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Smoking in young adulthood among African Americans: Interconnected effects of supportive parenting in early adolescence, proinflammatory epitype, and young adult stress

STEVEN R. H. BEACH,[a] MAN KIT LEI,[a] GENE H. BRODY,[a] GREGORY E. MILLER,[b] EDITH CHEN,[b] JELANI MANDARA,[b] AND ROBERT A. PHILIBERT[c]
[a]*University of Georgia;* [b]*Northwestern University; and* [c]*University of Iowa*

Abstract

We examined two potentially interacting, connected pathways by which parental supportiveness during early adolescence (ages 1–13) may come to be associated with later African American young adult smoking. The first pathway is between parental supportiveness and young adult stress (age 19), with stress, in turn, predicting increased smoking at age 20. The second pathway is between supportive parenting and tumor necrosis factor (*TNF*) gene methylation (i.e., *TNF*m), a proinflammatory epitype, with low levels indicating greater inflammatory potential and forecasting increased risk for smoking in response to young adult stress. In a sample of 382 African American youth residing in rural Georgia, followed from early adolescence (age 10–11) to young adulthood (age 20), supportive parenting indirectly predicted smoking via associations with young adult stress, IE = –0.071, 95% confidence interval [–0.132, –0.010]. In addition, supportive parenting was associated with *TNF*m measured at age 20 ($r = .177$, $p = .001$). Further, lower *TNF*m was associated with a significantly steeper slope ($b = 0.583$, $p = .003$) of increased smoking in response to young adult stress compared to those with higher *TNF*m ($b = 0.155$, $p = .291$), indicating an indirect, amplifying role for supportive parenting via *TNF*m. The results suggest that supportive parenting in early adolescence may play a role in understanding the emergence of smoking in young adulthood.

Smoking is the leading preventable cause of morbidity and mortality in the United States, with complications of smoking causing nearly half a million US deaths per year as well as higher population rates of preventable, serious illness (Centers for Disease Control [CDC], 2011, 2014; Mokdad, Marks, Stroup, & Gerberding, 2004). Unfortunately, many youths currently between the ages of 12 to 20 will initiate cigarette smoking, comprising a large portion of the 1.5 million individuals initiating daily smoking each year. As a consequence, it is expected that 5.6 million children who are alive today will experience premature death attributable to cigarette smoking (CDC, 2014). Cigarette smoking has also become increasingly tied to socioeconomic status (SES), making it a primary driver of SES-related health disparities (e.g., Fagan, Moolchanm, Lawrence, Fernander, & Ponder, 2007), with those most economically disadvantaged at greatest risk for smoking-related illness. At the same time, in the United States, people of African descent suffer worse outcomes for smoking-related illnesses (CDC, 2010; Haiman et al., 2006), even after adjusting for covariates such as SES and healthcare access, suggesting that the need for attention to smoking prevention is particularly acute among African American youth for whom it could have especially pronounced health benefits.

Family Influences and Stress

Family influences have emerged as a key element in models identifying potential points of intervention for early prevention of smoking (e.g., Ennett et al., 2001). Parenting is a significant predictor of all forms of substance use across early to late adolescence (Piko & Balazs, 2012; Ryzin, Fosco, & Dishion, 2012), the time during which the vast majority of adult smokers initiated smoking (Brynin, 1999). Increases in smoking across this age range also predict development of substance abuse disorders, poor psychosocial functioning, and poorer mental health outcomes (e.g., Conger, Ge, Elder, & Lorenz, 1994; Simons, Burgeson, Carlton-Ford, & Blyth, 1987; Windle & Windle, 2009, 2012). Accordingly, there are many reasons for a continuing focus on ways that families influence smoking outcomes during adolescence.

Another well-studied predictor of cigarette smoking among young adults is the experience of stress (Cerbone & Larison, 2000; Sinha, 2001; Wills, 1990). Level of stress experienced by young adult African Americans as they tran-

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Address correspondence and reprint requests to: Steven R. H. Beach, Center for Family Research, University of Georgia, 1095 College Station Road, Athens, GA 30602-4527; E-mail: srhbeach@uga.edu.

sition into adult roles is predictive of their smoking (Aseltine & Gore, 2005; Brody, Chen, Kogan, Smith, & Brown, 2010; Paschall, Flewelling, & Faulkner, 2000). This is not surprising given the well-documented effect of stress on increased desire to smoke (Buchmann et al., 2010; Colamussi, Bovbjerg, & Erblich, 2007; Erblich, Boyarsky, Spring, Niaura, & Bovbjerg, 2003; Niaura, Shadel, Britt, & Abrams, 2002). Given the effect of young adult stress on smoking, one potential pathway connecting supportive parenting to smoking may be from early adolescent parenting to later experience of stress in young adulthood, and then ultimately to later cigarette smoking.

Inflammation and epigenetics

The emergence of a literature on potential biological effects of parenting suggests an additional potential pathway from supportive parenting to smoking. Recent work has identified ways in which experiences in childhood or early adolescence may "get under the skin," becoming biologically embedded risk factors that manifest later in adulthood (Brody, Chen, & Kogan, 2010; De Bellis, 2002; Gordon, 2002; Sinha, 2008). Predictive-adaptive response models (Cole, Hawkley, Arevalo, & Cacioppo, 2011; Gluckman, Hanson, & Spencer, 2005; Rickard & Lummaa, 2007), in particular, suggest that social adversity and perceived threat in childhood or early adolescence should lead to greater proinflammatory propensities. From an evolutionary perspective this shift is thought to be adaptive because it prepares the individual for potentially elevated risk for tissue damage across the life span, with effects on proinflammatory potential that persist into young adulthood. The presence of chronic stressors, including poverty, neighborhood crime, and discrimination, may increase the importance of supportive parenting as a protective factor.

Building on this broad foundation, an integrative model recently put forward by Nusslock and Miller (2015) posits that biologically embedded changes developed by youth growing up in difficult circumstances may be linked to later behavioral outcomes, such as smoking, in part, because they are linked to broader neuro–hormonal–immune system network changes that serve to amplify links between inflammatory propensities on the one hand and the neural circuitry of anxiety and reward on the other. On this view, if biologically embedded vulnerabilities are triggered by young adult stressors, they may amplify resulting behavioral effects. For example, such vulnerabilities might amplify increases in cigarette use in young adulthood in response to stress.

