Variation in *CYP2A6* and tobacco dependence throughout adolescence and in young adult smokers

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Abstract

Background—Smoking is influenced by genetic factors including variation in *CYP2A6* and *CYP2B6*, which encode nicotine-metabolizing enzymes. In early adolescence, *CYP2A6* slow nicotine metabolism was associated with higher dependence acquisition, but reduced cigarette consumption. Here we extend this work by examining associations of *CYP2A6* and *CYP2B6* with tobacco dependence acquisition in a larger sample of smokers followed throughout adolescence.

Methods—White participants from the Nicotine Dependence in Teens cohort that had ever inhaled ($n=421$) were followed frequently from age 12–18 years. Cox's proportional hazards models compared the risk of ICD-10 tobacco dependence acquisition (score 3+) for *CYP2A6* and *CYP2B6* metabolism groups. Early smoking experiences, as well as amount smoked at end of follow-up, was also computed. At age 24 ($N=162$), we assessed concordance between self-reported cigarette consumption and salivary cotinine.

Results—In those who initiated inhalation during follow-up, *CYP2A6* slow (vs. normal) metabolizers were at greater risk of dependence (hazards ratio (HR)=2.3; 95% CI=1.1, 4.8); *CYP2B6* slow (vs. normal) metabolizers had non-significantly greater risk (HR=1.5; 95% CI=0.8, 2.6). Variation in *CYP2A6* or *CYP2B6* was not significantly associated with early smoking symptoms or cigarette consumption at end of follow-up. At age 24, neither gene was significantly associated with dependence status. Self-reported consumption was associated with salivary cotinine, a biomarker of tobacco exposure, acquired at age 24 ($B=0.37$; $P<0.001$).

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Conclusions—Our findings extend previous work indicating that slow nicotine metabolism mediated by *CYP2A6*, and perhaps *CYP2B6*, increases risk for tobacco dependence throughout adolescence.

Keywords

nicotine; addiction; adolescent; smoking; genetics; *CYP2A6*; *CYP2B6*

1. Introduction

Approximately 90% of smokers begin smoking in adolescence (O’Loughlin et al., 2014b; U.S. Department of Health and Human Services, 2012). A substantial proportion (~40–75%) of smoking behaviour is influenced by genetics (Broms et al., 2006; Vink et al., 2005). *CYP2A6* inactivates nicotine, the principle psychoactive compound in cigarette smoke, to cotinine (Nakajima et al., 1996). Genetic variation in *CYP2A6* that reduces the rate of nicotine metabolism is associated with lower cigarette consumption (Malaiyandi et al., 2006; Wassenaar et al., 2011), dependence scores (Schnoll et al., 2014; Sofuoglu et al., 2012; Wassenaar et al., 2011), brain response to smoking cues (Tang et al., 2012), and greater cessation (Gu et al., 2000; Lerman et al., 2006; Schnoll et al., 2009), even in adolescence (Chenoweth et al., 2013). In adolescents, *CYP2A6* slow nicotine metabolism was also associated with an increased risk of tobacco dependence acquisition at young ages (from age 12–16 years) (Al Koudsi et al., 2010 ; O’Loughlin et al., 2004), but slower escalation in nicotine dependence (Audrain-McGovern et al., 2007) and reduced cigarette consumption (O’Loughlin et al., 2004). In young adults, *CYP2A6* slow (vs. normal) metabolizers were less likely to be smokers (Schoedel et al., 2004). Together these findings suggest that while *CYP2A6* slow metabolism increases the risk of becoming a smoker in younger adolescence, slow metabolism also increases cessation, and reduces cigarette consumption in dependent smokers. However, it is not known whether *CYP2A6* slow metabolism increases smoking acquisition in later adolescence, a period during which a substantial amount of smoking uptake occurs (O’Loughlin et al., 2014b).

A small proportion (~10%) of nicotine’s metabolism to cotinine occurs via a second enzyme, *CYP2B6* (Al Koudsi and Tyndale, 2010). The *CYP2B6**6 allele, a prevalent haplotype (~25% frequency in Whites (Rotger et al., 2007)) is associated with lower *CYP2B6* hepatic protein levels (Al Koudsi and Tyndale, 2010) and slower *CYP2B6*-mediated metabolism of bupropion and efavirenz (reviewed in (Thorn et al., 2010)). In adult smokers, *CYP2B6**6 was associated with lower abstinence rates in the placebo arm of a bupropion smoking cessation clinical trial; 15% of individuals with one or two copies of *CYP2B6**6 achieved abstinence, compared to 32% of *CYP2B6**1/*1 individuals (Lee et al., 2007a). In a separate study, the *CYP2B6**6 allele was more frequent in nicotine dependent individuals compared to those that were not dependent (32% vs. 22%, respectively) (Riccardi et al., 2015). Whether *CYP2B6**6 also influences the risk for acquiring nicotine dependence in adolescence is not known.

Here we examined associations for *CYP2A6* and *CYP2B6* with tobacco dependence acquisition in a larger (n>400) sample of adolescent smokers assessed four times each year

across the entire adolescent period (age 12–18 years). We hypothesized that *CYP2A6* slow (vs. *CYP2A6* normal), and that *CYP2B6* slow (i.e., individuals with one or two copies of *CYP2B6*6*) (vs. *CYP2B6* normal) metabolizers would be at increased risk of acquiring dependence. We also hypothesized that a larger proportion of slow (vs. normal) metabolizers for each gene would report early smoking experiences, which are associated with the development of nicotine dependence (DiFranza et al., 2004). We also assessed cigarette consumption at the end of follow-up among dependent smokers, hypothesizing that *CYP2A6* slow (vs. *CYP2A6* normal) metabolizers would smoke fewer cigarettes; no association was expected between *CYP2B6* genotype groups. For both *CYP2A6* and *CYP2B6*, we further hypothesized that slow (vs. normal) metabolizers would be more likely to be dependent at end of follow-up. At age 24, we expected *CYP2A6* slow (vs. normal) metabolizers to be at lower risk of dependence, as *CYP2A6* slow metabolizers are less likely to be dependent smokers (vs. non-smokers) as adults (Schoedel et al., 2004). Finally, we hypothesized that *CYP2B6* slow (vs. normal) metabolizers would be more likely to be dependent at age 24, consistent with the higher frequency of *CYP2B6*6* in dependent (vs. non-dependent) adults (Riccardi et al., 2015).

