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The Role of Nicotinic Receptor Genes (*CHRN*) in the Pathways of Prenatal Tobacco Exposure on Smoking Behavior among Young Adult Light Smokers

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Abstract

Background: Prenatal tobacco exposure (PTE) is associated with more frequent smoking among young, light smokers. Little is known about how nicotinic acetylcholine receptor (*CHRN*) genes may contribute to this relationship.

Methods: Data were drawn from a longitudinal cohort of young light smokers of European ancestry ($N=511$). Three single nucleotide polymorphisms (SNPs) among offspring, rs16969968 and rs6495308 in *CHRNA5A3B4* and rs2304297 in *CHRNA3A6*, were analyzed with respect to whether they 1) predict PTE status; 2) confound the previously-reported effects of PTE on future smoking; 3) have effects on youth smoking frequency that are mediated through PTE; and 4) have effects that are moderated by PTE.

Results: rs2304297 and rs6495308 were associated with increased likelihood and severity of PTE, respectively. In a path analysis, rs16969968 directly predicted more frequent smoking in young adulthood ($B=1.50$, $p=.044$); this association was independent of, and not mediated by, PTE. The risk of rs16969968 ($IRR=1.07$, $p=.015$) and the protective effect of rs2304297 ($IRR=0.84$, $p<.001$) on smoking frequency were not moderated by PTE. PTE moderated the effect of rs6495308, such that these alleles were protective against later smoking frequency only among non-exposed youth ($IRR=0.85$, $p<.001$).

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Conclusions: The association between offspring *CHRNA5A3B4* and PTE is a novel finding. The risk of rs16969968 on youth smoking is independent and unrelated to that of PTE among young, light smokers. PTE moderates the protective effect of rs6495308 on youth smoking frequency. However, PTE's pathway to youth smoking behavior was not explained by these genetic factors, leaving its mechanism(s) of action unclear.

Keywords

Smoking; prenatal tobacco exposure; genetics; structural equation modeling

1. Introduction

Maternal smoking is a prominent risk factor for smoking among offspring.^{1–4} This finding is robust across different types of maternal smoking, including smoking during pregnancy^{5–8} and lifetime smoking status, regardless of its timing.⁹ Prenatal tobacco exposure (PTE) warrants special attention in light of some evidence that it may *directly* harm the developing fetus.^{10,11} Some have posited that PTE could alter the dopaminergic system and prime the brain for later nicotine dependence.¹² However, intrauterine mechanisms do not appear to explain PTE's association with smoking outcomes,^{13,14} but may explain other behavioral outcomes.¹⁵

Previous studies from our group found that mother's lifetime smoking and PTE were each *independently* associated with more frequent smoking among a cohort of light adolescent smokers.⁶ Further, path analysis compared possible mediating pathways of each factor, and found that PTE and mother's lifetime smoking acted through different pathways: PTE was directly associated with more frequent smoking in *young adulthood*, while maternal lifetime smoking acted through increased nicotine dependence and smoking frequency *during adolescence*.⁶

Genetic variants known to directly affect smoking heaviness may in part explain these relationships. Plausible candidates for genetic transmission include the *CHRN** genes that encode nicotinic acetylcholine receptors (*nAChR*).¹⁶ In particular, the *CHRNA5A3B4* and *CHRNA5A3B4* gene clusters are strongly associated with smoking behavior,^{17,18} and for this reason motivate the genotypes selected in the current follow-up study.⁶ Specifically, among European populations, *CHRNA5A3B4* has four common haplotypes that are tagged by single nucleotide polymorphisms (SNPs). In adult smokers, rs16969968 and rs1051730 tag haplotype A (HA) which is reliably associated with increased smoking heaviness; and rs6495308 and rs569207 tag haplotype C (HC) which is associated with *decreased* smoking heaviness.^{19,20} Additionally, rs2304297 tags a variant in *CHRNA5A3B4* that is protective against smoking heaviness in adult smokers.^{21,22}

The risk conveyed by *CHRNA5A3B4* HA also extends to PTE specifically: HA is associated with heavier and continued smoking during pregnancy.^{23,24} Thus, it is possible that PTE impacts smoking heaviness in offspring via inheritance of HA rather than via an intrauterine effect. Alternatively, a preclinical study demonstrated that intrauterine nicotine exposure in mice produced long-lasting changes in prefrontal neuronal morphology and activity that were moderated by *Chrna5*.²⁵ Thus, a teratogenic nicotine effect mediated by *CHRNA5*

must be considered. We are not aware of previous studies of the association between *CHRNA5A3B4* and PTE.