Parenting and biological embedding

Because supportive parenting may counter the impact of life stress on behavioral outcomes (Luthar, 2006), and parental emotional support buffers youths' physiological stress reactions, supportive parenting may be particularly important for minority youth growing up in challenging environments. In keeping with this view, supportive parenting may ameliorate hormonal, metabolic, inflammatory, and cardiovascular risk following childhood and early adolescent adversity (Brody et al., 2014; Chen, Miller, Kobor, & Cole, 2011), potentially protecting against the development of proinflammatory epigenetic changes (Beach, Lei, Brody, Dogan, & Philibert, 2015), accelerated weathering (Geronimus, Hicken, Keene, & Bound, 2006), and development of vulnerabilities to poorer health in young adulthood (Beach et al., 2016). In contrast, parenting that is harsh or abusive may amplify inflammatory profiles later in life (Dube et al., 2009; Miller & Chen, 2010). Together, these finding suggest that supportive parenting during early adolescence may protect against development of a proinflammatory epitype, providing another indirect pathway of influence from supportive parenting to later smoking by youth, in this case, by reducing the impact of later stress.

Why examine epigentic markers of vulnerability? The way in which protective parenting during early adolescence can be turned into biological changes with health consequences for young adulthood is likely complex (cf. Hertzman, 1999). However, one likely mechanism mediating such effects is epigenetic programming of immune cells (Miller, Chen, & Parker, 2011). Methylation of specific cytosine nucleotide–phosphate–guanine nucleoside (CpG) sites (i.e., regions of DNA in which cytosine occurs next to guanine separated by only one phosphate bond) can influence access to key regulatory elements controlling the rate of gene transcription, and thus influence downstream effects. Because methylation associated with the first exon is particularly predictive of gene expression (e.g., Brenet et al., 2011; Plume, Beach, Brody, & Philibert, 2012), characterizing individual differences in methylation of inflammation-related genes in the region of the first exon may be particularly informative, and provides a useful starting point for examination of potential epigenetic mediators. Quantifying the expectation of larger effects when methylation occurs in the first exon, Brenet et al. (2011) found that DNA methylation of the first exon was more tightly linked to transcriptional silencing than was methylation elsewhere in the genome (e.g., compared to effects for methylation of introns, internal exons, and last exons, and even methylation of the promoter region), with hypomethylation of the first exon producing a large effect on the log odds ratio (LOR) for gene expression (LOR = –2.8). Follow-up work to examine the impact of experimentally manipulated demethylation confirmed these conclusions (Brenet et al., 2011). In terms of Cohen effect sizes, an LOR of –2.8 can be characterized as a large effect size (Chen, Cohen, & Chen, 2010), suggesting that a focus on methylation of the first exon is a good starting point for characterization of epigenetic effects.

Why focus on tumor necrosis factor gene methylation (TNFm) as a proinflammatory epitype? Supportive parenting has been shown to be associated with epigenetic effects on cell-signaling processes generally (Beach et al., 2016) and

has been shown to predict greater methylation of the first exon of *TNF* (*TNF*m; Beach et al., 2015). *TNF* is the gene that encodes TNF-α. We assessed *TNF*m as a window on a proinflammatory epitype because *TNF* is a key regulator of the inflammatory response (Bradley, 2007; Dhama et al., 2013). For example, it stimulates production of inflammatory cytokines (e.g., interleukin [IL]-1β and IL6) that are prominent in many types of pathology, and it activates proinflammatory transcription factors such as nuclear factor kappa light-chain enhancer of activated B cells (NF-κB; Bradley, 2007). Further, vascular endothelial cells demonstrate several proinflammatory changes in response to TNF, and it has been linked to autoimmune and inflammatory conditions (Bradley, 2007; Dhama et al., 2013). Treatment of many inflammatory disorders involves medication designed to block the action of TNF (Bradley, 2007; Dhama et al., 2013), which is seen as central to inflammatory control efforts and a specific target of medical intervention. Accordingly, we focused on methylation of the first exon of *TNF* to identify individuals whose relatively greater methylation in this location would indicate downregulation of TNF production and decreased production of TNF relative to those with lower methylation. In humans TNF is commonly produced by activated macrophages, that is, monocytes that have migrated from blood to tissues and differentiated. Accordingly, as a central element of the innate inflammatory response, epigenetic change via shifts in the level of methylation of *TNF* (i.e., *TNF*m) is a mechanism that has strong theoretical links to existing theory, and that we can access using a readily available peripheral tissue to characterize individual differences.

Better understanding of the way family processes during early adolescence contribute to, or protect against, inflammation is particularly relevant for African Americans, who tend to show higher levels of inflammatory markers (Chyu & Upchurch, 2011; Geronimus et al., 2006; Paalani, Lee, Haddad, & Tonstad, 2011) than do whites. As a result, better understanding family processes, like supportive parenting, that potentially protect against a proinflammatory epitype (i.e., low *TNF*m), may be particularly useful in identifying risk and protective factors relevant to African American health and health behavior in young adulthood.

Heuristic model of parenting, inflammation, young adult stress, and smoking

The foregoing considerations suggest that supportive parenting in early adolescence may be important for understanding initiation and escalation of smoking among African American youth growing up in the rural southeast, a context that is challenging in many respects. In particular, African American youth residing in the Southern coastal plain are often exposed to economic disadvantage and SES-related risks, setting the stage for a proinflammatory shift in transcriptional responses (Cole, 2010, 2014; Cole et al., 2012; Kiecolt-Glaser et al., 2003, Miller et al., 2008; Ranjit, Diez-Roux, Shea, Cushman, & Seeman, 2007). In this context, supportive parenting that conveys a sense of safety and reduced stress could be powerful in protecting against the proinflammatory processes that might otherwise be engendered (cf. Gruenewald, Cohen, Matthews, Tracy, & Seeman, 2009; Loucks et al., 2010).

Among youth who are more stressed and/or develop a proinflammatory epitype, we hypothesized increased smoking for two reasons. First, as noted above, for smokers, there is a well-documented effect of stress on increased desire to smoke. This is partially attributble to the increased reward value of smoking in the context of stressful experiences (Childs & de Witt, 2010). Second, as suggested by Nusslock and Miller (2015), for youth raised in more difficult circumstances, proinflammatory processes may lead as well to long-lasting changes in reward processing (cf. Gianaros et al., 2011; Maier & Watkins, 1988; Miller, Maltic, & Raison, 2009), resulting in blunted reward sensitivity and greater nicotine craving among smokers (Peechatka, Whitton, Farmer, Pizzagalli, & Janes, 2015). This suggests that a proinflammatory epitype could contribute to more rapid escalation of smoking in the context of heightened stress because nicotine temporarily normalizes blunted reward responsiveness (Janes et al., 2015), enhancing smoking's attractiveness for those with a proinflammatory response pattern.

These expectations are represented in Figure 1. It can be seen that parenting during early adolescence is predicted to be associated with both *TNF*m and with young adult stress. Young adult stress is assumed to be an important driver of increased smoking across the young adult years, leading to an indirect effect of early adolescent parenting with young adult smoking, but the effects of young adult stress on smoking are predicted to be greater among those with a proinflammatory profile for *TNF*m.