Finally, an adjunct biochemical analysis to assess the validity of the self-reported cigarette consumption data was undertaken. We examined the construct-related validity of self-reported cigarette consumption against salivary cotinine, widely used as an objective biomarker of tobacco consumption (Connor Gorber et al., 2009), and also assessed its relationships with nicotine dependence and withdrawal scores.

2. Methods

2.1. Study population and data collection

As previously described (O’Loughlin et al., 2014a), 1294 adolescents from 10 secondary schools in Quebec were recruited in 1999 for the Nicotine Dependence in Teens (NDIT) cohort study (Montreal, Quebec, Canada). Self-report questionnaires were administered every three months during the 10-month school year over the five years of secondary school (grade 7–11), for a total of 20 survey cycles. Data from these 20 surveys for n=421 ever smoking Whites were included in the current analyses of tobacco dependence acquisition and cigarette consumption. Previous analyses in this population included data only up to survey cycle 16 (age 15–16 years) for only 281 smokers (O’Loughlin et al., 2004). Two additional surveys (survey cycles 21 and 22) were administered three and six years, respectively, after high school graduation (O’Loughlin et al., 2014a). Data from survey 22, completed when participants were aged 24 years on average, were used, along with salivary cotinine, for additional analyses. Parents or guardians provided written informed consent and participants provided assent at baseline. Participants (who had attained legal age) provided informed consent during post-high school survey cycles. The study was approved by McGill University (Quebec, Canada), the Centre de recherche du Centre hospitalier de l’Université de Montréal (Quebec, Canada), and the University of Toronto research ethics board (Toronto, Canada).

2.2. Determination of *CYP2A6* and *CYP2B6* genotype

DNA was extracted from saliva or blood samples, and participants were genotyped for four *CYP2A6* alleles which occur at relatively high frequency (~1–8%) in Whites and have an established impact on reducing nicotine metabolism: *CYP2A6**2, *CYP2A6**4, *CYP2A6**9, and *CYP2A6**12 (Benowitz et al., 2006; Chenoweth et al., 2013). Participants were grouped into *CYP2A6* normal, intermediate, or slow nicotine metabolism groups based on the predicted metabolic impact of each *CYP2A6* variant allele (Chenoweth et al., 2013). Participants were also genotyped for the *CYP2B6**6 allele, using a haplotyping method described previously (Lee et al., 2007a; Mwenifumbo et al., 2005); individuals with the *CYP2B6**1/*1 genotype were grouped as normal metabolizers, while those with one or two copies of the *CYP2B6**6 alleles were grouped as slow metabolizers. For genetic analyses, the sample was restricted to White ever-smokers in order to minimize possible effects of population stratification. *CYP2A6* genetic data were available for 421 White ever-smokers, while *CYP2B6* genetic data were available for 391 White ever-smokers.

2.3. Study variables

Data on early smoking experiences (symptoms of nausea and dizziness), tobacco/nicotine dependence (measured by the International Classification of Diseases (ICD)-10 and the modified Fagerstrom Tolerance Questionnaire (mFTQ)) were collected, as were data on nicotine withdrawal, other nicotine dependence symptoms, and self-medication scores (Chenoweth et al., 2013). Briefly, nicotine withdrawal, other nicotine dependence symptoms, and self-medication scores were measured in six, 14, and five individual items, respectively, and assessed symptoms of withdrawal including irritability, restlessness, anxiety, craving frequency, and endorsement of statements that smoking improves energy level, affect, and stress (Chenoweth et al., 2013). Scores were pro-rated if there were fewer than half the items missing for an individual score. If half or more of the items were missing, the participant was assigned a missing value.

Three measures of cigarette consumption were used. The mean number of cigarettes smoked/month in the 3-month interval preceding each survey cycle was assessed by multiplying the average number of cigarettes smoked/day by the average number of days smoked/month in each of the three months and calculating the average monthly consumption (O'Loughlin et al., 2014c). The number of cigarettes smoked in the past week was assessed at survey cycle 22 (approximately age 24) by summing the number of cigarettes smoked on each of the preceding seven days. Finally, the number of cigarettes smoked in the past 24 hours was assessed at survey cycle 22, indicated by the number of cigarettes smoked on the day prior to administration of the survey.

2.4. Determination of salivary cotinine level

Cotinine levels were determined from saliva samples collected from current smokers at survey cycle 22, when participants were 24 years of age on average. The level of cotinine was assessed using liquid chromatography-tandem mass spectrometry (Chenoweth et al., 2014; St Helen et al., 2012; Tanner et al., 2015). In total, cotinine levels were available for n=162 White self-reported current smokers with genetic data. The limit of quantification for cotinine was 1 ng/ml. Four participants had cotinine levels below the limit of quantification;

as per convention, the cotinine value was replaced with the limit of quantification (1 ng/ml) divided by the square root of 2, yielding a value of 0.7 ng/ml (Kalkbrenner et al., 2010).

