Short Communication

Genomic DNA Methylation among Women in a Multiethnic New York City Birth Cohort

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Abstract

One plausible mechanism for the environment to alter cancer susceptibility is through DNA methylation. Alterations in DNA methylation can lead to genomic instability and altered gene transcription. Genomic DNA methylation levels have been inversely associated with age, suggesting that factors throughout life may be associated with declines in DNA methylation. Using information from a multiethnic New York City birth cohort (born between 1959 and 1963), we examined whether genomic DNA methylation, measured in peripheral blood mononuclear cells, was associated with smoking exposure and other epidemiologic risk factors across the life course. Information on prenatal and childhood exposures was collected prospectively through 1971, and information on adult exposures and blood specimens were collected in adulthood from 2001 to 2007. Methylation levels of leukocyte DNA were determined using a [^{3}H]-methyl acceptance assay where higher values of disintegrations per minute per microgram DNA indicate less DNA methylation. Genomic methylation of leukocyte DNA differed by ethnicity (66% of Blacks, 48% of Whites, and 29% of Hispanics were above the median level of disintegrations per minute per microgram DNA; $P = 0.03$). In multivariable modeling, DNA methylation was statistically significantly associated with maternal smoking during pregnancy, longer birth length, later age at menarche, nulliparity, and later age at first birth. These data, if replicated in larger samples, suggest that risk factors across the life course may be associated with DNA methylation in adulthood. Larger studies and studies that measure within-individual changes in DNA methylation over time are a necessary next step. (Cancer Epidemiol Biomarkers Prev 2008;17(9):2306–10)

Introduction

One plausible mechanism for the environment to alter cancer susceptibility is through epigenetic effects on somatic cells, leading to activation or silencing of key genes in critical pathways. DNA methylation, one type of epigenetic change, may play an important role in causing cancer by silencing tumor suppressor genes through hypermethylation or activating oncogenes through hypomethylation (1, 2). In addition to gene-specific DNA methylation, global or genome-wide aberrant DNA methylation (hypomethylation) in regions that are normally methylated such as repeats or transposable elements can lead to genomic instability and altered gene transcription, impacting normal growth and development in target tissues (3, 4). Lower levels of DNA methylation or genomic DNA hypomethylation have been seen in a number of cancer types, including breast, colorectal, gastric and prostate cancers, bladder, head and neck cancers, and blood cancers such as chronic lymphocytic leukemia (5-9). There is increasing evidence that DNA methylation patterns and histone packaging are altered not only within tumors but also in precursor lesions in some cases or systemically (10-13). Genomic DNA hypomethylation measured in blood samples may therefore be a potential biomarker; such measures have already been associated with risk for bladder, head, and neck cancers (6, 9).

Larger differences in DNA methylation among older compared with younger identical twins suggest that both endogenous and exogenous factors might influence epigenetic changes throughout life (14). Few epidemiologic studies, however, have examined the association between early life exposures and adult levels of DNA methylation, although adult risk factors such as alcohol and tobacco have been associated with DNA methylation levels in tumor tissues (15). In this study, we were interested in whether exposure to tobacco smoke and other risk factors across the life course was associated with adult levels of DNA methylation measured in the peripheral blood mononuclear cells of women born between 1959 and 1963 in a multiethnic New York City birth cohort.

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Materials and Methods

Study Participants. We used epidemiologic data and blood samples collected from the adult follow-up study of the New York site of the National Collaborative Perinatal Project. The National Collaborative Perinatal Project started in 1959 at 12 institutions throughout the United States (including Columbia Presbyterian Medical Center in New York City) and collected detailed prospective data from pregnancy to childbirth and from childhood to age 7 years (16). In 2001, we started the adult follow-up of all girls from the New York site of the National Collaborative Perinatal Project who were followed to age 7 years (n = 841). We were able to successfully trace 44.5% of the eligible cohort of 841 (n = 374), and 70.1% of the women traced (n = 262) completed the questionnaire part of the study.

Early Life Data. At enrollment, the mothers were asked to provide information on age, height, parity, smoking, race, and prepregnancy weight. Birth weight was obtained within 1 hour of delivery by the National Collaborative Perinatal Project observer of labor and delivery using calibrated scales, and birth length was obtained using a standardized procedure within 24 hours of birth and measured crown to heel. In addition to the physical measurements, socioeconomic status was determined from data on maternal and paternal education, occupation, and income at birth and at 7 years old.

Adult Data. Women self-reported adult exposures using a mailed questionnaire that included information on self-reported race, body weight, height, age at menarche, parity, smoking history, alcohol intake, and report of exposure to family members' household smoking.