In this research, we extend our previous findings on PTE and smoking outcomes among young, light smokers⁶ by incorporating known genetic markers for smoking heaviness (as described above, rs16969968 to tag *CHRNA5A3B4* HA, rs6495308 to tag *CHRNA5A3B4* HC, and rs2304297 to tag the protective *CHRNA5A3B4* variant). Specifically, we test 1) whether offspring genotype predicts PTE status; 2) a “confounding hypothesis” that offspring genotypes inherited from the mother partially or fully account for the relationship between PTE and youth smoking frequency in young adulthood (approximately age 24); 3) a “mediation hypothesis” that the possible genetic effects on young adult smoking frequency are partially or fully mediated through PTE; and 4) a “moderation hypothesis” that PTE interacts with the genetic effects on young adult smoking frequency.

2. Methods

2.1. Sample

The study sample was drawn from the Social and Emotional Contexts of Adolescent Smoking Patterns (SECASP) Study, a cohort of 1263 novice/light smokers and nonsmokers studied longitudinally for 8 years starting in 9th/10th grade,^{6,9} and surveyed approximately annually. Ethical approval was obtained through the University of Illinois at Chicago IRB. The mean age at baseline was 15.7 years ($SD=0.61$) and at the 8-year follow-up was 23.6 years ($SD = 0.61$), and 316 (62%) were female. A parent questionnaire was completed for 81% of the sample ($N=1022$) at baseline. Eighty percent of the youth participants ($N=1027$) were asked to participate in SECASP’s genetics component (5-year wave). Of these, nearly all ($N=1019$) agreed to participate and provided a saliva sample for DNA extraction.

Selection criteria for the current study were as follows. Retention at the 8-year assessment was 79.8% ($N=1007$), of which $N=922$ had provided genetic data. Self-reported never-smokers ($N=85$) at the 8-year wave were excluded. To reduce population stratification, the current analyses were limited to non-Hispanics of European descent ($N=511$) as determined by a cluster analysis of 64 ancestry informative genetic markers.²⁶ Finally, all analyses except path analysis were limited to valid data on maternal smoking ($N=439$).

2.2. Measures

2.2.1. Phenotypes—Smoking frequency self-reported as the number of days smoked in the past 30 days. Responses were collected on a 9-point scale, and were coded as the midpoint of each category.⁶ In addition, an intermediate variable was created to characterize each participant’s trend in smoking frequency across adolescence (from the 6-month follow-up through age 18, the legal age to purchase cigarettes in Illinois). Intermediate smoking frequency trends were estimated as each individual’s per-year change in smoking frequency, based on the random slopes of a linear mixed model (random intercept and slope) examining the trends in smoking frequency over all such intermediate time points (for most participants, the 6-month through 2-year follow-ups).

Nicotine dependence was assessed using a shortened, 10-item version of the Nicotine Dependence Syndrome Scale (NDSS)²⁷ that has been validated for use in adolescents.²⁸ Each item was answered on a 4-point scale, and all items were averaged to obtain a total NDSS score. In addition to the baseline value, an intermediate variable representing the trend of nicotine dependence throughout adolescence was calculated as described above for smoking frequency.