2.5. Statistical analyses

Analysis 1. Tobacco dependence acquisition, early smoking experiences, the level of smoking in adolescence, and dependence status at end of follow-up (Surveys 1–20)—Data from surveys 1–20 (age 12–18 years) were included in this analysis; surveys 21 and 22, both collected in young adulthood (ages 20 and 24 years, respectively), were not included in this analysis due to the much less frequent data collection interval. Using bootstrap-based multiple imputation to manage missing data, 10 imputed datasets were created as previously described (Chenoweth et al., 2013). Cox's proportional hazards models were used to compare the risk of acquiring ICD-10 tobacco dependence (score of ≥ 3) in adolescence (age 12–18 years) between CYP2A6 normal, intermediate, and slow metabolizers who had ever inhaled on a cigarette, and between CYP2B6 normal and slow metabolizers who had ever inhaled on a cigarette. Models were stratified according to whether the participant had inhaled at cohort inception; separate models examined all inhalers (i.e., those who inhaled prior to cohort inception + those who inhaled during follow-up) and those who first inhaled during follow-up (i.e., incident inhalers), as data covering the entire smoking career were available in this latter group. Time was measured in days from time zero in all participants who ever inhaled on a cigarette, which corresponded to the day participants who had inhaled prior to cohort inception joined NDIT, or the day incident smokers first inhaled during NDIT follow-up. Participants were followed from time zero until they became dependent or were censored (i.e., were lost to follow-up or the follow-up period ended). Once smokers entered the analysis, all surveys were considered to be 'at-risk' periods. Early smoking experiences (nausea and dizziness) were assessed in the first survey completed for prevalent inhalers, and in the first survey completed following smoking initiation for incident inhalers. Past-month cigarette consumption at end of follow-up (i.e., each participant's last available survey from surveys 1 to 20) was compared between CYP2A6 genotype groups and between CYP2B6 genotype groups, with and without covariates (sex, age, and duration of smoking) using linear regression analysis. Dependence status at end of follow-up was also assessed for each genotype group.

Analysis 2. Associations for CYP2A6 and CYP2B6 with tobacco dependence status in young adulthood (Survey 22)—Using the same study population from analysis 2, we performed chi-square tests to compare the frequency of ICD-10 tobacco dependence (score of ≥ 3) across genotype groups.

Analysis 3. Construct-related validity of self-reported smoking behaviour (cigarette consumption) against cotinine in young adults (Survey 22, age 24)—Cotinine was not normally distributed. Therefore, the strength of the association between self-reported cigarette consumption and cotinine level was assessed using Spearman's rho and linear regression analysis. We also explored the relationships between cotinine and nicotine dependence scores (i.e., ICD-10, mFTQ dependence scores, withdrawal, other nicotine dependence symptoms, and self-medication) and smoking indicators using

Spearman's rho and by conducting a separate univariate linear regression analysis for each smoking or nicotine dependence indicator.

3. Results

Participant characteristics at baseline are shown in Table 1. In all participants (n=421), 78.9%, 14.5%, and 6.7% were *CYP2A6* normal, *CYP2A6* intermediate, and *CYP2A6* slow metabolizers, respectively. In those for whom *CYP2B6* genetic data was available (n=391), 58.3% were *CYP2B6* normal (n=228), while 41.7% were *CYP2B6* slow (n=163), metabolizers. The frequency of the *CYP2B6**6 allele was 24%, consistent with a previous report in Caucasians (Rotger et al., 2007). Eleven individuals are expected to be both *CYP2A6* and *CYP2B6* slow metabolizers (6.7% x 41.7% x N=391); twelve were identified which does not deviate substantially from the expected value.

The median follow-up time was 1574 days. We first examined the risk of tobacco dependence acquisition according to *CYP2A6* metabolism group in incident inhalers and in all inhalers. In the incident inhalers, *CYP2A6* slow metabolizers were more likely to acquire tobacco dependence than *CYP2A6* normal metabolizers, with a hazards ratio (HR) of 2.3 (95% confidence interval (CI) = 1.1, 4.8) (Table 2). Similar findings were observed in all inhalers (HR = 1.8, 95% CI = 1.0, 3.3 for tobacco dependence acquisition in *CYP2A6* slow vs. normal metabolizers) (Table 2). In the incident inhalers, *CYP2B6* slow metabolizers were no more likely than *CYP2B6* normal metabolizers to acquire tobacco dependence (HR = 1.5, 95% CI = 0.8, 2.6). Similar findings were observed in all inhalers (HR = 1.2, 95% CI = 0.8, 1.7 for tobacco dependence acquisition in *CYP2B6* slow vs. normal metabolizers) (Table 2). We were underpowered to test for potential interactions between *CYP2A6* and *CYP2B6* on tobacco dependence acquisition. No associations for *CYP2A6* or *CYP2B6* with the frequency of nausea or dizziness during early smoking were found (Table 3).

We next investigated the level of cigarette consumption in incident smokers at the end of follow-up in adolescence between *CYP2A6* and *CYP2B6* metabolism groups according to dependence status, with and without covariates. In the unadjusted and adjusted models, dependent *CYP2A6* slow metabolizers smoked 108 and 74 fewer cigarettes/month, respectively, compared to dependent *CYP2A6* normal metabolizers, however neither finding was significant (Table 4). In the unadjusted and adjusted models, dependent *CYP2B6* slow metabolizers smoked 67 and 55 more cigarettes/month, respectively, compared to dependent *CYP2B6* normal metabolizers, which was not significant (Table 4). At the end of follow-up in adolescence, a greater proportion of *CYP2A6* slow (vs. normal) metabolizers were dependent (46% vs. 23%, respectively), but this did not reach significance (median P value from 10 imputed datasets = 0.09). *CYP2B6* slow metabolizers were no more likely than *CYP2B6* normal metabolizers to be dependent at end of follow-up (21% vs. 30%, respectively; P=0.15).

At age 24, 29% of *CYP2B6* normal metabolizers (*CYP2B6**1/*1; n=82) were ICD-10 dependent compared to 45% of *CYP2B6* slow metabolizers (*CYP2B6**1/*6 + *6/*6; n=65), however this was not significant (P=0.05). There was no difference in dependence status between *CYP2A6* genotype groups at age 24; 35% of *CYP2A6* normal metabolizers

(N=125) were dependent (score 3+), compared to 42% and 56% of intermediate (N=24) and slow (N=9) metabolizers, respectively ($P=0.44$). The low number of *CYP2A6* slow metabolizers could have significantly limited statistical power for this cross-sectional analysis.

