimentation among susceptible youth.

Gene variants in serotonin receptors are logical candidates for studying inter-individual variation in smoking initiation, as the neurotransmitter plays a role in modulating social behavior. The 5-hydroxytryptamine (serotonin) receptor HTR1B variant, rs6297, was associated with an increased risk of new experimentation among the full sample (OR = 1.89) and just more than a 2-fold increased risk of new experimentation among the committed never-smokers. This synonymous exonic SNP has been associated with aggressive behavior in children (59), and impulsivity, although not consistently (60).

The SNAP complex mediates presynaptic vesicle trafficking and participates in the release of dopamine and other neurotransmitters (61). The SNAP25 (rs8119844) variant exhibited the strongest association with new experimentation of any of the genes and psychosocial factors we examined in the overall analysis and among committed never-smokers. Committed never-smokers who carry the minor AA allele are almost 5 times more likely to have tried cigarettes than their peers. To the best of our knowledge, the relationship between SNAP25 and experimenting with cigarettes has not been reported in the literature before. However, SNAP25 is associated with negative effect (61) and with ADHD (62), both of which are associated with an increased risk for smoking among adolescents (63, 64).

The current study has both strengths and limitations. The prospective design allowed us to examine experimentation at follow-up among participants who had not experimented at baseline and to test for moderation effects. Furthermore, participants were from a population-based cohort and included roughly equal numbers of girls and boys. All psychosocial constructs were assessed using validated measures, and data were collected using personal digital assistants to ensure participant privacy and quality of the data. The participants represent a large ethnically homogeneous and predominantly low-income sample of Mexican origin youth, an understudied population. A final strength is the high retention rate—87% of the youth provided data on all 5 contacts.

Conversely, the main limitation of this study is the lack of an independent replication sample; thus, we must consider our findings preliminary. However, given the large number of SNP tested, we did adjust the significance level for each SNP included in this analysis using a BFDP approach (30). Although the percentage of SNPs failing the Hardy–Weinberg test is higher than expected, we did not observe a systematic pattern with regard to the SNPs that failed suggesting that it did not impact our results. A third limitation is that participants were all of Mexican origin and results may not generalize to other ethnicities. In addition, we did not have biochemical validation of the participants’ smoking status (e.g., salivary cotinine). However, we informed participants during the consent process that they might be selected to provide a saliva sample to check their smoking status; this “bogus pipeline” procedure has been shown to increase the validity of self-reported smoking status (65).

Conclusion

This is one of the first studies to examine the role of both genetic and non-genetic factors associated with the transition from never smoking to cigarette experimentation among Mexican origin youth. The results confirm and extend previous findings (e.g., the strong association between SNAP25 and experimenting) and suggest that the impact of both psychosocial and genetic factors differs depending upon the susceptibility status of the adolescents. Our results need to be confirmed with an independent sample of Mexican origin youth (in particular, with regard to SNAP25; as in general, with recessive genes, the number of risk SNPs is smaller than dominant) and among youth of other ethnic backgrounds, as they have implications for development of culturally specific interventions. In conclusion, with regard to the genes we identified, which are related to the very beginning of the smoking trajectory, when youth are contemplating whether to try cigarettes (i.e., risk of experimentation), there appear to be differences in genotype between youth who think they will try cigarettes compared with their peers who think they will not try cigarettes in the future.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References


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