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A Role for Epigenetics in Broadening the Scope of Pediatric Care in the Prevention of Adolescent Smoking

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Abstract

Adolescence presents a critical opportunity to support the development of healthy patterns of behavior and prevent future health problems. Unfortunately, there is not yet a well-developed prevention model that allows pediatricians to initiate and monitor prevention efforts for the substantial minority of individuals who engage in risky behavior during adolescence. We suggest that recently developed epigenetic technologies may provide a bridge to a new prevention paradigm in which pediatricians screen for indicated smoking prevention services. Specifically, since currently available tobacco use screening measures are insensitive to low levels of use, newly developed methylation based approaches may enhance the sensitivity of the initial screening for nascent smoking behavior as well monitoring of outcomes. We conclude that the incorporation of Next Gen screening technologies into standard pediatric evaluations may allow for more effective referral to prevention programming for tobacco use. In addition to the potential direct impact on long-term enhancement of health outcomes secondary to smoking prevention, well-established links between smoking and other risk behaviors suggest that expanded referral for nascent smoking may also create opportunities to address other risky adolescent behaviors, and so decrease several interrelated drivers of long-term health care costs.

Keywords

Aryl hydrocarbon receptor repressor; cotinine; DNA methylation; pediatrics; smoking prevention

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CONFLICT OF INTEREST

Dr. Philibert is the Chief Scientific Officer and a stockholder for Behavioral Diagnostics, Inc (Iowa City, USA). The use of cg05575921 for the detection of smoking is covered by U.S. patent 8,637,652 which is co-owned by Drs. Philibert and Anup Madan. Dr. Philibert also has pending intellectual property rights with respect to use of DNA methylation for the detection of smoking. The other authors have no conflicts to report.

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ADOLESCENCE IS THE TIME FOR PREVENTION PROGRAMMING

The potential for pediatric care to have increased impact on the prevention of future health problems by preventing risky behavior in adolescence is considerable. However, prevention models that facilitate an expanded role for pediatricians in this area are not currently well developed. In this commentary we focus on methylation based approaches to the detection of smoking initiation as a new epigenetic technology that could help expand pediatric care for adolescents and more effectively address a highly consequential, risky behavior that typically begins in adolescence and has long-term health consequences. Because most risky behaviors with health damaging potential, like smoking, have complex determinants that render them difficult targets for direct biological intervention, the role of pediatricians in addressing these problems has been limited. For example, it is well appreciated that genetic factors play a role in shaping risky behaviors [1, 2], and this has sometimes led to the assumption that little can be done to alter early tendencies toward risky behavior such as smoking. However, the same genetically informed designs that demonstrate the importance of genetic factors in smoking have also demonstrated interactions between social environment and genetic influences, including interaction with family context [3]. This suggests the likely mutability of many heritable effects through carefully targeted prevention programming. Unfortunately, once established, many of these patterns of risky behavior, particularly tobacco use, become more difficult to modify. This means that by the time pediatricians typically become aware of adolescent smoking, the optimal window of opportunity for successful intervention already may have passed. A better, potentially more effective, model would allow referral at an earlier stage, before the pattern of use is well established. That is, early, reliable identification of nascent smoking is critical if pediatricians are to play a pivotal role in preventing new cases of nicotine addiction and so help prevent the myriad health problems that follow in the wake of this pattern of behavior.

Early detection of smoking and referral for indicated preventive intervention is a particularly attractive target for expansion of pediatric care because of the important role played by tobacco use in a range of adverse health outcomes. Over the past two decades, there has been increasing appreciation of the relationship of adolescent smoking to the manifestation of later onset adult disorders such as type II diabetes (T2DM), coronary artery disease (CAD) and lung disease [4]. Vulnerability to each of these disorders is markedly increased by cigarette smoking. Given the high cost of treating each of these disorders and the fact that smoking is the largest preventable cause of mortality of these and other complex disorders, there has been increased interest in developing better methods to treat and ideally, prevent early onset substance use, with a particular interest in preventing smoking. Likewise, there has been interest in prevention of smoking as a potential avenue for broader prevention of substance use (for reviews see [5–7]).