We also undertook an adjunct analysis to determine the construct-related validity of using self-reported smoking behaviour as an indication of tobacco dose. We first focused on the relationship between self-reported cigarette consumption (number of cigarettes/month), and salivary cotinine level (available for $n=162$ self-reported current smokers at age 24). The measures were strongly correlated ($Rho=0.71$, $P<0.001$) (Figure 1a). Using linear regression, self-reported cigarette consumption was associated with salivary cotinine level ($B=0.37$, $P<0.001$) (Figure 1a). We next examined the relationship between past-week and past-day self-reported cigarette consumption and salivary cotinine level, and these measures were also strongly correlated ($Rho=0.75$ and 0.73 , respectively; $P<0.001$). Both past-week and past-day self-reported cigarette consumption were also associated with cotinine in linear regression analyses ($B=1.8$ and 11.2 , respectively, $P<0.001$). We also observed correlations between cotinine and nicotine dependence scores (i.e., ICD-10 and mFTQ dependence scores, withdrawal, other nicotine dependence symptoms, and self-medication; Figure 1b–f; $Rho=0.28–0.61$, all $P<0.001$). In linear regression analyses, ICD-10 dependence ($B=23.2$, $P=0.001$), mFTQ dependence ($B=37.6$, $P<0.001$), withdrawal ($B=7.9$, $P<0.001$), other nicotine dependence symptoms ($B=8.1$, $P<0.001$), and self-medication scores ($B=15.1$, $P=0.008$) were all significantly associated with cotinine (Figure 1b–f).

4. Discussion

In this longitudinal study in adolescent smokers, we extend previous findings (O’Loughlin et al., 2004) of an increased risk for tobacco dependence acquisition among *CYP2A6* slow nicotine metabolizers relative to *CYP2A6* normal metabolizers, by demonstrating that this elevated risk occurs throughout adolescence. Despite their increased risk of acquisition, *CYP2A6* slow metabolizers are also more likely to quit smoking throughout adolescence and adulthood (Chenoweth et al., 2013; Gu et al., 2000); thus depending on the point in time during the transition from adolescence to adulthood, or in smoking dependence trajectory, slow metabolizers may be over- or under-represented in smokers versus non-smokers. This may contribute to the observed lower risk among slow (vs. normal) metabolizers for being a smoker versus non-smoker in young adulthood (Schoedel et al., 2004). While the precise mechanism(s) underlying the association between *CYP2A6* slow metabolism and increased risk for tobacco dependence acquisition in adolescence is unknown, our data suggest that experiencing early nausea or dizziness may not contribute. The effect of slower nicotine metabolism on nicotine-mediated reward was recently characterized in nicotine-naïve adult mice (Bagdas et al., 2014). Slower nicotine metabolism, achieved through methoxsalen-mediated inhibition of CYP2A5 (the murine ortholog of human CYP2A6), caused animals to display a preference for nicotine in the conditioned place preference paradigm (Bagdas et al., 2014), suggestive of greater reward (Tzschentke, 2007). If these findings extend to novice smokers, those with slow nicotine metabolism may also experience greater reward during initial smoking experiences, potentially due to greater nicotine exposure, increasing their risk for developing nicotine dependence.

Despite their higher risk of acquiring tobacco dependence, once dependent, the level of cigarette consumption appeared to be lower in *CYP2A6* slow (vs. normal) metabolizers, however this was not significant. Slow nicotine metabolism, measured by *CYP2A6* genotype or NMR, is associated with lower cigarette consumption among dependent adults smoking a mean of 18+ cigarettes/day (Chenoweth et al., 2014; Malaiyandi et al., 2006; Wassenaar et al., 2011). Future investigations will clarify whether dependent adolescent smokers, even at comparatively lower levels of cigarette consumption versus adults, titrate their level of nicotine intake according to their rate of nicotine metabolism in order to maintain desirable levels (Ashton et al., 1979; Hill and Marquardt, 1980), with *CYP2A6* slow metabolizers needing to smoke fewer cigarettes to achieve these levels. In contrast to *CYP2A6*, variation in *CYP2B6* does not substantially alter the rate of peripheral nicotine metabolism or cigarette consumption (Lee et al., 2007b); we also showed a lack of significant association between *CYP2B6* and adolescent cigarette consumption.

In a cross-sectional study of adolescent smokers (<5 cigarettes/day) aged 13 to 17 years (mean = 16 years), NMR was negatively associated with mFTQ dependence scores and cigarette consumption, where slower metabolizers displayed both higher dependence and higher consumption (Rubinstein et al., 2013). It is possible that this study captured an earlier time-point in smoking history than our study, where the *CYP2A6* slow metabolizers were more likely to be dependent (and have higher dependence scores), but had not yet begun to titrate their nicotine intake according to their rate of nicotine metabolism (i.e., had not begun to smoke fewer cigarettes).

Many studies of adolescent smoking, including our own, utilize self-report questionnaires (Ary and Biglan, 1988; Rigotti et al., 2000). Misclassification in self-report data can occur in epidemiological studies, particularly in studies of substance use behaviour and in younger cohorts (Brener et al., 2003; Clarke et al., 2014; Fendrich et al., 2005; Morral et al., 2000). There may be issues stemming from recall bias, as well as the desire to conform with social norms (Brener et al., 2003). In order to help minimize recall bias, we collected data frequently (every three months) in adolescence (O'Loughlin et al., 2014a), and assessed tobacco dependence acquisition in all inhalers as well as in those who initiated inhalation during follow-up (i.e., incident inhalers). We also assessed the accuracy of self-reported cigarette consumption; there was strong agreement between self-reported cigarette consumption and salivary cotinine, supporting the construct validity of the questionnaire data. A limitation of our study is a lack of available biomarker data during adolescence. In addition, self-reported smoking is not always concordant with salivary cotinine levels in adolescents (Kandel et al., 2006). The associations between cotinine and the other smoking and dependence measures at age 24 suggest that these measures are also related to tobacco dose. Associations between dependence measures and salivary cotinine levels have been previously demonstrated in young adolescent smokers with an average age of 15 years (Rubinstein et al., 2007). Dependence measures including the mFTQ, nicotine dependence syndrome scale, timing of craving in the morning, and self-rated level of addiction were all correlated with cotinine level (Rubinstein et al., 2007). In these young adolescent smokers, the mean cotinine level and cigarette consumption were both lower than in our sample at age 24 (~44 vs. ~139 ng/ml, and ~4 vs. ~8 cigarettes/day, respectively). Together these findings

suggest that dependence measures are related to tobacco dose across a range of exposures and ages (Rubinstein et al., 2007).