PTE was assessed in the parent questionnaire. Of the current study's analytic sample of $N=511$, the parent questionnaire was completed for $N=439$; this comprises 392 mothers and 61 fathers, who proxy-reported on the mother's prenatal smoking behavior. Accuracy of partners' contemporaneous reports is high²⁹ but is unknown for retrospective proxy reports. PTE was reported as the amount smoked (5 categories ranging from 0 to >20 cigarettes/day)³⁰ during each of three trimesters; this method increases reliability of retrospective self-reports.¹⁵ Consistent with previous research on this sample,⁶ PTE was defined as persistent smoking during pregnancy, i.e., any smoking during 2+ trimesters vs. 1 trimester. Among the 439 mothers with valid PTE data, 75 (17%) smoked 2+ trimesters. A majority (26/30) of mothers smoking only 1 trimester did so in their first trimester, and these offspring did not differ from those with 0 trimesters of PTE;⁶ this is consistent with mothers spontaneously quitting as a protective action for their babies, leading to corollary developmental advantages.³¹

Mother's lifetime smoking status was proxy-reported by adolescents at baseline. The original responses of current smoker, ex-smoker, and never-smoker were dichotomized into lifetime smoking vs. never smoking, consistent with previous research on this sample.^{6,9} Among the full sample of $N=511$, 266 (54%) had mothers who ever smoked.

2.2.2. Genotypes—SNP genotyping from genomic DNA isolated from youth saliva samples was carried out as previously described.²² The tag SNP for the *CHRNA3A6* protective haplotype was rs2304297, with an allele frequency (count) of G:0.759 (666), C:0.241 (212). The tag SNP for the *CHRNA5A3B4* HA risk haplotype was rs16969968 with an allele frequency (count) of G:0.687 (603), A:0.313 (275), and the tag SNP for the *CHRNA5A3B4* HC protective haplotype was rs6495308 with an allele frequency (count) of T:0.743 (651), C:0.257 (225).

2.3. Analyses

2.3.1. PTE Associations—Group differences between PTE status and each other variable were examined by chi-square tests (categorical variables) and Wilcoxon signed-rank tests (quantitative variables) to accommodate skewed variables. Of particular interest was whether offspring genotype is a proxy signal (see Supplementary Material S1) for PTE.

2.3.2. Path Analysis (Confounding and Moderation Tests)—A path analysis model was derived from a preceding study⁶ and updated to include more recent data (including youth genotype) and to remove nonsignificant variables (smoking quantity and other tobacco use) for the sake of parsimony. Father's smoking was excluded based on previous non-significance.⁹ Mother's lifetime smoking was included despite being non-significant, because it significantly alters PTE's effect estimates, indicating confounding.

This base model replicated several of our previously-reported findings:⁶ namely, PTE's independent, direct effect on youth smoking, and maternal lifetime smoking's mediated effect through intermediate nicotine dependence.

For the current study, multiple final models were created by adding each SNP to the base model (Figure 1). The outcome of smoking frequency at the 8-year wave was examined as a function of stable characteristics (youth SNP, mother's lifetime smoking status, and sex) and baseline smoking frequency and nicotine dependence. For each predictor, the model included pathways for both direct and indirect (via intermediate smoking frequency and nicotine dependence) effects. PTE was modeled as a potential mediator of the effects of the SNP and mother's lifetime smoking status on 8-year smoking frequency.

AMOS version 23.0.0 was used to run the path analysis. AMOS accommodates missing data through full-information maximum likelihood estimation, and automatically corrects for multiple comparisons (pathways and nested models). First, model fit was assessed using several fit indices, since the conventional χ^2 goodness-of-fit is overly sensitive to sample size; good fits are indicated by adjusted χ^2 ($\chi^2/\text{d.f.}$) ≤ 5 ,³² Comparative Fit Index (CFI) ≥ 0.95 ,³³ Tucker-Lewis Index (TLI) ≥ 0.90 ,³⁴ and the root mean square error of approximation (RMSEA) ≤ 0.05 .³⁵

The confounding hypothesis was tested by evaluating whether PTE's effect on 8-year smoking frequency was weakened or eliminated after including the SNP. Specifically, a nested model was created in which all pathways representing SNP effects were constrained to 0, and the coefficient of PTE's direct effect was compared across models. Confounding (a change of $\geq 10\%$) would indicate that the SNP accounts for some of the variance in smoking frequency predicted by PTE. Alternatively, if both the SNP and the measures of maternal smoking have significant pathways, that indicates separate and statistically independent effects on youth smoking. Finally, if the SNP pathway is not significant, that indicates that the SNP has no additional effect on youth smoking over and above the other terms in the model.