Preliminary and supplemental analyses

In addition to the direct tests of the model described below, as a preliminary step we first described the pattern of change in smoking from early adolescence to early adulthood for the current sample of African American youth (see Figure 2), and this pattern informed our decision to control smoking through age 14. Among our control variables are factors capturing cell-type variation. Our derivation of these control variables is more extensively described in the online-only supplementary materials (Table S.1). We also provide supplemental material examining the individual residues that comprise *TNF*m and show the high consistency of their individual ranges and mean values (Table S.2). Because our key outcome, smoking reports at age 20, is skewed and overdispersed, we used negative binomial regressions to better represent the dependent variable when examining factors influencing young adult smoking. Supporting this decision, we provide supplemental material reporting comparisons of residuals for the negative binomial with alternative statistical models appropriate for count data. Specifically, we compare residuals for negative binomial distributions with Poisson and zero-inflated analytic models (Table S.3). To examine the robustness of effects to changes in our measurement of

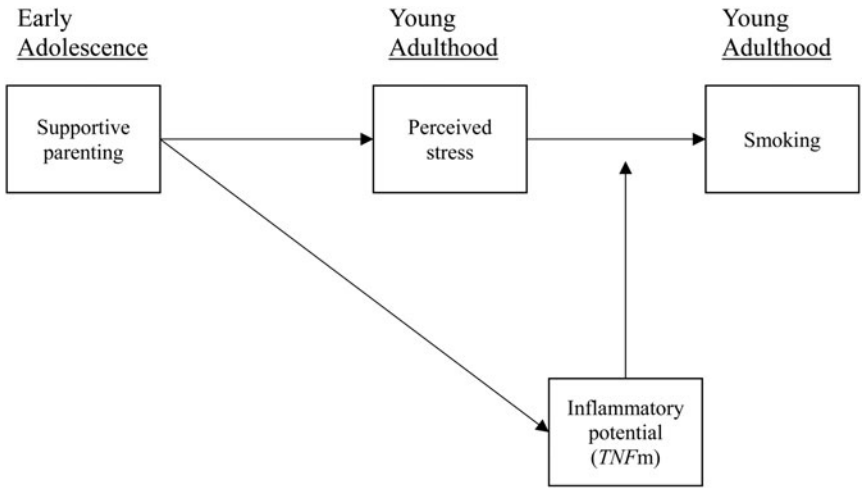

Figure 1. Theoretical model showing effects of early adolescent parenting on tumor necrosis factor methylation (*TNF*m), young adult stress, and smoking in young adulthood in response to stress.

young adult stress, we provide supplemental analyses using an alternative, broader, characterization of young adult stress comprising the average of standardized stress measures for ages 17, 18, and 19. This analysis shows an equivalent pattern of results to those resulting from a focus on stress at age 19 only (see Table S.4). Stress measures were significantly, but only moderately, correlated across waves and measures (average $r = .196$). To examine robustness with regard to concurrent use of alcohol and marijuana, we also conducted a supplemental analysis including concurrent alcohol and marijuana use as control variables, and found that including them did not change the observed pattern (see Table S.5). Finally, we also provide supplemental material showing the association of *TNF*m with methylation genomewide using gominer to describe broader patterns of methylation associated with the index (Table S.6).

Hypotheses to Be Tested

The primary hypotheses derived from the model displayed in Figure 1 are that supportive parenting may influence young adult smoking in two ways. First, it may influence young

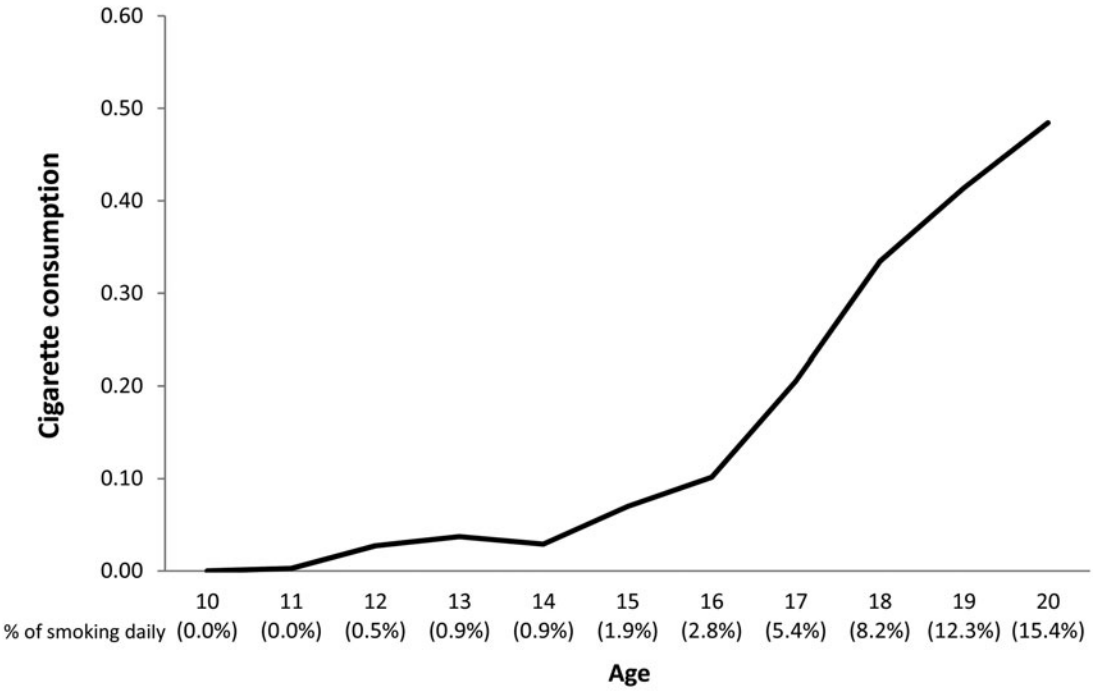

Figure 2. African American youth smoking from ages 10 to 20. The curve shows little increase in average consumption or daily smoking prior to age 14, but substantial escalation thereafter.

adult smoking by influencing young adult stress, and second, it may influence smoking by influencing *TNF*m. In turn, we hypothesize that higher *TNF*m, resulting from more supportive parenting, will reduce the effect of young adult stress on smoking at age 20. We examine whether lower *TNF*m is associated with increases in smoking in response to stress beyond effects attributable to other circulating inflammatory markers, such as C-reactive protein (CRP; a marker of current systemic inflammation known to be associated with body mass index), as well as individual differences in cell-type composition that could be responsive to initiation of smoking. To address these issues, we examine the following specific hypotheses:

1. Supportive parenting during early adolescence (ages 11–13), indexed by reports from both adolescents and their primary caregiver, will be associated with less perceived stress and greater *TNF*m in young adulthood.
   a. This association will be reflected in significant associations between early adolescent supportive parenting and both young adult stress (negative association) and *TNF*m (positive association).
   b. In addition, both associations will be robust to controls for sex, inflammatory cytokine level (CRP), and cell-type variation.
2. Young adult stress will predict smoking at age 20, controlling for early smoking (prior to age 14).
   a. The effect of stress on smoking at age 20 will be robust to controls for sex, early smoking, inflammatory cytokine levels (CRP), and cell-type variation.
   b. There will be a significant indirect association of supportive parenting with smoking through young adult stress.
3. Level of *TNF*m will be consequential with regard to later onset of smoking in response to stress.
   a. Lower *TNF*m will be associated with a greater impact of stress on cigarette smoking at age 20, controlling for gender, CRP, level of SES risk across early adolescence (ages 11–13), variation in blood cell type, and early smoking.
   b. The effect of young adult stress on smoking at age 20 will be significant for those with low *TNF*m and significantly greater for those lower in *TNF*m than for those higher in *TNF*m.

Method

Participants

At the first assessment, 667 families were selected randomly from lists of fifth-grade students residing in nine rural counties in Georgia, using names that schools provided (for a full description, see Brody et al., 2004). From the sample of 561 available at the age 18 data collection (a retention rate of 84%), 500 emerging adults were selected randomly to continue participating in biological assessments going forward. Costs associated with proposed biological assessments necessitated the drawing of the subsample. From this subsample, 398 (79.8% of the original sample) provided blood samples for genome-wide methylation analyses around age 20 (for additional details, see Brody et al., 2014). Of these, 16 did not provide data at age 19, leaving an effective sample of $N = 382$ for the current analyses. Comparisons with participants who did not provide blood samples or complete all study measures did not reveal any significant differences on any variables at baseline.

Youth lived in small towns and communities with poverty and unemployment rates among the highest in the United States (Dalaker, 2001), and all youth self-described as African American. Based on feedback from local communities and focus groups of rural African American community members (Brody et al., 2004), community liaisons were used to aid in the recruitment and retention of participants. At the first assessment, primary caregivers in the sample worked full time, on average, for an average of 39.9 hr per week, but 42.3% lived below federal poverty standards, with a majority living below 150% of the poverty threshold. Median monthly family income was $1,644 at age 19 and $1,840 at age 20. In this and other regards they are representative of the Georgia counties in which they reside (Boatright & Bachtel, 2003).

Youth mean age was 10.66 years at the first wave of assessment; 20.46 years, on average, at the time of the blood draw used for epigenetic analyses; and 20.43 years, on average, when reporting on Wave 9 cigarette smoking. Of the young adults whose outcomes are the focus of the investigation, 45.4% are male and 54.6% are female. Approximately one-quarter (24%) had less than a 12th-grade education. The current sample has been the focus of prior research described in Beach et al. (2014, 2015, 2016).

Procedure

A standardized assessment protocol lasting 2 hr, on average, collected in participants' residences, was used at each wave of data collection. Self-report questionnaires were administered to youth in an interview format. Each interview was conducted privately, with no other family members present or able to overhear the conversation. Youth reported on their primary caregiver's supportive parenting, their own smoking behavior, and also provided blood for epigenetic assessments. Primary caregivers reported on their parenting and family SES. To further enhance rapport with participants, African American students and community members served as home visitors to collect data at all visits.

Primary caregivers consented to their own and the youths' participation in the study, and the youths under 18 assented to their own participation and then consented when they participated as adults. All procedures were approved by the University of Georgia Institutional Review Board.

Measures

Parenting. Supportive parenting was assessed using target youth and parent reports when targets were 10.7, 12.4, and

13.2, on average. The short form of the Interaction Behavior Questionnaire (IBQ; Prinz, Foster, Kent, & O'Leary, 1979) was used to assess both youth and parent report. The short form includes the items with the highest phi coefficients and the highest item-total correlations among the 75 original items. It is correlated 0.96 with the full-length scale. The 15 true–false items comprising the scale ask about listening, understanding, enjoying, and getting along. Cronbach α was over 0.70 for each reporter and at each wave (i.e., youth IBQ: 0.76 at age 10.7 (first wave), 0.79 at age 12.4 (second wave), and 0.82 at age 13.2 (third wave). Parent IBQ had Cronbach α of 0.84 at Wave 1, 0.86 at Wave 2, and 0.88 at Wave 3. Parenting total scores were standardized and summed across youth and parent report and across ages 11 to 13 to form an overall index of supportive parenting during early adolescence (i.e., ages 11–13). Primary caregiver and target reports were correlated significantly at each wave ($r = .232$, .188, and .245, at Waves 1, 2, and 3, respectively).

SES risk. Caregiver reports collected when youth were 10.7 to 13.2 (Waves 1–3) were used to create our measure of SES risk. Early adolescent cumulative SES risk was assessed across six indicators. Each indicator was scored dichotomously (0 if absent, 1 if present). Cumulative SES risk was defined as the average number of risk factors across the three assessments, yielding an index with a theoretical range of 0 to 6 ($M = 2.33$, $SD = 1.35$). The six risk indicators were (a) family poverty, defined as being below the poverty level, taking into account both family income and number of family members; (b) primary caregiver noncompletion of high school or an equivalent; (c) primary caregiver unemployment; (d) single-parent family structure; (e) family receipt of Temporary Assistance for Needy Families; and (f) income rated by the primary caregiver as not adequate to meet all needs.

Perceived stress. Participating young adults responded to 10 items from the Perceived Stress Scale (Cohen, Kamarck, & Mermelstein, 1983) when targets were 19.1 years old, on average. The response format ranged from 1 (*never*) to 5 (*very often*). An example item is "How often in the past 30 days have you been upset because of something happening unexpectedly." Cronbach α was 0.81, with higher scores indicating greater perceived stress. As noted above, a parallel analysis is provided in the Supplementary Material showing that a combination of stress measures collected across ages 17.1 and 18.5 (Waves 6 and 7) combined with the measure of perceived stress used at age 19.1 (Wave 8) yields the same pattern of results (see online-only Table S.4).