In a recent study of young smokers, the influence of *CYP2A6* variation on smoking outcomes varied throughout the 6-year follow-up period (Cannon et al., 2015). At age 16, *CYP2A6* intermediate metabolism (vs. normal and slow metabolism) was associated with the highest nicotine dependence syndrome scale (NDSS) score; in contrast, as young adults (age 22), *CYP2A6* normal metabolizers had the highest NDSS score (Cannon et al., 2015). We did not replicate these findings in our sample at age 24. Differences between our findings and those from the longitudinal investigation (Cannon et al., 2015) may stem from differences in study design (cross-sectional vs. longitudinal), variation in assessment of *CYP2A6* activity (categorical grouping of metabolism vs. use of a metabolism metric), and/or differences in smoking phenotypes examined (ICD-10 dependence status vs. NDSS score). Our small sample size for slow metabolizers (n=9) may have also affected statistical power. Future longitudinal investigations will help to clarify the factors, including the role of *CYP2A6*, associated with smoking in adolescence, and as youth transition to adulthood. A better understanding of these factors may lead to targeted interventions that help reduce the risk for lifelong smoking.

While there was some indication that *CYP2B6* slow metabolizers were at greater risk of acquiring tobacco dependence in adolescence, this did not reach significance. When we assessed the participants in young adulthood (age 24), there was a trend toward higher dependence rates in *CYP2B6* slow (vs. normal) metabolizers. These findings are consistent with a recent report in Italians where the frequency of the *CYP2B6**1/*6 genotype was significantly higher in individuals with nicotine dependence (FTND score 4+) compared to those who were not dependent (36% vs. 21%, respectively), which was not seen for those with the normal *CYP2B6**1/*1 genotype (20% vs. 33%, respectively) (Riccardi et al., 2015). Together with our findings, these data suggest that the *CYP2B6**6 allele is associated with a higher risk of nicotine dependence in adulthood; over adolescence we speculate that the risk conferred by *CYP2B6* slow metabolism increases. Data from an animal model suggests variable brain CYP2B activity may influence nicotine metabolism within the brain, in turn affecting nicotine-mediated behaviours (Garcia et al., 2015). In rats, the inhibition of brain CYP2B activity through intracerebroventricular injection of a selective CYP2B inhibitor led to higher rates of acquisition of nicotine self-administration behavior but no difference in level of responding among dependent animals; this was performed without altering peripheral nicotine levels or metabolism (Garcia et al., 2015). It is possible that *CYP2B6* variation within human brain may lead to altered central metabolism of nicotine, which may account for the observed differences in nicotine dependence (Riccardi et al., 2015) and cessation outcomes (Lee et al., 2007a), while having no effect on the level of consumption as observed here.

Overall, we have shown that throughout adolescence, *CYP2A6* slow nicotine metabolizers are at a higher risk of developing tobacco dependence. The role of genetic variation in *CYP2B6* in smoking acquisition and dependence remains to be clarified, but may increase over time. The adjunct analysis validating our measure of smoking supports these conclusions. The findings highlight the role of genetic risk factors in nicotine addiction in

adolescence, a development period in which the contribution of genetics to smoking behaviours has been studied only infrequently to date.

References

- Al Koudsi N, O'Loughlin J, Rodriguez D, Audrain-McGovern J, Tyndale RF. The genetic aspects of nicotine metabolism and their impact on adolescent nicotine dependence. *Journal of Pediatric Biochemistry*. 2010; 1:105–123.
- Al Koudsi N, Tyndale RF. Hepatic CYP2B6 is altered by genetic, physiologic, and environmental factors but plays little role in nicotine metabolism. *Xenobiotica*. 2010; 40:381–392. [PubMed: 20307138]
- Ary DV, Biglan A. Longitudinal changes in adolescent cigarette smoking behavior: onset and cessation. *J Behav Med*. 1988; 11:361–382. [PubMed: 3236377]
- Ashton H, Stepney R, Thompson JW. Self-titration by cigarette smokers. *Br Med J*. 1979; 2:357–360. [PubMed: 486932]
- Audrain-McGovern J, Al Koudsi N, Rodriguez D, Wileyto EP, Shields PG, Tyndale RF. The role of CYP2A6 in the emergence of nicotine dependence in adolescents. *Pediatrics*. 2007; 119:e264–274. [PubMed: 17130279]
- Bagdas D, Muldoon PP, Zhu AZ, Tyndale RF, Damaj MI. Effects of methoxsalen, a CYP2A5/6 inhibitor, on nicotine dependence behaviors in mice. *Neuropharmacology*. 2014; 85:67–72. [PubMed: 24859605]
- Benowitz NL, Swan GE, Jacob P 3rd, Lessov-Schlaggar CN, Tyndale RF. CYP2A6 genotype and the metabolism and disposition kinetics of nicotine. *Clin Pharmacol Ther*. 2006; 80:457–467. [PubMed: 17112802]
- Brener ND, Billy JO, Grady WR. Assessment of factors affecting the validity of self-reported health-risk behavior among adolescents: evidence from the scientific literature. *J Adolesc Health*. 2003; 33:436–457. [PubMed: 14642706]
- Broms U, Silventoinen K, Madden PA, Heath AC, Kaprio J. Genetic architecture of smoking behavior: a study of Finnish adult twins. *Twin Res Hum Genet*. 2006; 9:64–72. [PubMed: 16611469]
- Cannon DS, Medina TR, Mermelstein RJ, Hedeker D, Bakian AV, Coon H, Cook EH, Hamil C, Weiss RB. CYP2A6 Longitudinal Effects in Young Smokers. *Nicotine Tob Res*. 2015 Epub ahead of print.
- Chenoweth MJ, Novalen M, Hawk LW Jr, Schnoll RA, George TP, Cinciripini PM, Lerman C, Tyndale RF. Known and Novel Sources of Variability in the Nicotine Metabolite Ratio in a Large Sample of Treatment-Seeking Smokers. *Cancer Epidemiol Biomarkers Prev*. 2014; 23:1773–82. [PubMed: 25012994]
- Chenoweth MJ, O'Loughlin J, Sylvestre MP, Tyndale RF. CYP2A6 slow nicotine metabolism is associated with increased quitting by adolescent smokers. *Pharmacogenet Genomics*. 2013; 23:232–235. [PubMed: 23462429]
- Clarke P, Sastry N, Duffy D, Ailshire J. Accuracy of self-reported versus measured weight over adolescence and young adulthood: findings from the national longitudinal study of adolescent health, 1996–2008. *Am J Epidemiol*. 2014; 180:153–159. [PubMed: 24944288]
- Connor Gorber S, Schofield-Hurwitz S, Hardt J, Levasseur G, Tremblay M. The accuracy of self-reported smoking: a systematic review of the relationship between self-reported and cotinine-assessed smoking status. *Nicotine Tob Res*. 2009; 11:12–24. [PubMed: 19246437]
- DiFranza JR, Savageau JA, Fletcher K, Ockene JK, Rigotti NA, McNeill AD, Coleman M, Wood C. Recollections and repercussions of the first inhaled cigarette. *Addict Behav*. 2004; 29:261–272. [PubMed: 14732415]
- Fendrich M, Mackesy-Amity ME, Johnson TP, Hubbell A, Wislar JS. Tobacco-reporting validity in an epidemiological drug-use survey. *Addict Behav*. 2005; 30:175–181. [PubMed: 15561458]
- Garcia KL, Coen K, Miksys S, Le AD, Tyndale RF. Effect of Brain CYP2B Inhibition on Brain Nicotine Levels and Nicotine Self-Administration. *Neuropsychopharmacology*. 2015; 40:1910–1918. [PubMed: 25652250]