The mediation hypotheses was tested by examining PTE as a mediator of each SNP's possible effect on 8-year smoking frequency. If the SNP is significantly associated with 8-year smoking frequency, partial mediation is indicated by the joint presence of 1) a significant path from the SNP (a proxy for maternal genotype) to PTE and 2) a significant path from PTE to 8-year smoking frequency. Further, if the direct effect of the SNP on smoking behavior is no longer significant after including this mediation pathway, this indicates *full* mediation.

2.3.3. Gene-by-Environment Interactions—The moderation hypothesis was examined through analyses of gene-by-environment interactions. Zero-inflated Poisson (ZIP) regressions were utilized due to the distribution of smoking frequency. ZIP analyses simultaneously fit a logistic model (any vs. no past-month smoking) and a count model (number of days smoked in the past month) with the assumption that the counts and the excess zeros arise from separate processes. Gene-by-environment analyses were run by including an interaction term between PTE and each SNP in separate models, and significant

interactions were examined using follow-up subgroup analyses stratified by PTE ($N=75$ with PTE and $N=364$ without).

3. Results

3.1. PTE Associations

PTE was associated with more frequent smoking over the course of this longitudinal study, both at baseline and throughout adolescence, and exposed youth were more nicotine-dependent throughout adolescence (Table 1). Offspring rs2304297 was inversely associated with the *likelihood* of PTE (Table 1), but none of the genetic variants were associated with the *heaviness* of PTE (Supplementary Material S1).

3.2. Path Analysis (Confounding and Moderation Tests)

Table 2 presents the results for all genetic pathways. All fit indices indicated an excellent fit, despite the significant χ^2 . The number of rs16969968 minor alleles had a direct, positive effect on 8-year smoking frequency (unstandardized coefficient (B)=1.502, standard error (SE)=0.746, $p=.044$). However, neither rs2304297 nor rs6495308 were significantly associated with 8-year past-month smoking frequency. None of the SNPs were significantly associated with intermediate smoking frequency or nicotine dependence. rs2304297 was associated with a lower chance of PTE ($B=-0.70$, $SE=0.026$, $p=.007$).

First, the confounding hypothesis was evaluated. None of the three SNP's confounded the effect of PTE on 8-year smoking frequency: including rs16969968 changed PTE's coefficient from 4.077 ($SE=1.560$) to 4.035 ($SE=1.553$); including rs2304297 changed PTE's coefficient from 4.077 ($SE=1.560$) to 3.928 ($SE=1.571$); and including rs6495308 changed PTE's coefficient from 4.077 ($SE=1.560$) to 4.159 ($SE=1.558$). This indicates rs16969968's effect on more frequent smoking in young adulthood is unrelated to PTE and mother's lifetime smoking.

Next, the mediating hypothesis was evaluated. Since rs2304297 and rs6495308 were not significantly associated with 8-year smoking frequency (either with or without including PTE as a mediator), by definition they have no effects that could be mediated by PTE. The effect of rs16969968 was not mediated through PTE, as indicated by the non-significant pathway between rs16969968 and PTE. The non-significant relationship between rs16969968 and PTE (both its occurrence and severity) was confirmed in separate regression models (see Supplementary Material S1). Together, these results fail to support PTE as a mediator of these genetic effects on smoking frequency.

3.3. Gene-by-Environment Interactions

Finally, ZIP regressions evaluated the moderation hypothesis. None of the logistic models were significant, so this component is not presented. Neither rs16969968 nor rs2304297 showed a significant interaction by PTE. In the count portion of the ZIP models, the frequency of smoking at the 8-year assessment was positively associated with rs16969968 (incident rate ratio (IRR)=1.07, $CI=1.01-1.13$, $p=.015$) and inversely related to rs2304297 ($IRR=0.84$, $CI=0.79-0.89$, $p<.001$) among the whole group, with no interaction by PTE.