DETECTION OF ADOLESCENT SMOKING IDENTIFIES A LONG-TERM RISK AS WELL AS RISK FOR ENGAGING IN OTHER HEALTH RISK BEHAVIORS

Further enhancing interest in smoking prevention in adolescence is the fact that most nicotine addiction starts during this developmental stage. Indeed, more than 3/4ths of all

adult cigarette smokers began smoking during adolescence [8]. At the same time, there is a period at the very beginning of nicotine addiction during which nascent smokers may be less driven by physiological or psychological dependence. Most smokers experience initial exposure (i.e. first cigarettes), which is followed by experimental smoking (irregular, contextually dependent, periods of smoking), then by regular smoking (recurrent smoking in specific contexts), and then finally dependent smoking (smoking driven by physiological or psychological cues) [9]. Once fully established, smoking behaviors are fairly stable though most smokers experience multiple cycles of quitting and relapsing even if they finally achieve complete abstinence [10]. Because efforts to quit smoking often fail and individuals may incur irreversible medical consequences before they quit, there is a strong belief throughout the medical community that the best way to stop smoking is to intervene early in its course before it becomes established, i.e. during the phase of experimental, irregular smoking. If pediatricians had an accurate screening tool for the identification of nascent smoking, they would be well situated to play an expanded role in addressing this problem as an extension of routine care, providing a cost-effective service that would benefit both patients and the health care system.

In addition to directly addressing the problem of long-term costs of smoking, the identification of nascent smoking has the potential to identify the likely presence of other risk behaviors as well. In a landmark study of 2001 subjects from the National Health and Nutrition Survey (NHANES) between the ages of 12 and 16, Hanna and colleagues found that those adolescents who had experimented with smoking were 6.5 times more likely to be current drinkers. Those with an established pattern of smoking were 18 times more likely to be current drinkers [11]. Among the females, current smokers were 12 times more likely to experience pregnancy than non-smokers [11]; they were also 10 times more likely to have school problems and five times more likely to use marijuana, crack or cocaine. Similar results have been found in work by our research team, linking smoking with drug use, drinking, and risky sex among African American (AA) adolescents in the Family and Community Health Study (FACHS) [12, 13]. These studies affirm the results of prior smaller prospective studies [14, 15] and indicate that early detection of adolescent smoking may also serve to identify a group at risk for a range of other risky behaviors with the potential for severe negative health outcomes in the future [16]. In short, adolescent smoking is a serious health risk behavior in its own right and is associated with a number of other risk-taking behaviors including risky sex, illicit drug use, and drinking. Accordingly, if the technology were available to identify adolescent smokers who are still in the experimental stage of smoking, i.e. nascent smokers, pediatricians could refer them to prevention programs to interrupt one of the most important preventable causes of morbidity and premature death. At the same time, a referral of this sort could provide an occasion for more careful assessment of other risky behaviors, potentially further amplifying the benefit of the intervention.

METHODS TO PREVENT AND DECREASE THE ESCALATION OF RISK TAKING BEHAVIORS

One factor contributing to the current low level of enthusiasm for referral to prevention programs is the clear need for improvement in the efficacy of smoking prevention programs.

The efficacy of school-based interventions has been examined the most frequently and the results are somewhat disappointing. A meta-analysis of the outcomes of 207 school based substance prevention studies demonstrated that lecture-based prevention programs that emphasize drug knowledge have small but significant effects while those which foster the development of interpersonal skills typically have greater effects [17]. Less sanguinely, a series of meta-analyses and reviews concluded that currently available informational or affective based programs do not change behavior even in the short-run [18], and meta-analyses of long-term outcomes by two separate investigative groups have raised significant questions as to the long-term effectiveness of any of the programs [19, 20].

A second approach for prevention is interactive programming. A recent review by Flay and colleagues (2012) concluded that smoking prevention studies, particularly interactive skill based approaches, *are effective in the short-term* with the degree of long term effectiveness decaying as a function of time from treatment [21], however, these effects are less than might be hoped. But small effect sizes and relapse are commonly observed in the development of most treatment innovations for complex disorders. The fact that the interactive interventions work at all strongly suggests that their effects can be improved. Furthermore, the simplest solution to preventing relapse for almost all medical disorders is generally better physician monitoring.

Finally, there are family based approaches. Our group is one of a number of consortiums to have developed successful family-based prevention interventions for smoking and smoking related behaviors that focus on empowering parents to work more effectively with each other and with their child. Because the initiation of smoking co-sorts with the likelihood of initiating other behaviors, the SAAF interventions can have broad effects on many domains of risk taking behavior (smoking, drinking, risky sex) [22–24]. A recent analysis of follow-up data from 502 families who participated in randomized examination of the SAAF-T intervention, a version of the program developed for teenagers, showed significant reductions in substance use, depressive symptoms and conduct disorder spectrum symptoms two years after enrolling in the program [25].