- Gu DF, Hinks LJ, Morton NE, Day IN. The use of long PCR to confirm three common alleles at the CYP2A6 locus and the relationship between genotype and smoking habit. *Ann Hum Genet.* 2000; 64:383–390. [PubMed: 11281276]
- Hill P, Marquardt H. Plasma and urine changes after smoking different brands of cigarettes. *Clin Pharmacol Ther.* 1980; 27:652–658. [PubMed: 7371362]
- Kalkbrenner AE, Hornung RW, Bernert JT, Hammond SK, Braun JM, Lanphear BP. Determinants of serum cotinine and hair cotinine as biomarkers of childhood secondhand smoke exposure. *J Expo Sci Environ Epidemiol.* 2010; 20:615–624. [PubMed: 20237497]
- Kandel DB, Schaffran C, Griesler PC, Hu MC, Davies M, Benowitz N. Salivary cotinine concentration versus self-reported cigarette smoking: three patterns of inconsistency in adolescence. *Nicotine Tob Res.* 2006; 8:525–537. [PubMed: 16920650]
- Lee AM, Jepson C, Hoffmann E, Epstein L, Hawk LW, Lerman C, Tyndale RF. CYP2B6 genotype alters abstinence rates in a bupropion smoking cessation trial. *Biol Psychiatry.* 2007a; 62:635–641. [PubMed: 17223085]
- Lee AM, Jepson C, Shields PG, Benowitz N, Lerman C, Tyndale RF. CYP2B6 genotype does not alter nicotine metabolism, plasma levels, or abstinence with nicotine replacement therapy. *Cancer Epidemiol Biomarkers Prev.* 2007b; 16:1312–1314. [PubMed: 17548706]
- Lerman C, Tyndale R, Patterson F, Wileyto EP, Shields PG, Pinto A, Benowitz N. Nicotine metabolite ratio predicts efficacy of transdermal nicotine for smoking cessation. *Clin Pharmacol Ther.* 2006; 79:600–608. [PubMed: 16765148]
- Malaiyandi V, Lerman C, Benowitz NL, Jepson C, Patterson F, Tyndale RF. Impact of CYP2A6 genotype on pretreatment smoking behaviour and nicotine levels from and usage of nicotine replacement therapy. *Mol Psychiatry.* 2006; 11:400–409. [PubMed: 16402128]
- Morrall AR, McCaffrey D, Iguchi MY. Hardcore drug users claim to be occasional users: drug use frequency underreporting. *Drug Alcohol Depend.* 2000; 57:193–202. [PubMed: 10661670]
- Mwenifumbo JC, Myers MG, Wall TL, Lin SK, Sellers EM, Tyndale RF. Ethnic variation in CYP2A6*7, CYP2A6*8 and CYP2A6*10 as assessed with a novel haplotyping method. *Pharmacogenet Genomics.* 2005; 15:189–192. [PubMed: 15861044]
- Nakajima M, Yamamoto T, Nunoya K, Yokoi T, Nagashima K, Inoue K, Funae Y, Shimada N, Kamataki T, Kuroiwa Y. Role of human cytochrome P4502A6 in C-oxidation of nicotine. *Drug Metab Dispos.* 1996; 24:1212–1217. [PubMed: 8937855]
- O'Loughlin J, Dugas EN, Brunet J, DiFranza J, Engert JC, Gervais A, Gray-Donald K, Karp I, Low NC, Sabiston C, Sylvestre MP, Tyndale RF, Auger N, Mathieu B, Tracie B, Chaiton M, Chenoweth MJ, Constantin E, Contreras G, Kakinami L, Labbe A, Maximova K, McMillan E, O'Loughlin EK, Pabayo R, Roy-Gagnon MH, Tremblay M, Wellman RJ, Hulst A, Paradis G. Cohort Profile: The Nicotine Dependence in Teens (NDIT) Study. *Int J Epidemiol.* 2014a Epub ahead of print.
- O'Loughlin J, Paradis G, Kim W, DiFranza J, Meshefedjian G, McMillan-Davey E, Wong S, Hanley J, Tyndale RF. Genetically decreased CYP2A6 and the risk of tobacco dependence: a prospective study of novice smokers. *Tob Control.* 2004; 13:422–428. [PubMed: 15564629]
- O'Loughlin JL, Dugas EN, O'Loughlin EK, Karp I, Sylvestre MP. Incidence and determinants of cigarette smoking initiation in young adults. *J Adolesc Health.* 2014b; 54:26–32. e24. [PubMed: 23992758]
- O'Loughlin JL, Sylvestre MP, Dugas EN, Karp I. Predictors of the occurrence of smoking discontinuation in novice adolescent smokers. *Cancer Epidemiol Biomarkers Prev.* 2014c; 23:1090–1101. [PubMed: 24895443]
- Riccardi LN, Carano F, Bini C, Ceccardi S, Ferri G, Pelotti S. CYP2B6 gene single-nucleotide polymorphisms in an Italian population sample and relationship with nicotine dependence. *Genet Test Mol Biomarkers.* 2015; 19:103–107. [PubMed: 25489907]
- Rigotti NA, Lee JE, Wechsler H. US college students' use of tobacco products: results of a national survey. *JAMA.* 2000; 284:699–705. [PubMed: 10927777]
- Rotger M, Tegude H, Colombo S, Cavassini M, Furrer H, Decosterd L, Blievernicht J, Saussele T, Gunthard HF, Schwab M, Eichelbaum M, Telenti A, Zanger UM. Predictive value of known and novel alleles of CYP2B6 for efavirenz plasma concentrations in HIV-infected individuals. *Clin Pharmacol Ther.* 2007; 81:557–566. [PubMed: 17235330]