However, rs6495308 did interact with PTE ($p < 0.001$), such that this minor allele was associated with reduced smoking frequency among those who were not prenatally exposed to tobacco ($IRR=0.82$, $CI=0.78-0.87$, $p < .001$) but not in those who were exposed ($IRR=1.07$, $CI=0.98-1.19$, $p=.117$).

4. Discussion

The current study builds upon a previously published model of maternal smoking's influence on youth smoking,⁶ and incorporates newly available data to examine the potential role of genetic variants (*CHRNA5A3B4* rs16969968 and rs6495308, and *CHRNA3A6* rs2304297) in confounding, mediating, and moderating the relationship between PTE and smoking frequency in young adult, light smokers. Offspring rs2304297 was associated with a lower likelihood of having had PTE. Offspring rs16969968 was associated with increased smoking frequency at the 8-year follow-up, while rs2304297 and rs6495308 were associated with decreased smoking frequency. The direct effect of rs16969968 represents an additional risk for heavier smoking behavior that is independent of, and not mediated through, PTE. Finally, we identified a significant PTE-by-SNP interaction for rs6495308, in which its protective effect on less frequent smoking in young adulthood was observed only for those who were not prenatally exposed to tobacco.

The direct effect of rs16969968 on increased smoking frequency among young adult, light smokers was statistically independent of, and did not confound, the effects of maternal smoking behavior or PTE. Thus, rs16969968 represents an independent, additional risk on smoking in this adolescent cohort of light smokers. In particular, HA conveys a direct risk on heavier smoking for the mother and offspring simultaneously, and this genetic risk seems to be unrelated to intrauterine mechanisms, as suggested previously.^{12,14,36} Meanwhile, the mechanisms of PTE's risk could be environmental in nature, e.g. through behavioral modeling,³⁷ perceived approval of smoking,³⁸ or perceived availability of cigarettes.³⁹ Alternatively, epigenetic effects may explain these effects of maternal smoking; for example, PTE seems to alter DNA methylation which may cause or signal a risk for smoking¹¹ and other adverse outcomes.⁴⁰ Other hereditary effects not included in this study, such as behavioral disinhibition or other personality traits,⁴¹ could also explain these findings.

A related finding to the lack of mediation by PTE was the non-significant relationship between rs16969968 and PTE. This was unexpected, given previous evidence that HA is associated with heavier smoking during pregnancy.^{23,24} Our contradictory result may be due to the characteristics of our sample, namely the exclusion of heavier smokers at baseline (see Limitations), or by the measurement error introduced by proxy-coding of maternal genotype.

However, offspring rs2304297 was inversely associated with PTE, which is a novel finding to our knowledge. Considering the reduction in effect size introduced by genotyping-by-proxy, the current findings suggest this effect on mothers' ability to quit smoking during pregnancy may be even stronger than the effect that has been reported for rs1051730/rs16969968.^{23,42} Further prospective studies are needed to investigate rs2304297 as a potential genetic marker for PTE, either directly or by proxy, in the general population.

The gene-by-environment analysis showed a significant interaction between rs6495308 and PTE when modeling smoking frequency in young adulthood, such that it showed a protective effect of reduced smoking frequency only for non-exposed youth. Though the mechanisms for this are unknown, it is noteworthy that the mouse experiment examining neural circuitry²⁵ observed that *Chrna5* genotype can determine the direction of teratogenic changes in the prefrontal cortex due to developmental nicotine exposure. In contrast, PTE did not alter the risk of rs16969968 or the protective effect of rs2304297 on youth smoking rates, indicating that PTE does not moderate these genetic risks.