Blood Collection. Daughters who completed the questionnaire were asked to provide us with access to their existing mammographic films and to provide a small blood sample. Of the 262 daughters, 92 (35%) completed the blood portion of the study. Blood collection was primarily completed by mail by sending blood kits to all daughters who consented to give blood and having them get their blood drawn by their physician or local laboratory (n = 62). Blood samples were collected for the remaining 30 daughters by study phlebotomists at Columbia (n = 19) and through home visits (n = 11). Participants provided five 10-mL vacutainers of blood. Processing, storage, and DNA isolation were done at the Herbert Irving Comprehensive Cancer Center Biomarkers Core Facility at Columbia University. Mononuclear cells were collected by centrifugation over Ficoll. Daughters who completed the blood portion of the study were similar to those who did not on all demographic variables (age, race, socioeconomic status) and maternal [age, prepregnancy body mass index (BMI), pregnancy weight gain, pregnancy smoking], infant and childhood anthropometry, and adult factors (parity, alcohol intake, smoking, and oral contraceptive use; data not shown).

Laboratory Analyses. Eighty-five samples had DNA of sufficient quality to measure genomic hypomethylation, which we did by using the [^{3}H]-methyl acceptance assay as described by Balaghi and Wagner (17) and Pilsner et al. (18). The DNA was incubated with [^{3}H] *S*-adenosylmethionine in the presence of the *Sss*I prokaryotic methylase enzyme, which indiscriminately methylates all unmethylated CpG sequences. Therefore, the ability of DNA to incorporate [^{3}H] methyl groups *in vitro* is inversely related to endogenous DNA methylation. Briefly, 200 ng of DNA was incubated with 3 U of *Sss*I methylase (New England Biolabs); 3.8 μmol/L (1.1 μCi) ^{3}H-labeled *S*-adenosylmethionine (Perkin-Elmer); and EDTA, DTT, and Tris-HCL (pH 8.2) in a 30-μL mixture and incubated for 1 hour at 37°C. The reaction was terminated on ice and 15 μL of the reaction mixture applied onto Whatman DE81 filter paper. The filter was washed on a vacuum filtration apparatus three times with 5 mL of 0.5 mol/L sodium phosphate buffer (pH 8.0), followed by 2 mL each of 70% and 100% ethanol. Dried filters were each placed in a vial with 5 mL of scintillation fluid (Scintisafe, Fisher) and analyzed by a Packard Tri-Carb 2100TR liquid scintillation analyzer. Each DNA sample was processed in duplicate, and each processing run included samples for background (reaction mixture with all components except *Sss*I enzyme), a hypomethylation control (HeLa cell DNA), and a quality control sample (DNA extracted from a whole-blood sample) to determine the intra-assay CV. Intra- and interassay coefficients of variation (CVs) were 2.0 and 3.9, respectively. To quantify the amount of double-stranded DNA in each reaction, an aliquot of the assayed DNA was used to determine DNA concentrations using PicoGreen double-stranded DNA quantitation reagent (Molecular Probes). All disintegrations per minute values were expressed per microgram DNA as quantified by PicoGreen. All laboratory analyses were conducted blinded to epidemiologic data.

Statistical Analyses. We first compared differences in DNA methylation by race using both the race reported in the birth records and self-reported race reported in adulthood by questionnaire. We used univariable linear regression to examine associations between life course variables and DNA methylation. We transformed the outcome variable (disintegrations per minute per microgram) by taking the natural logarithm. We first focused on race, prenatal maternal smoke exposure, and adult smoking exposure to examine in multivariable models. We then included any variable that confounded any of these three variables by more than 10%. Adult BMI, parental smoking exposure in childhood, birth length,

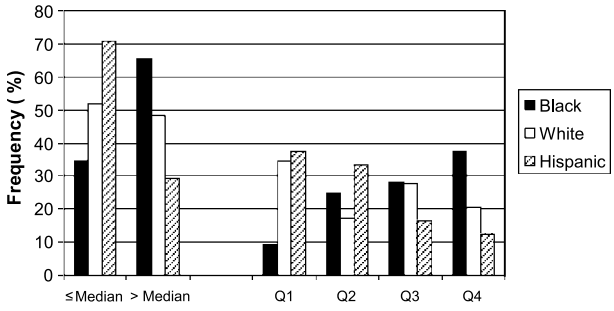

Figure 1. DNA methylation (disintegrations per minute per microgram) by race, New York Women's Birth Cohort (higher values indicate less DNA methylation).