Cigarette consumption. At each wave of data collection, subjects were asked, "In the past month, how much did you smoke cigarettes?" Response options included 0 = *none at all*, 1 = *less than 1 cigarette a day*, 2 = *1 to 5 cigarettes a day*, 3 = *about a half a pack a day*, 4 = *about a pack a day*, 5 = *about 1 and a half packs a day*, and 6 = *about 2 packs a day*. This allowed us to chart changes across adolescence and early adulthood as well as to identify those smoking daily. Changes in smoking responses as a function of age (not wave of assessment) is provided in Figure 2. The percentage of the sample reporting "daily smoking" is also provided for each age. As can be seen, the graph is relatively flat from 11 to 14 and then rises thereafter continuously until 15.4% of the sample is smoking daily at the final wave of assessment, age 20. Examination of self-reported smoking at age 20.4 (Wave 9) indicated that reports of smoking are overdispersed ($M = 0.484$, variance = 1.185, skewness = 2.680, kurtosis = 7.848). Accordingly, we used a negative binominal regression to predict age 20 smoking (alternative models compared for residuals are shown in supplementary Table S.3, indicating that they provide inferior fit).

CRP. Certified phlebotomists went to each participant's home to draw blood when participants were 20.46 on average. At time of the blood draw, one tube of blood was drawn into a serum separator tube by the certified phlebotomist, and this tube was frozen and delivered to the Psychiatric Genetics Lab at the University of Iowa for assaying. Serum levels of CRP were determined using a Duo Set Kit (DY1707; R&D Systems, Minneapolis, MN) according to the manufacturer's directions. A normal concentration of CRP in healthy human serum is usually lower than 10 mg/L. No participants had CRP levels outside the normal range. Because CRP is characterized by a skewed distribution (skewness = 1.90, kurtosis = 2.94), we applied a log transformation to normalize the readings, resulting in substantial improvement of the distribution (skewness = 0.91, kurtosis = –0.31 after the transformation).

Methylation. Certified phlebotomists also drew whole blood (30 ml) from each participant and shipped it to a lab in Iowa the same day for preparation. At the lab, the blood tubes were inspected to ensure anticoagulation and aliquots of blood were diluted, mononuclear cell pellets were separated from the diluted blood specimen by density-gradient centrifugation, and the mononuclear cell layer was removed from the tube using a transfer pipette, resuspended, and frozen at –80 °C until use. Genomic DNA was prepared using a QiaAmp (Qiagen, Germany) according to manufacturer's directions. A typical DNA yield for each mononuclear cell pellet was between 10 and 15 μg.

The Illumina (San Diego, CA) HumanMethylation450 Beadchip was used to assess genome-wide DNA methylation. Participants were randomly assigned to 12 sample slides/chips with groups of 8 slides being bisulfite converted in a single batch, resulting in five batches/plates. A replicated sample of DNA was included in each plate to aid in assessment of batch variation and to ensure correct handling of specimens. The replicated sample was examined for average correlation of beta values between plates, resulting in average correlations greater than 0.99. Prior to normalization, methylation data were filtered based on these criteria: (a) samples containing 1% of CpG sites with detection $p > .05$ were removed, (b) sites were removed if a bead count of <3 was present in 5% of samples, and (c) sites

with a detection $p > .05$ in 1% of samples were removed. More than 99.76% of the 485,577 probes yielded statistically reliable data.

Quantile normalization of methylation data. Quantile normalization methods with separate normalization of Type I and Type II assays in the Illumina array produce marked improvement in detection of relationships by correcting distributional problems inherent in the manufacturers default method for calculating β (i.e., $\beta = M/[M + U + 100]$; where M and U are methylated and unmethylated signal intensities, respectively; Pidsley et al., 2013). Accordingly, in the current investigation all loci across all plates were quantile normalized concurrently, separating methylated and unmethylated intensities, and using the wateRmelon (2013) R package (Bioconductor, 2012) to institute the *dasen* function recommended by Pidsley et al. (2013). This method equalizes the backgrounds of Type I and Type II probes prior to normalization and conducts between-array normalization of Type I and Type II probes separately.

Identifying and correcting for chip and batch effects. As demonstrated by Sun et al. (2011), quantile normalization typically reduces, but may not eliminate, batch and chip effects. Accordingly, after cleaning and quantile normalizing the data, all samples were examined for batch and chip effects. The distribution of quantile normalized average β values for all samples in each chip and batch were contrasted with all others using a box and density plot to indicate both the mean and the confidence intervals around the mean in each case. The results of this examination indicated that both batch and chip effects were eliminated through quantile normalization. Absence of plate effects was confirmed via direct examination of the sample replicated across plates.

Assessing and controlling proportion of cell types in mixed cell populations. Mononuclear cell pellets of the sort used in the current investigation are composed of several different cell types (e.g., primarily T-helper and cytotoxic cells, monocytes, B cells, and natural killer cells; Reinius et al., 2012). Accordingly, we controlled for individual differences in cell types by using a regression calibration approach similar to that developed by Houseman et al. (2012), except we used Illumina HumanMethylation 450K BeadChip data to identify the 100 sites best differentiating to the five cell types of interest. A locus determined to be on the X chromosome was dropped from subsequent analyses. Then, we performed a principal components analysis to identify principle components characterizing dimensions of individual variability in cell type in the current sample. Regressions linking each factor with proportion of cell types can be found in the supplementary material (see supplementary Table S.1 for details).

TNFm. An index of proinflammatory tendencies was created by examining degree of methylation of the first exon of *TNF* (*TNF*m). Eight CpG sites were identified as being associated with the first exon of *TNF* based on the manufacturer's documentation. Greater methylation of the first exon of *TNF* in cells capable of expressing TNF-α should result in less expression of this gene product and, all other things being equal, lower proinflammatory response. The intercorrelation of the eight CpG values on exon one was examined (rs = .736 to .942; all ps < .00001). A factor analysis of the eight CpGs identified a single factor with all loadings above 0.85. Accordingly, to index overall methylation of the first exon of *TNF* (*TNF*m), βs for CpGs on the first exon were standardized prior to creating an average score with a Cronbach α of 0.98. *TNF*m averaged 0.265 (range = 0.14–0.43) with all loci showing similar distributions (see supplementary Table S.2 for details).

Results

Hypothesis 1: Effect of supportive parenting on stress and TNFm

As can be seen in Table 1, primary study variables were correlated at a zero-order level. Supportive parenting was significantly (and negatively) associated with SES risk in early adolescence. Only SES risk and later smoking at age 20 were significantly associated with very early onset smoking and only supportive parenting in early adolescence (ages 11–13) and sex were significantly associated with (age 19) reports of young adult stress. Only supportive parenting was significantly associated with *TNF*m. All zero-order effects of interest were small to medium in size.

To further explicate the association of early adolescent parenting (ages 11–13) with *TNF* methylation and young adult stress, thereby examining the first stage of the theoretical mode (Figure 1), we examined these associations, introducing multivariate controls, including sex, SES risk, log (CRP), and cell type. As can be seen in Table 2, the association of early adolescent supportive parenting (ages 11–13) with *TNF*m and young adult stress were robust to the introduction of these controls, supporting the first step of the theoretical model presented in Figure 1.