The findings that both rs2304297 and rs6495308 were associated with reduced smoking frequency confirms previous findings that *CHRNA6* and HC have protective effects against smoking behavior.¹⁸ Previous work has shown protective effects early in smoking history among this sample,^{21,22} and the current findings show that this effect extends into young adulthood. This protective effect may occur through subjective experiences with smoking⁴³ such as less dizziness at first inhalation,⁴⁴ a risk factor for heavier subsequent smoking. However, the gene-by-environment interaction found for rs6495308 warrants future research on the mechanisms of this effect.

On the other hand, rs2304297 and rs6495308 did not show the expected robust effects. Though they were significant in the ZIP analyses, they were not significantly associated with young adult smoking frequency in the path analysis. A likely reason for this difference is lower statistical power in the path analysis: eliminating the baseline and intermediate variables resulted in stronger albeit nonsignificant trends for these variables (not shown). Thus, these genetic effects may become significant in a larger sample.

This study more generally confirms robust findings that rs16969968 poses a risk for heavier smoking behavior.¹⁹ According to animal models of the $\alpha 5$ *nAChR* subunit, this effect is likely related to a disrupted aversive response to nicotine at high doses,^{45,46} suggesting that it is primarily relevant to heavy post-initiation smoking behavior. In human smokers, the rs16969968 risk allele also reduces subjective aversive effects of an acute dose of intravenous nicotine.⁴⁷ Interestingly, an effect of the rs16969968 risk allele has not been identified previously in this sample using the 6-year wave, with the explanation being that this sample is not smoking heavily enough to activate this effect.^{21,22} However, this explanation does not appear to be supported by the current results: rs16969968 is significantly associated with smoking frequency at the 7-year (not shown) and 8-year follow-ups, despite *lighter* smoking relative to the 6-year wave (not shown). Instead, the significant association of rs16969968 only at the latest time points could suggest an age-varying genetic effect that manifests only in young adulthood, which has been identified in other contexts.^{18,48} Further research is needed to examine the fine-grained timing of these effects.

4.1. Limitations

These findings should be interpreted in the context of the study's limitations. The current sample was selected primarily based on novice and light smoking, and may not be representative of other populations for several reasons. First, the selection process excluded heavier smokers at baseline, possibly resulting in different genetic profiles for this sample

relative to larger populations. Since rs16969968 poses a risk for heavier smoking behavior, if the screening process has reduced its prevalence in this sample, then its effect may be weakened in the current findings relative to populations of heavier smokers. Similarly, the SNPs examined here are primarily relevant for *post-initiation* smoking behavior,^{17,22} but not other smoking outcomes such as initiation. Additionally, current findings may not generalize to non-European race/ethnic groups. Regarding analytical limitations, the path analysis imposes a causal model on the data, but given the observational nature of the data, causality cannot be definitively concluded. Survey data are also subject to the limitations of self-report, including retrospective and proxy reports for PTE and mother's lifetime smoking. Finally, the exact physiological and/or psychological mechanisms underlying these relationships cannot be determined here.

4.2. Strengths

We report significant and robust HA effects on young adult smoking frequency, as well as the $\alpha 6$ protective effect on PTE. Additionally, the current data are rich with respect to both long-term follow-up and its range of detailed variables on youth and maternal smoking behavior and youth genetic information. Path analysis allowed a rigorous examination of mediating pathways, and the parsing out of simultaneous pathways affecting youth smoking behavior. This study substantially contributes to the existing literature by directly examining how genetic factors contribute to the effect of maternal smoking behavior on youth smoking outcomes and how these processes intersect with vulnerability pathways beginning even before birth.

4.3. Conclusion

Offspring SNPs from the *CHRNA3B6* and *CHRNA5A3B4* genes represent risk or protective factors against smoking behavior in young adulthood, independently of prenatal smoking behavior. We also identify a gene-by-environment interaction between PTE and a protective SNP. These findings further research on the mechanisms underlying the effect of maternal smoking on youth smoking, and motivate further research that tests specific genetic, physiological, and psychological mechanisms of this effect.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1:

Base Path Analysis Model. The outcome of smoking frequency at the 8-year follow-up (right) is examined as a function of all stable characteristics and baseline measures of tobacco use and dependence (left). For each of these predictors, both direct effects on the outcome and mediated effects (middle) through intermediate trends in smoking behavior and dependence were modeled. Intermediate variables represent the per-year change between the 6-month follow-up and age 18 (for most participants, this includes the 2-year follow-up). Straight lines represent pathways, which are equivalent to regressions. Curved lines represent covariances between predictors. SNP: single nucleotide polymorphism; placeholder for the specific SNP's tested in each model.