Table 1. Unadjusted differences and 95% CIs for the association between DNA methylation by prenatal, early life, adolescent, and adult variables (higher values indicate less DNA methylation)

	Mean (SD)	Range, minimum, maximum	DPM/μg ≤ 78,670 (n = 43)	DPM/μg > 78,670 (n = 42)	log DPM/μg β (95% CI)
Prenatal					
Prenatal smoke exposure (cigarettes smoked/d)					
Unexposed	122,158 (114,004)	(45,872, 660,800)	26	29	
Exposed	96,480 (88,765)	(40,032, 536,595)	16	13	−0.17 (−0.41, 0.08)
Race					
White	103,917 (81,256)	(40,032, 407,086)	15	14	
Black	131,220 (131,296)	(64,985, 660,800)	11	21	0.17 (−0.11, 0.44)
Hispanic	99,144 (93,132)	(45,872, 466,774)	17	7	−0.08 (−0.37, 0.21)
Family socioeconomic status					−0.005 (−0.01, 0.00)
Early life					
Birth weight (kg)					
≤3.12	109,842 (89,544)	(45,872, 536,595)	20	23	−0.04 (−0.27, 0.18)
>3.12	115,926 (120,854)	(40,032, 660,800)	23	19	
Birth length (cm)					
≤50	125,793 (111,726)	(45,872, 536,595)	21	24	−0.05 (−0.10, −0.002)
>50	98,286 (97,549)	(40,032, 660,800)	22	18	
Adolescent					
Age at menarche (y)					
<13	128,563 (126,851)	(40,032, 660,800)	18	22	
≥13	100,348 (82,819)	(48,082, 536,595)	23	20	−0.14 (−0.38, 0.10)
Childhood passive smoke exposure					
Unexposed	124,461 (140,825)	(48,082, 660,800)	10	9	
Exposed	109,506 (94,145)	(40,032, 536,595)	33	33	−0.04 (−0.32, 0.24)
Adult					
Age at interview (y)					
≤42.28	105,210 (107,781)	(40,032, 660,800)	25	17	
>42.28	120,310 (104,111)	(48,082, 536,595)	18	25	0.03 (−0.03, 0.09)
Current BMI (kg/m^2)					
<25	116,683 (104,058)	(45,872, 536,595)	16	21	
≥25	111,550 (112,165)	(40,032, 660,800)	25	19	−0.05 (−0.29, 0.19)
Smoking status					
Never	111,913 (96,772)	(45,872, 536,595)	20	22	
Former	106,470 (94,724)	(40,032, 466,774)	14	12	−0.06 (−0.33, 0.21)
Current	124,914 (142,420)	(59,672, 660,800)	9	8	0.05 (−0.26, 0.36)
Parity					
Parous	121,295 (119,662)	(45,872, 660,800)	29	29	
Nulliparous	93,660 (65,240)	(40,032, 316,790)	14	12	−0.23 (−0.49, 0.02)
Age at first birth	—	—	—	—	−0.02 (−0.04, −0.004)

Abbreviation: DPM, disintegrations per minute.

birth weight, and family socioeconomic status confounded at least one of these variables by >10% and were included in the multivariable model 1. We then added any additional variables that were associated with the outcome but did not operate as confounders, which included nulliparity and age at first birth (model 2). Age at first birth was modeled by centering about the mean age at first birth and including an indicator variable for nulliparity.

Results

Figure 1 shows that DNA methylation by overall quartiles of the distribution differed by race or ethnicity recorded in the birth record (38% of Blacks, 21% of Whites, and 13% of Hispanics were in the highest quartile of disintegrations per minute per microgram DNA, indicating less DNA methylation, compared with 9% of Blacks, 34% of Whites, and 38% of Hispanics in the lowest quartile; P = 0.08). These racial differences are further highlighted in Fig. 1, with DNA methylation levels cut at the overall median (66% of Blacks, 48% of Whites, and 29% of Hispanics are above the median disintegrations per minute per microgram DNA compared with 24% of Blacks, 52% of Whites, and 71% of Hispanics below the median; P = 0.03).

The univariable associations between DNA methylation (log disintegrations per minute per microgram DNA) and life course exposure variables are reported in Table 1. Compared with Whites, Blacks had higher log disintegrations per minute per microgram DNA [β, 0.17; 95% confidence interval (95% CI), −0.11, 0.44], although this result was not statistically significant when DNA methylation was considered as a continuous variable. Current smoking was positively associated, and prenatal smoke exposure was negatively associated with log disintegrations per minute per microgram DNA, although these associations were not statistically significant. Birth length was associated with lower log disintegrations per minute per microgram DNA, indicating that longer babies had higher levels of DNA methylation (β, −0.05 per cm; 95% CI, −0.10, −0.002). Later age at first birth was associated with lower disintegrations per minute per microgram DNA, indicating that older mothers had higher levels of DNA methylation (β, −0.02 per year; 95% CI, −0.04, −0.004).