- Rubinstein ML, Shiffman S, Moscicki AB, Rait MA, Sen S, Benowitz NL. Nicotine metabolism and addiction among adolescent smokers. *Addiction*. 2013; 108:406–412. [PubMed: 22823143]
- Rubinstein ML, Thompson PJ, Benowitz NL, Shiffman S, Moscicki AB. Cotinine levels in relation to smoking behavior and addiction in young adolescent smokers. *Nicotine Tob Res*. 2007; 9:129–135. [PubMed: 17365743]
- Schnoll RA, George TP, Hawk L, Cinciripini P, Wileyto P, Tyndale RF. The relationship between the nicotine metabolite ratio and three self-report measures of nicotine dependence across sex and race. *Psychopharmacology (Berl)*. 2014; 231:2515–2523. [PubMed: 24402139]
- Schnoll RA, Patterson F, Wileyto EP, Tyndale RF, Benowitz N, Lerman C. Nicotine metabolic rate predicts successful smoking cessation with transdermal nicotine: a validation study. *Pharmacol Biochem Behav*. 2009; 92:6–11. [PubMed: 19000709]
- Schoedel KA, Hoffmann EB, Rao Y, Sellers EM, Tyndale RF. Ethnic variation in CYP2A6 and association of genetically slow nicotine metabolism and smoking in adult Caucasians. *Pharmacogenetics*. 2004; 14:615–626. [PubMed: 15475735]
- Sofuoglu M, Herman AI, Nadim H, Jatlow P. Rapid nicotine clearance is associated with greater reward and heart rate increases from intravenous nicotine. *Neuropsychopharmacology*. 2012; 37:1509–1516. [PubMed: 22334123]
- St Helen G, Novalen M, Heitjan DF, Dempsey D, Jacob P 3rd, Aziziyeh A, Wing VC, George TP, Tyndale RF, Benowitz NL. Reproducibility of the nicotine metabolite ratio in cigarette smokers. *Cancer Epidemiol Biomarkers Prev*. 2012; 21:1105–1114. [PubMed: 22552800]
- Tang DW, Hello B, Mrozwicz M, Fellows LK, Tyndale RF, Dagher A. Genetic variation in CYP2A6 predicts neural reactivity to smoking cues as measured using fMRI. *Neuroimage*. 2012; 60:2136–2143. [PubMed: 22342802]
- Tanner JA, Novalen M, Jatlow P, Huestis MA, Murphy SE, Kaprio J, Kankaanpää A, Galanti L, Stefan C, George TP, Benowitz NL, Lerman C, Tyndale RF. Nicotine Metabolite Ratio (3-hydroxycotinine/cotinine) in Plasma and Urine by Different Analytical Methods and Laboratories: Implications for Clinical Implementation. *Cancer Epidemiol Biomarkers Prev*. 2015; 24:1239–1246. [PubMed: 26014804]
- Thorn CF, Lamba JK, Lamba V, Klein TE, Altman RB. PharmGKB summary: very important pharmacogene information for CYP2B6. *Pharmacogenet Genomics*. 2010; 20:520–523. [PubMed: 20648701]
- Tzschentke TM. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. *Addict Biol*. 2007; 12:227–462. [PubMed: 17678505]
- U.S. Department of Health and Human Services. Preventing tobacco use among youth and young adults: a report of the Surgeon General. Atlanta, GA: US Department of Health and Human Services, Centers for Disease Control and Prevention, National Center for Chronic Disease Prevention and Health Promotion, Office on Smoking and Health; 2012.
- Vink JM, Willemsen G, Boomsma DI. Heritability of smoking initiation and nicotine dependence. *Behav Genet*. 2005; 35:397–406. [PubMed: 15971021]
- Wassenaar CA, Dong Q, Wei Q, Amos CI, Spitz MR, Tyndale RF. Relationship between CYP2A6 and CHRNA5-CHRNA3-CHRNA4 variation and smoking behaviors and lung cancer risk. *J Natl Cancer Inst*. 2011; 103:1342–1346. [PubMed: 21747048]

Figure 1.

Self-reported smoking behaviours are strongly associated with salivary cotinine levels in young adults. Correlation between self-reported cigarettes/month and cotinine, demonstrating construct-related validity, is shown in (a). Correlations between cotinine and ICD-10 (b) and mFTQ (c) dependence scores, withdrawal (d), other nicotine dependence symptoms (e), and self-medication (f) scores are also shown in all White self-reported current smokers ($n=162$).

Table 1

Selected baseline characteristics of genotyped White ever-smokers, according to whether they had inhaled at cohort inception. NDIT 1999–2012.