Table 1:

Characteristics of the sample

Measure	PTE (N=75)	No PTE (N=364)	<i>p</i> -value	Number of missing cases
<i>CHRN</i> SNP MAF's				
rs16969968_A	0.32	0.31	.845	0
rs2304297_C	0.17	0.26	.023	0
rs6495308_C	0.25	0.26	.837	0
Baseline smoking frequency ^a	1.0 (0.0 – 12.3)	0.0 (0.0 – 2.5)	.029	0
Intermediate trend in smoking frequency ^b	1.1 (0.3 – 5.2)	2.2 (0.2 – 13.7)	.038	7
8-year smoking frequency ^a	12.0 (0.0 – 30.0)	0.0 (0.0 – 20.0)	.002	0
Baseline nicotine dependence (NDSS score)	1.0 (0.8 – 1.9)	1.0 (0.8 – 1.6)	.023	8
Intermediate trend in nicotine dependence ^c	0.9 (0.8 – 1.4)	1.1 (0.9 – 1.7)	.019	7
Female sex	49 (65.3%)	220 (60.4%)	.508	0

Note. Summaries are presented as *N* (valid %) for categorical variables and *median* (*interquartile range*) for quantitative variables. PTE: Prenatal tobacco exposure. *CHRN*: Nicotinic acetylcholine receptor. SNP: Single nucleotide polymorphism. NDSS: Nicotine Dependence Syndrome Score. MAF = Minor Allele Frequency. *p*-values are based on logistic regressions for SNP's, chi-square tests for categorical variables and Wilcoxon signed-rank tests for quantitative variables. Bold: $p < .05$.

^aSmoking frequency is defined as the number of days smoked in the past 30 days. These summaries include those who smoked 0 days out of the past 30 (PTE group: $N=30$ (40.0%); No PTE group: $N=192$ (52.7%))

^bQuantified as the per-year change in smoking frequency after baseline and before age 18 (approximately 2 years).

^cQuantified as the per-year change in NDSS score after baseline and before age 18 (approximately 2 years).

Table 2:

Path analysis results for genetic pathways

Offspring SNP	Fit indices of main model	SNP → 8-year Smoking frequency	SNP → PTE	PTE → 8-year Smoking frequency
rs16969968	$\chi^2(38)=27.16$, $p=.040$ adjusted $\chi^2=1.70$ CFI=.992 TLI=.977 RMSEA=.037	1.502 (0.746) , $p=.044$	0.006 (0.025), $p=.821$	4.035 (1.553) , $p=.009$
rs2304297	$\chi^2(38)=30.08$, $p=.018$ adjusted $\chi^2=1.880$ CFI=.990 TLI=.971 RMSEA=.042	-0.775 (0.808), $p=.338$	-0.70 (0.026) , $p=.007$	3.928 (1.571) , $p=.012$
rs6495308	$\chi^2(38)=32.05$, $p=.010$ adjusted $\chi^2=2.00$ CFI=.988 TLI=.966 RMSEA=.044	-0.959 (0.808), $p=.235$	0.017 (0.027), $p=.525$	4.159 (1.558) , $p=.008$

Note. Results for each pathway are presented as unstandardized coefficient (B) (standard error (SE), p -value. Only genetic pathways from the path model (Figure 1) relevant to mediation through PTE are presented. PTE: Prenatal tobacco exposure. SNP: single nucleotide polymorphism. χ^2 : chi-square goodness-of-fit statistic. Adjusted χ^2 : χ^2 /degrees of freedom. CFI: Comparative Fit Index. TLI: Tucker-Lewis Index. RMSEA: Root-mean-square error of approximation. Bold: $p<.05$.