These three methods are not the only approaches and new methods continue to develop [26]. However, a commonality to each of these approaches is the need to identify those most in need and, once abstinence is achieved, to grasp that achievement tightly. Accordingly, the effects of these approaches to prevention may become stronger when coupled with both earlier referral for the intervention and better physician-based monitoring of outcomes.

Conceptually, this approach can be easily integrated into current medical practices. Pediatricians and allied healthcare providers routinely see adolescents for well health exams. We propose to increase the value of those visits by implementing routine screening for substance use (see Fig. 1). Those individuals with positive screens should have their biopsychosocial histories reviewed to determine which intervention approach may be best suited to their presentation. After intervention is complete, the success of the intervention should be monitored with both clinical and biometric measures. Those failing initial intervention or relapsing should be referred for more intensive treatments.

CURRENT METHODS FOR DETECTING NASCENT SMOKING

The key to making this new approach work is to devise a method through which to sensitively and specifically detect substance use. Currently the most daunting aspect of implementation of indicated prevention models in the context of standard pediatric care is determining when to refer. Because self-report by high risk adolescents, who may wish to escape detection, is unreliable, a simple self-report of smoking is unlikely to identify all of those in need. Supporting the value of self-report, in 1994, Patrick and colleagues reported that the average sensitivity and specificity of self-report with respect to smoking verified by cotinine assessment was near 90% in epidemiological samples [27]. However, this finding has been increasingly challenged. In 2005, Fendrich and colleagues surveyed 627 individuals between the ages of 18 and 40 using an audio computer-assisted self-interview procedure and found self-report sensitivity estimates well below the levels previously cited with the lack of sensitivity increasing as a function of time and being particularly notable among minority groups [28]. In 2007, Harrison and colleagues examined the correlation between smoking self-report and cotinine levels in 2203 subjects from the general population who were between ages 12 and 25 and found a kappa of only 0.643 with a marked insensitivity for periodic smokers [29]. Finally, Gorber and colleagues (2009) reviewed 54 studies that compared self-reported with biochemical measures and found an overall sensitivity of ~80% in epidemiological samples, most of which focused exclusively on those 18 years and older [30]. Accordingly, self-report alone is not likely to be an acceptable foundation for referral within an indicated prevention model, particularly one focused on the detection of nascent smoking.

Two methods to better identify early smokers using biochemical screening are currently available [31]. The first is *exhaled CO* and the second is salivary, urine or serum *cotinine*. Both approaches have considerable strengths, particularly in the context of assessment of regular smokers. The greatest strength of the CO approach is ease of measurement [31]. Unfortunately, because the half-life of CO in the blood is only 4–5 hours and there are other potential environmental sources of CO, this marker is not able to distinguish light or periodic smokers from non-smokers [32, 33], and would be relatively easy to manipulate if an adolescent wanted to avoid detection. Hence, it is not well suited to identify the nascent smokers of greatest interest from the standpoint of indicated preventive intervention. An alternative, cotinine, has emerged as the principal biomarker for the assessment of smoking and has greater potential. *Cotinine*, the principal metabolite of nicotine, can be assessed in urine, serum, hair or saliva [34]. As opposed to CO, it has a longer half-life (15–19 hrs) and is relatively specific for tobacco. When used for the assessment of regular smokers, it is both sensitive and specific [35]. However, as a stand-alone tool for assessment of sporadic or nascent smokers it is much less sensitive and specific [36]. Moreover, the detection of low level smoking is made more difficult by substantial individual variation in the metabolism of nicotine and the clearance of cotinine among African Americans, Whites, and Asians [35, 37, 38]. Spurred by earlier observations of this potential confounder, Benowitz and colleagues analyzed the relation between cotinine levels and ethnicity in adolescent smokers, and recommended a set of cotinine cutoff levels that vary nearly threefold with respect to ethnicity [35]. Because families are becoming increasingly interracial, this ethnic sensitivity

also poses a problem for widespread use of a detection method that relies entirely on cotinine.

The problems with cotinine assessments suggest that supplemental, perhaps less expensive, and perhaps more sensitive, assessments of nascent smokers will be important to provide optimal guidance if an expanded role for pediatricians is to become a reality.