	All inhalers (inhaled before cohort inception or during follow-up) n=420–421 ^a	Incident inhalers (inhaled during follow-up) n=214
Male, % ^a	36.2–36.3	35.5
Mean age in years (SD)	12.7 (0.5)	12.6 (0.4)
Francophone, % ^a	18.8–19.1	14.5
Single parent family, % ^a	9.3–9.5	5.6
Parent(s) university/college educated, % ^a	50.0–52.4	57.0–61.2
Parent(s) smoke, % ^a	42.9–43.3	28.5–29.0
Friend(s) smoke, % ^a	48.8–49.1	30.4–30.8

^aRanges in values represent the values derived from the 10 imputed datasets

Table 2

Hazard ratios (HR) and 95% confidence intervals (CI) for ICD-10 tobacco dependence in adolescent smokers according to *CYP2A6* and *CYP2B6* metabolism groups. NDIT 1999–2012.

Metabolism group	HR (95% CI) All inhalers ^a	HR (95% CI) Incident inhalers
<i>CYP2A6</i>		
Normal (reference)	1.0; n=330–333	1.0; n=162–165
Intermediate	0.8 (0.4, 1.4); n=60–63	1.0 (0.4, 2.4); n=29–32
Slow	1.8 (1.0, 3.3); n=27–28	2.3 (1.1, 4.8); n=20–21
<i>CYP2B6</i>		
Normal (reference)	1.0; n=228	1.0; n=121
Slow	1.2 (0.8, 1.7); n=163	1.5 (0.8, 2.6); n=82

^aIncludes prevalent (i.e., those who had inhaled before cohort inception) and incident (i.e., those who first inhaled during follow-up) inhalers

Table 3

Frequency of early smoking experiences among participants who inhaled, according to *CYP2A6* and *CYP2B6* metabolism group. NDIT 1999–2012.

Metabolism group	All inhalers ^a		Incident inhalers	
	% (n) Experiencing Nausea ^b	% (n) Experiencing Dizziness ^b	% (n) Experiencing Nausea ^b	% (n) Experiencing Dizziness ^b
<i>CYP2A6</i>				
Normal	34.5% (114)	13.7% (45)	33.7% (55)	13.6% (22)
Intermediate	37.7% (23)	18.0% (11)	36.7% (11)	13.3% (4)
Slow	40.7% (11)	14.8% (4)	40.0% (8)	15.0% (3)
P value ^c	0.75	0.67	0.83	0.98
<i>CYP2B6</i>				
Normal	33.6% (76)	14.2% (32)	33.3% (40)	13.3% (16)
Slow	33.7% (55)	16.0% (26)	34.1% (28)	14.8% (12)
P value ^c	0.98	0.61	0.90	0.77

^aIncludes prevalent (i.e., those who had inhaled before cohort inception) and incident (i.e., those who first inhaled during follow-up) inhalers

^bNausea and dizziness was indicated by the response “a lot” or “a bit” (vs. “not at all”) when participants were asked about experiencing these symptoms the first time they smoked.

^cP values are derived from chi-square tests.

Table 4

Linear regression analysis of past month cigarette consumption at end of follow-up among participants who initiated inhalation during follow-up, according to dependence status and *CYP2A6* and *CYP2B6* metabolism group. NDIT 1999–2012.

		ICD-10 Tobacco Dependent	
	All	Yes	No
	Coefficient (95% CI) ^a	Coefficient (95% CI) ^a	Coefficient (95% CI) ^a
Unadjusted Model			
<i>CYP2A6</i> Normal ^c	64.2 (39.6, 88.7); n=162–165	222.3 (146.4, 298.1); n=36–38	17.9 (2.2, 33.6); n=126–127
<i>CYP2A6</i> Intermediate	1.9 (–66.1, 70.0); n=29–32	1.2 (–187.4, 189.7); n=6–8	2.0 (–54.9, 58.9); n=23–24
<i>CYP2A6</i> Slow	–13.1 (–100.3, 74.0); n=20–21	–108.4 (–303.8, 86.9); n=9–10	–17.6 (–72.3, 37.0); n=11–12
Adjusted Model ^b			
<i>CYP2A6</i> Normal ^c	66.9 (43.1, 90.7); n=162–165	221.8 (149.1, 294.4); n=36–38	18.8 (3.2, 34.5); n=126–127
<i>CYP2A6</i> Intermediate	–5.8 (–72.5, 60.7) ; n=29–32	–41.4 (–228.5, 145.6); n=6–8	–4.6 (–61.9, 52.8); n=23–24
<i>CYP2A6</i> Slow	–30.5 (–115.5, 54.6); n=20–21	–74.0 (–269.4, 121.4); n=9–10	–17.4 (–71.6, 36.8); n=11–12
Unadjusted Model			
<i>CYP2B6</i> Normal ^c	46.3 (15.0, 77.6); n=121	178.8 (78.3, 279.3); n=25	11.8 (–8.7, 32.2); n=96
<i>CYP2B6</i> Slow	47.2 (0.3, 94.2); n=82	66.6 (–66.1, 199.2); n=25	15.1 (–17.0, 47.3); n=57
Adjusted Model ^b			
<i>CYP2B6</i> Normal ^c	48.7 (18.3, 79.2); n=121	184.5 (86.7, 282.3); n=25	12.6 (–8.4, 33.6); n=96
<i>CYP2B6</i> Slow	41.2 (–4.5, 86.9); n=82	55.1 (–74.5, 184.8); n=25	13.1 (–20.5, 46.7); n=57

Abbreviations: CI, confidence interval

^aThe regression coefficients and 95% confidence interval are reported, derived from 10 imputed datasets.

^bCovariates included: sex, age, time since first inhalation

^cIntercept in regression

Note: Coefficients for *CYP2A6* intermediate and *CYP2A6* slow metabolizers are expressed relative to *CYP2A6* normal metabolizers, while coefficients for *CYP2B6* slow metabolizers are expressed relative to *CYP2B6* normal metabolizers. For example, in the unadjusted model in the dependent smokers, *CYP2A6* slow metabolizers smoked 108 fewer cigarettes compared to *CYP2A6* normal metabolizers.