Hypothesis 2: Stress effects on smoking and mediation of supportive parenting

We next examined the association of young adult stress (age 19) with smoking in young adulthood (age 20). We controlled for the effect of very early smoking 11–14 (i.e., Waves 1 to 4), as well as sex, SES risk, log (CRP), and cell type. As can be seen in Table 3, Model 1, the association of young adult stress (age 19) with smoking (age 20) remained significant after including controls. As predicted, there was evidence of a significant indirect pathway from early parenting to change in young adult smoking through associations with young adult perceived stress (indirect effect [IE] = –0.071, 95% confidence interval [–0.132, –0.010]). Examination of the incidence rate ratio column indicates that a standard deviation increase in

Table 1. *Correlation matrix and means and standard deviations for primary study variables*

	1	2	3	4	5	6	7	8
1. Supportive parenting (ages 11–13)	—							
2. Perceived stress (age 19)	−.202**	—						
3. Cigarette consumption (ages 11–14)	−.109*	.029	—					
4. Cigarette consumption (age 20)	−.100†	.093†	.122*	—				
5. *TNF*m	.177**	−.071	−.054	−.045	—			
6. Log of CRP	−.004	.055	−.096†	−.007	−.064	—		
7. Sex (1 = males)	.081	−.177**	.081	.289**	.064	−.282**	—	
8. SES risk (ages 11–13)	−.196**	.033	.144**	.095†	−.063	.039	−.014	—
Mean	−0.021	27.374	0.026	0.484	0.007	−1.131	0.456	6.869
SD	0.688	5.967	0.161	1.088	0.947	2.464	0.499	3.981

Note: $N = 382$. Factors 1–4 are the four principle components reflecting cell-type variation in the current data. *TNF*m, tumor necrosis factor gene methylation; CRP, C-reactive protein; SES, socioeconomic status.
†$p \leq .10$. *$p \leq .05$. **$p \leq .01$. Two-tailed tests.

perceived stress at Wave 8 was associated with an increase in the expected response regarding cigarette consumption at Wave 9 by 47.30%, holding all other variables, including smoking prior to age 14, constant. This relatively large effect is put in context by the even larger effect of sex (>6), indicating that smoking was reported much more frequently by males.

There was no evidence of multicollinearity in the regression. Diagnostic variance inflation factor scores for all variables in the regression were below 10, ranging from 1.056 to 3.097, indicating no evidence of multicollinearity among the study variables.

Table 2. *Regression models indicating supportive parenting as a predictor of TNFm (age 20) and young adult perceived stress (age 19)*

	*TNF*m		Stress	
	b	β	*b*	β
Supportive parenting (ages 11–13)	0.055* (0.026)	0.058	−1.107** (0.289)	−0.185
Sex (1 = males)	0.022 (0.061)	0.012	−1.904** (0.677)	−0.159
SES risk (ages 11–13)	0.041 (0.09)	0.043	−0.053 (0.294)	−0.009
Factor 1 cell type	0.569** (0.031)	0.601	−0.171 (0.305)	−0.029
Factor 2 cell type	0.489** (0.032)	0.517	−0.048 (0.328)	−0.008
Factor 3 cell type	−0.104** (0.028)	−0.110	0.218 (0.337)	0.036
Factor 4 cell type	−0.139** (0.036)	−0.147	−0.007 (0.290)	−0.001
Log of CRP	−0.034 (0.025)	−0.036	0.031 (0.291)	0.005
Constant	−0.003 (0.036)		28.242** (0.424)	
R^2	.677		.069	

Note: $N = 382$. Unstandardized (*b*) and standardized coefficients (β) are provided (robust standard errors); supportive parenting (ages 11–13), SES risk (ages 11–13), factors cell type, and CRP are standardized by *z* transformation (mean = 0, *SD* = 1). *TNF*m, tumor necrosis factor gene methylation; SES, socioeconomic status; CRP, C-reactive protein.
*$p \leq .05$. **$p \leq .01$. Two-tailed tests.

H3: Amplification of stress by TNFm

In Model 2 of Table 3 we examined the potential role of *TNF*m as a moderator of stress effects on increases in smoking by adding *TNF*m and the interaction term created by the product of *TNF*m with young adult stress to the regression. As can be seen in Model 2, the effect of stress remained significant, and there was no significant main effect of *TNF*m, but the interaction term reflecting the joint impact of *TNF*m and young adult stress was significant, indicating significant moderation. To explicate the significant interaction effect, we graphed slopes for high (1 *SD* above the mean) and low (1 *SD* below the mean) *TNF*m. As can be seen in Figure 3, greater *TNF*-related inflammatory potential (i.e., low *TNF*m) was associated with significantly increased impact of young adult stress (age 19) on cigarette use at age 20. For low *TNF*m (high inflammatory potential), the association was significant ($b = 0.583$, $p = .003$) whereas for high *TNF*m (low inflammatory potential), the association was not significant ($b = 0.155$, $p = .291$). A standard deviation increase in perceived stress at Wave 8 for respondents with low *TNF*m was associated with a 79.14% increase in reported cigarette consumption at Wave 9, holding all other variables constant. Conversely, among those with high *TNF*m, a standard deviation increase in stress was associated with a nonsignificant (16.77%) increase in smoking. Accordingly, the IE of supportive parenting during early adolescence on change in young adult smoking was significant only among youth with lower *TNF*m, IE = –0.039; 95% confidence interval [–0.084, –0.008]. To assess whether smoking at Wave 8 (age 19) or Wave 9 (age 20) might predict *TNF*m or CRP, potentially suggesting a role for smoking in predicting inflammatory potential, rather than the reverse, we examined a series of simple correlations. As can be seen in Table 4, there is

Table 3. *Negative binomial regression models depicting the joint effect of perceived stress and TNFm on cigarette consumption at age 20*

	Cigarette Consumption			
	Model 1		Model 2	
	b	IRR	*b*	IRR
Perceived stress (age 19)	0.387** (0.137)	1.473	0.369** (0.137)	1.447
*TNF*m			−0.331 (0.221)	0.718
Perceived Stress (age 19) × *TNF*m			−0.214* (0.103)	0.807
Supportive parenting (ages 11–13)	−0.076 (0.113)	0.926	−0.087 (0.109)	0.917
Sex (1 = males)	1.928** (0.258)	6.877	1.925** (0.255)	6.854
SES risk (ages 11–13)	0.193 (0.129)	1.213	0.180 (0.118)	1.197
Cigarette consumption (ages 11–14)	0.141 (0.175)	1.152	0.157 (0.190)	1.171
Factor 1 cell type	0.173 (0.111)	1.189	0.393* (0.167)	1.481
Factor 2 cell type	−0.078 (0.110)	0.925	0.131 (0.164)	1.140
Factor 3 cell type	0.089 (0.093)	1.093	0.029 (0.093)	1.030
Factor 4 cell type	0.061 (0.097)	1.063	0.020 (0.107)	1.021
Log of CRP	0.192† (0.106)	1.211	0.168 (0.107)	1.182
Constant	−2.006** (0.227)		−2.049** (0.222)	
−2LL	614.638		609.992	
$\Delta\chi^2$ ($df = 1$)			4.646*	