AN EPIGENETIC APPROACH TO DETECTING NASCENT SMOKING

The above data indicate the need for alternative approaches to detect and quantify cigarette smoking in adolescents. Ideally, the new approach should be easy to use and should address the shortcomings of the current measures by being able to capture intermittent use and quantify total use. One method through which this could be accomplished is through measuring smoking associated changes in DNA methylation. Over the past several years, we and a number of other groups have demonstrated that smoking is associated with changes in DNA methylation [39–45]. Whereas the initial examinations were performed using single locus bisulfite sequencing, the most recent of these examinations have taken advantage of the extensive genome wide coverage afforded by the Illumina 450K Human Methylation Bead array. These studies have shown that chronic smoking in older adults is associated with extensive remodeling of the DNA methylation signature with particular changes of DNA methylation at loci associated with inflammation or toxin metabolism. However, the extent of the genome wide remodeling is dose dependent with smaller changes, mostly confined to be aryl hydrocarbon receptor repressor (AHRR), being noted in smokers in their teens or early 20s.

Because of their cost, using genome wide approaches to screen for incipient smoking is not currently a clinical option. Fortunately, we and others have also shown that single or multiple locus measures of DNA methylation may fill that clinical need. For example, using bisulfite sequencing, Shenker and colleagues have shown that the data from the quantitative pyrosequencing of four genomic loci (two at 2q37, one each at 6p21 and AHRR) could be combined to form a sensitive and specific test for determining cigarette smoking history in adults [46].

This approach may not work for detecting smoking in adolescents however. In our studies of 19 yr old smokers, of the four loci identified by Shenker, only the AHRR locus (cg23576855) shows reasonably significant changes in methylation (uncorrected p-values of 5×10^{-6} , FDR corrected p-value of 0.55 [40]. The other three loci are significantly more weakly associated with smoking in this younger group with the nominal p-values for the two 2q37 loci (cg06644428 and cg21566642) and the 6p21 locus (cg06126421) being $p < 0.37$, $p < 0.54$ and $p < 0.002$, respectively.

In contrast, our work with the neighboring AHRR locus, cg05575921, which is consistently among the top loci in most if not all of the adult studies, shows that it is exquisitely sensitive to smoking in adolescents. The reason for this sensitivity is illustrated in Fig. (2) and takes advantage of the fact that tobacco smoke is laden with cytotoxins dioxin and poly aromatic hydrocarbons (PAH). When smoke is inhaled, these substances and other toxins are readily

absorbed by the blood as it passes through the lung. Sensing these chemicals, catabolically competent cells which include hepatocytes, white blood cells and pulmonary macrophages attempt to inactivate these toxins *via* the xenobiotic pathway. The activity in the xenobiotic pathway is regulated by the aryl hydrocarbon receptor (AHR). In brief, after it binds dioxin or PAH, AHR dimerizes with its conjugate nuclear receptor partner, the AHR nuclear translocator (ARNT), and then translocates to the nucleus. In the nucleus, this dimer binds to hexameric motifs referred to as xenobiotic response elements (XRE) that are found in the promoters of key genes in the xenobiotic pathway including CYP1A1 and CYP1A2. Unfortunately, because unrestrained activation of this pathway can also catabolize key nutrients and lead to cancer, firm control of transcriptional activity in this pathway is necessary [47, 48]. This is accomplished by the concurrent AHR/ARNT dimer activation of AHR transcription that results in feedback inhibition of AHR stimulated gene transcription by competing for ARNT and by sterically inhibiting AHR action at XRE elements. The enhancer region surrounding cg05575921 is absolutely critical to this process with cofactors associated with gene activation preferentially binding to enhancer region surrounding the CpG residue when it is unmethylated [41].

This area may be the most sensitive region of the genome. In our genome wide surveys of 19 and 22-year-old African American subjects, only cg05575921 was consistently associated at the genome wide level (See Table 1) [39, 40]. In younger subjects, its measurement alone may suffice for assessing cigarette consumption. In the 22-year-old African American subjects, a model that used methylation at cg05575921 showed high correlation with serum cotinine levels (0.42) with other loci not contributing significantly to the predictive model. Secondary analyses of these data also demonstrate that serum cotinine and methylation measures may be useful for the detection of smoking with the best model for predicting self-reported smoking status in this population being a combination of both methylation and serum cotinine data.

