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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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HIGHLIGHTS

- Prenatal tobacco exposure (PTE) poses a risk for more frequent smoking among youth.
- PTE's effect is not explained by any of 3 selected CHRN* genetic variants.
- PTE does not moderate any of the CHRN* associations with youth smoking.
- A PTE-by-rs6495308 interaction has a protective effect for non-exposed youth.
- rs2304297 may be a novel genetic proxy marker for PTE.

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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 *CHRNB3A6*, 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 ($IRR = 0.85$, $p < .001$).

Conclusions: The association between offspring *CHRNB3A6* 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.

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1. Introduction

Maternal smoking is a prominent risk factor for smoking among offspring (Bricker, Peterson, Sarason, Andersen, & Rajan, 2007; Burchfiel, Higgins, Keller, Butler, & Donahue, 1989; Hu, Davies, & Kandel, 2006; Melchior, Chastang, Mackinnon, Galera, & Fombonne, 2010). This finding is robust across different types of maternal smoking, including smoking during pregnancy (Kardia, Pomerleau, Rozek, & Marks, 2003; Porath & Fried, 2005; Selya et al., 2013; Tehranifar, Liao, Ferris, & Terry, 2009) and lifetime smoking status, regardless of its timing (Selya, Dierker, Rose, Hedeker, & Mermelstein, 2012). Prenatal tobacco exposure (PTE) warrants special attention in light of some evidence that it may *directly* harm the developing fetus (Richmond, Simpkin, Woodward, et al., 2015; Wongtrakool, Wang, Hyde, Roman, & Spindel, 2012). Some have posited that PTE could alter the dopaminergic system and prime the brain for later nicotine dependence (Kandel, Wu, & Davies, 1994). However, intrauterine mechanisms do not appear to explain PTE's association with smoking outcomes (Rydell, Granath, Cnattingius, Magnusson, & Galanti, 2014; Taylor et al., 2014), but may explain other behavioral outcomes (Estabrook, Massey, Clark, et al., 2016).

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 (Selya et al., 2013). 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* (Selya et al., 2013).

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*) (Mineur & Picciotto, 2008). In particular, the *CHRNA5A3B4* and *CHRNB3A6* gene clusters are strongly associated with smoking behavior (Thorgeirsson, Gudbjartsson, Surakka, et al., 2010; Weiss, Baker, Cannon, et al., 2008), and for this reason motivate the genotypes selected in the current follow-up study (Selya et al., 2013). 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 (Liu, Tozzi, Waterworth, et al., 2010; Saccone, Culverhouse, Schwantes-An, et al., 2010). Additionally, rs2304297 tags a variant in *CHRNB3A6* that is protective against smoking heaviness in adult smokers (Cannon, Mermelstein, Hedeker, et al., 2014; Pugach, Cannon, Weiss, Hedeker, & Mermelstein, 2017).

The risk conveyed by *CHRNA5A3B4* HA also extends to PTE specifically: HA is associated with heavier and continued smoking during pregnancy (Chen, Baker, Piper, et al., 2014; Freathy, Ring, Shields, et al., 2009). 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* (Bailey, Tian, Kang, O'Reilly, & Lambe, 2014). Thus, a teratogenic nicotine effect mediated by *CHRNA5* must be considered. We are not aware of previous studies of the association between *CHRNB3A6* and PTE.

In this research, we extend our previous findings on PTE and smoking outcomes among young, light smokers (Selya et al., 2013) 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 *CHRNB3A6* 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 (Selya et al., 2012, 2013), 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 (Pritchard & Rosenberg, 1999). 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 (Selya et al., 2013). 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) (Shiffman, Waters, & Hickcox, 2004) that has been validated for use in adolescents (Sledjeski, Dierker, Costello, et al., 2007). 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 high (Hatch, Misra, Kabat, & Kartzmer, 1991) but is unknown for retrospective proxy reports. PTE was reported as the amount smoked (5 categories ranging from 0