Note: $N = 382$. Unstandardized (*b*) coefficients (robust standard errors) are provided; supportive parenting (ages 11–13), SES risk (ages 11–13), cigarette consumption (ages 11–14), factor cell type, and CRP are standardized by *z* transformation (mean = 0, *SD* = 1). Using Karlson–Holm–Breen methods (Breen, Karlson, & Holm, 2013), the test of the indirect effect of supportive parenting (ages 11–13) on cigarette consumption (age 20) through perceived stress (age 19) is significant, indirect effect = –0.071, 95% confidence interval [–0.132, −0.010]. *TNF*m, tumor necrosis factor gene methylation; IRR, incident rate ratio; SES, socioeconomic status; CRP, C-reactive protein; –2LL, –2 log likelihood.
†$p \leq .10$. *$p \leq .05$. **$p \leq .01$. Two-tailed tests.

no evidence of a significant association of smoking at age 19 or 20 with either *TNF*m or log (CRP). Only the association of body mass index with CRP was significant, as would be expected. Finally, to examine robustness with regard to concurrent use of alcohol and marijuana, we also conducted a supplemental analysis including concurrent alcohol and marijuana use as additional control variables in the negative binomial regression models examining the joint effect of perceived stress and *TNF*m on cigarette consumption, and found that including them did not change the observed pattern (see supplementary Table S.5).

Discussion

The results supported two pathways by which supportive parenting in early adolescence may have an impact on smoking in young adulthood. As indicted in the theoretical model presented in Figure 1, there was an indirect effect from early adolescent supportive parenting (ages 11–13) to young adult smoking (age 20) via associations with young adult stress (age 19). In addition, supportive parenting during early adolescence was associated with *TNF*m, indicating that supportive parenting provides protection that gets "under the skin," or prevents other stressors from doing so, and works against proinflammatory propensities. As predicted, those with the less protective epitype experienced an amplified effect of young adult stress on smoking by age 20. As a consequence, among African American youth growing up in economically disadvantaged circumstances, even after controlling for sex, circulating inflammatory cytokines, early SES related risk, and variation due to individual differences in cell types comprising the blood samples, more supportive parenting in early adolescence was associated with decreased smoking in young adulthood.

The results are consistent with theorizing by Nusslock and Miller (2015) and others (Cole et al., 2011; Gluckman et al., 2005) that experiences in childhood or early adolescence may contribute to biological vulnerabilities, particularly inflammation-related vulnerabilities, that can be maintained into adulthood and can be consequential for young adult health behavior. The current results indicate that supportive parenting may be protective against a proinflammatory epitype that amplifies the effect of later young adult stress, highlighting supportive parenting as a potential early target of intervention to prevent rapid escalation in smoking behavior among rural African American youth as they enter young adulthood. Although stress effects on smoking are clearly evident in the current results, they suggest that the impact of stress encountered in the transition into adulthood may be influenced by earlier, modifiable family factors that can confer some protection. In the current sample the impact of stress was moderated by level of *TNF*m. There was no significant increase in smoking in response to young adult stress among those with higher *TNF*m, and this protection was associated with reports of greater parental supportiveness provided by both youth and their primary caregiver in early adolescence. By looking at the association of stress with smoking across levels of parenting support and *TNF*m simultaneously, it can be seen that parenting support had no effect for those experiencing low stress but had a substantial effect for those experiencing higher stress, and the impact of high stress on smoking was particularly noticeable among those who also had low methylation of TNF (see online-only supplementary Figure S.1).

It has been shown previously that longer term patterns of smoking trigger an inflammatory response that is maintained over time (see Shaykhiev et al., 2009; Willemse, Postma, Timens, & ten Hacken, 2004). In particular, among adoles-

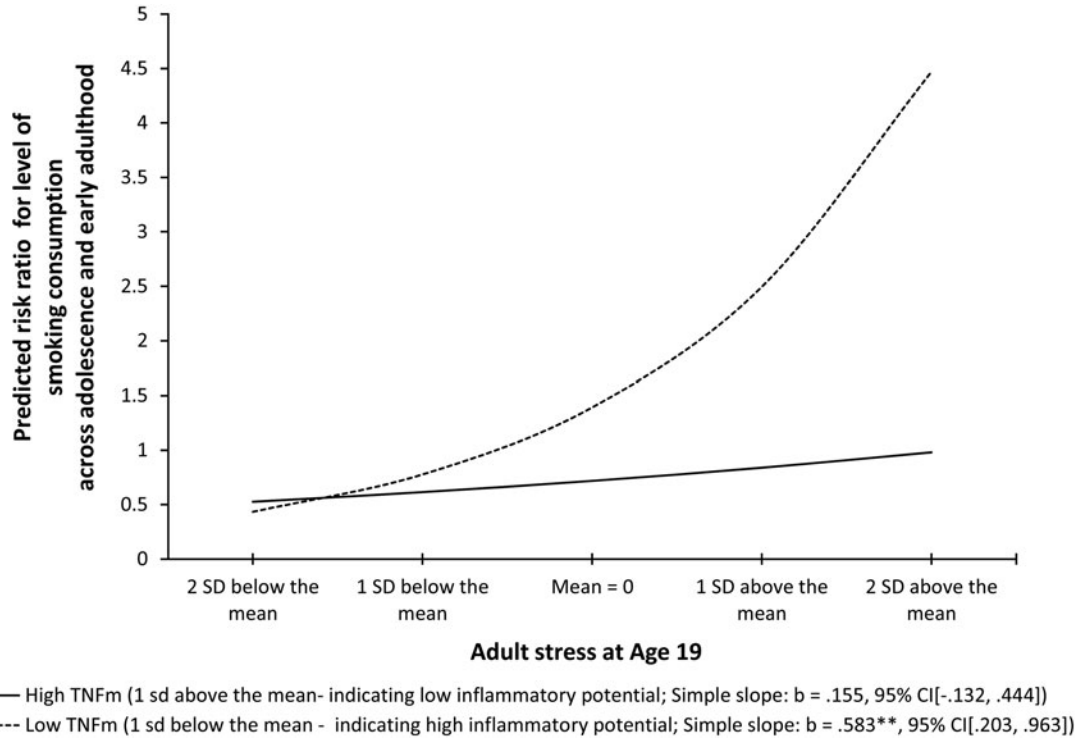

Figure 3. Explication of interaction between tumor necrosis factor gene methylation (*TNF*m) and stress in the prediction of change in smoking using a negative binomial regression model, controlling for sex, socioeconomic status risk (ages 11–13), cell type, and early cigarette consumption (ages 11–14). The lines represent the regression lines for different levels of *TNF*m (low = 1 *SD* below the mean, high = 1 *SD* above the mean). Simple slopes and confidence intervals are provided in parentheses.