Building on established approaches using cotinine assessment, a joint approach may also have significant promise with respect to detecting smoking status and level of smoking. Recently, our group has used a commercially available assay for this locus and serum cotinine values to assess smoking status in a sample of 650 high risk adult African-Americans and found that both measures independently and significantly contribute to the prediction of self-reported smoking status. (Todorov *et al.*, in preparation). Commercial Phase I testing of this technology for the detection of smoking in adults in the United States is scheduled to begin April 1, 2014 with additional testing of adolescents being proposed for 2015. Although additional validation is needed, the potential for an inexpensive, sensitive, and specific index of nascent smoking that could be used by pediatricians and other allied health care providers for routine screening and monitoring appears to be within reach.

A CALL FOR GREATER PROVIDER INVOLVEMENT

In conclusion, smoking and other risky behaviors are often initiated during adolescence and have the potential to strongly influence long-term health outcomes and risk for a number of adult onset disorders. Pediatricians are poised to play a greater role in the prevention of these harmful behaviors because nascent smoking is readily detectable using currently available

epigenetic techniques, particularly when they are combined with widely available supplemental assessments and information. Although further development is necessary, the processes outlined in our model (see Fig. 1) can be harnessed to substantially enhance the role of pediatric care in the prevention process by enabling physicians to confidently make indicated referrals for intervention to prevent the establishment of nascent smoking patterns. Doing so has the potential to reduce the onset of habitual smoking and may prove an effective entry point for addressing a range of other risk behaviors as well. The expansion of existing pediatric care to include referral for an indicated intervention to prevent nascent use of cigarettes from becoming habitual use, is one example of the way in which current research in epigenetics can enable physicians to play a greater role in preventive care, helping improve the nation's long-term health and decrease health care costs. The approach has potential to confer substantial benefits at the level of population health and if it can be used to stimulate attention to other risky behavior in adolescence, the long-term potential of the indicated prevention approach will grow as prevention efficacy increases across multiple problem areas.

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LIST OF ABBREVIATIONS

AHRR	Aryl hydrocarbon receptor
GxE	Gene-environment interaction
FACHS	Family and Community Health Studies
SAAF	Strong African American Families project
ELISA	Enzyme linked immunoassay
NHANES	National Health and Nutrition Examination Study
T2DM	Type 2 diabetes mellitus
CAD	Coronary artery disease
AIDT	Autoimmune disease of the thyroid
IL-6	Interleukin 6
NSAIDS	Non-steroidal anti-inflammatory drugs

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Fig. 1.

A new model for smoking prevention programming. In this new paradigm, earlier referral to prevention programming is facilitated by pediatric screening during routine well-child health assessments and immunization visits. After a positive initial screen is received (from either self report or biological assessments of cotinine or methylation status), the pediatrician assesses the patient for the appropriate intervention. After the intervention, the patient is periodically monitored to facilitate the early recognition of relapse and re-referral into more intensive services.

Fig. 2.

Exposure of cells to dioxins or polycyclic aromatic hydrocarbons (PAH) found in tobacco smoke results in the induction of the AHR nuclear receptor/xenobiotic metabolism pathway. Once AHR binds either dioxin or PAH, it binds to its heterodimer partner, the AHR Nuclear Translocator (ARNT). Together, they move to the nucleus and induce P450 genes such as CYP1A1 that are critical to the catabolism of tobacco related substances. Unchecked, this catabolic activity is detrimental. However, this activity also induces the demethylation of cg05575921. This demethylation allows the enhancer motif surrounding cg05575921 to bind factors that increase AHRR transcription. The resulting AHRR receptor provides feedback modulation of AHR induced transcription by competing with AHR for ARNT and by competing with AHR for xenobiotic receptor enhancer (XRE) motifs.

Top Five Probes from Genome Wide Comparison of Serum Cotinine Confirmed 22 Year Old Current Smokers and Self Reported Serum Negative Non-Smokers

Table 1

Probe ID	Average Methylation				T-Test	Corrected P-Value
	Gene	Smoker (n=37)	Non-Smoker (n=64)			
cg05575921	AHRR	0.74	0.85		4.92E-09	0.002
cg21161138	AHRR	0.69	0.73		1.18E-07	0.029
cg26703534	AHRR	0.64	0.69		4.72E-07	0.076
cg22132788	MYO1G	0.94	0.88		1.19E-06	0.144
cg17072268	PLD3	0.82	0.84		1.11E-05	0.999