cents with heavier past-month smoking, an association between smoking status and elevated CRP has been observed (O'Loughlin et al., 2008). Consequently, it was important to see if *TNF*m could be construed as a result of current smoking or if other inflammatory markers such as CRP might better capture observed associations. We examined smoking at age 19 and 20, and found that it was not associated with either *TNF*m or CRP in the current sample, suggesting that our observations occurred sufficiently early in the development of smoking patterns to avoid an effect of smoking on indicators of systemic inflammation.

There are several potential implications of the current results for the development of preventive interventions designed to reduce health problems among young adult African Americans. In addition to interventions to reduce young adult stress, it appears that interventions focused on increasing supportive parenting in early adolescence, before the onset of normative experimentation with smoking, may have the potential to modify epigenetic vulnerability and reduce vulnerability for later smoking in response to stress. Theoretically, we might also expect an epitype associated with greater inflammatory potential to be associated with health outcomes

Table 4. *Smoking is not predictive of either inflammatory potential (TNFm) or circulating inflammatory markers (CRP) at age 20*

	1	2	3	4	5
1. Log of CRP (age 20)	—				
2. BMI (age 20)	.519**	—			
3. *TNF*m (age 20)	−.064	−.088†	—		
4. Cigarette consumption (age 19)	−.017	−.031	−.067	—	
5. Cigarette consumption (age 20)	−.007	−.099†	−.045	.572**	—
Mean	−1.131	28.527	0.007	0.414	0.48
SD	2.464	8.268	0.947	1.010	1.088

*Note: TNF*m, tumor necrosis factor gene methylation; CRP, C-reactive protein; BMI, body mass index.
†$p \leq .10$. *$p \leq .05$. **$p \leq .01$.

at older ages. Accordingly, intervention to increase supportive parenting in early adolescence would appear to have substantial beneficial potential on later adult health. However, because the epigenetic vulnerability we examined appears to be correlated with parenting at ages 11–13, the earliest assessment for the current sample, it is not possible to discern with certainty at what age a focus on enhancing supportive parenting would have its maximum effect on *TNF*m. That is, it is possible that the association between epitype and parenting was established at an earlier age than those we assessed, suggesting that investigation of samples at earlier ages is warranted. Some theorists would suggest that parenting potentially influences the development of inflammation-related patterns of differential methylation beginning much earlier in childhood (cf. Miller & Chen, 2010; Miller et al., 2011). Accordingly, further research is needed to examine whether epigenetic vulnerabilities can be modified later in adolescence or in young adulthood.

Because youth can also exert influence on parenting behavior (e.g., Kiff, Lengua, & Zalewski, 2011), it is possible that, in addition to the nonspecific influence of environmental stressors, some of the observed impact of early adolescent supportive parenting on young adult outcomes may reflect early adolescent temperament and other behavioral characteristics that were not directly measured in the current study. Likewise, the current research depends on a single wave of assessment of methylation, reducing confidence in causal conclusions. It is to be hoped that future research, incorporating multiple assessments sufficiently powered to examine change in both differential methylation and parenting relationships, will further clarify the time course of differential methylation, the direction of effects, and perhaps identify the age at which maximum effects of suppportive parenting on differential methylation are obtained.

It is important to note that there are several indications that the *TNF*m proinflammatory epitype was not merely a response to cigarette smoking. First, it is a precursor variable rather than an inflammatory agent itself, making it less probable that it would vary as part of an inflammatory reaction to smoking. Second, there are significant correlations between *TNF*m and theoretically plausible precursors (i.e., early adolescent parenting) but no correlations with smoking at ages 19 or 20 (i.e., the ages that should have shown an effect if smoking was a driver of *TNF*m). Third, as was shown in Figure 2, increases in cigarette smoking began after age 14, later than the age for which parenting variables are predictive of *TNF*m. Thus, it seems unlikely that observed effects are attributable to an effect of smoking on *TNF*m. Nonetheless, direct measurement of *TNF*m at multiple ages, including assessment prior to the onset of smoking, would help solidify conclusions about causal direction.

Contrary to expectations, we did not find an effect of SES risk on *TNF*m. Although SES risk was associated significantly with suppportive parenting and early smoking, it was not associated with *TNF*m. This may be due in part to selection of a sample with relatively low variability in SES, with all participants experiencing a challenging economic context. In addition, results suggest a need to examine other proinflammatory changes that may be predicted by SES risk to see how they may differ from or interact with *TNF*m. Alternatively, it may be useful to examine broader networks of proinflammatory changes to better capture the impact of a range of childhood adversities and examine the role of supportive parenting in relation to each of them. To better characterize significantly enriched pathways using controls for multiple comparisons, we did an exporatory genome-wide examination of CpG sites, also on first exons, that were significantly associated with *TNF*m using GoMiner™. We used "all gene ontology" as the root category setting, and used the 105 genes reflecting the 128 CpG sites associated at a genome-wide significance level (i.e., $p < 10e\text{-}7$) as the "changed" gene set (Zeeberg et al., 2003). The top 10 pathways are reported online in supplementary Table S.6. *TNF*m was robustly associated with gene pathways linked to immune functioning and cell-type activation and signalling among others.

The limitations of the current investigation notwithstanding, the current research provides an initial step in explicating the complex and important ways in which early adversity and early family environment may become biologically embedded and set the stage for, or protect against, later health problems. As illustrated in the current investigation, biologically embedded consequences of early experience, reflected in differential methylation, may be consequential for later behavioral response to stress, helping explain the increased vulnerability of some youth to rapid escalation in smoking in young adulthood. In addition, the influence of early supportive parenting on the development of latent vulnerability to smoking in young adulthood has particular relevance for prevention programs because parenting practices are potentially modifiable (Brody, Chen, Kogan, et al., 2012; Brody et al., 2014; Chen et al., 2011), and they appear to provide promising potential points of intervention upon which smoking prevention programs can expand. As a consequence, the current research contributes to much needed translational research efforts that identify new potential points of preventive intervention for smoking.

Supplementary Material

To view the supplementary material for this article, please visit https://doi.org/10.1017/S0954579416000961.

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