Table 2 reports the multivariable linear models (models 1 and 2) of life course variables and log disintegrations per minute per microgram DNA. Associations between race and DNA methylation did not decrease after further adjusting for life course variables, suggesting that these selected variables did not explain the racial patterns in DNA methylation. Prenatal tobacco smoke exposure (β, −0.34; 95% CI, −0.65, −0.02) and increasing birth length (β, −0.11; 95% CI, −0.19, −0.02 per centimeter of length) were both associated with lower log disintegrations per minute per microgram DNA. In contrast, birth weight was positively, but not statistically significantly, associated with log disintegrations per minute per microgram DNA (for comparison, the last column of Table 2 reports standardized effect estimates). Both childhood smoke exposure and current smoke exposure were associated, but not significantly, with higher log disintegrations per minute per microgram DNA. These associations changed little when further adjusting for other variables that were statistically significantly associated with log disintegrations per minute per microgram DNA: later age at menarche (β, −0.07; 95% CI, −0.13, −0.004), nulliparity (β, −0.30; 95% CI, −0.57, −0.02), and age at first birth (β, −0.03; 95% CI, −0.06, −0.01). These results did not change materially when adjusting for socioeconomic status at age 7 years instead of socioeconomic status at birth. They also did not change when using self-reported adult race instead of race from the birth record.

Discussion

Among women drawn from a birth cohort followed until midlife, we observed differences in DNA methylation by race, with Blacks more likely to have lower levels of DNA methylation than Whites or Hispanics, although these findings were only statistically significant when comparing categories of DNA methylation based on the median. Lower levels of DNA methylation have also been observed in lung tumor samples of different histologic subtypes in Blacks compared with Whites (19). Adult lifestyle factors such as smoking and alcohol consumption were reported to be associated with lower levels of genomic DNA methylation in individuals with head and neck tumors (15). In our study, we were able to examine the association between passive and active smoke exposure across the life course. Both passive smoke exposure in childhood (measured by presence of household smoker) and current smoking were associated with lower levels of DNA methylation compared with nonsmokers; however, these associations were modest and nonsignificant. In contrast, exposure to prenatal smoke was statistically significantly associated with higher levels of DNA methylation. In multivariable linear models, increasing birth length, later age at menarche, nulliparity, and later age at first birth were also associated with higher DNA methylation levels in adulthood. These factors have not been assessed previously in studies of genomic DNA methylation, and larger studies are needed to determine if the associations can be replicated.

Given the geographic diversity of the adult members of our cohort, we relied on blood collection by mail rather than in-person, which lowered response rates. Responders and nonresponders did not differ on any of the epidemiologic variables we assessed in this study. However, given the low overall response rates for the blood collection, it is possible that the DNA methylation and risk factor associations for our subset represent biased estimates of the entire population. All prenatal and early life data were collected prospectively, and all laboratory assays were completed blinded to exposure data. Other data, including BMI, adult smoking, and childhood smoke exposure, were based on self-report. The reliability of the assay was extremely high, and the [^{3}H]-methyl acceptance assay has been used in a number of intervention studies (20–22). Reported limitations of the methyl acceptor assay include the relative instability of *S*-adenosylmethionine and *Sss*I methyltransferase

Table 2. Multivariable linear regression of DNA methylation (log DPM/μg) by prenatal, early life, adolescent, and adult variables

	Linear multivariate model 1	Linear multivariate model 2	Linear multivariate model 2
	β (95% CI)	β (95% CI)	Standardized β
Prenatal			
Prenatal smoke exposure	−0.34 (−0.65, −0.02)	−0.38 (−0.70, −0.07)	−0.32
Race			
Black-White	0.20 (−0.14, 0.54)	0.17 (−0.17, 0.51)	0.15
Hispanic-White	−0.14 (−0.45, 0.17)	−0.15 (−0.45, 0.16)	−0.12
Family socioeconomic status	−0.01 (−0.01, 0.001)	−0.01 (−0.01, 0.002)	−0.18
Early life			
Birth weight (kg)	0.33 (−0.06, 0.72)	0.28 (−0.11, 0.67)	0.26
Birth length (cm)	−0.11 (−0.19, −0.02)	−0.11 (−0.19, −0.03)	−0.46
Adolescent			
Age at menarche (y)		−0.07 (−0.13, −0.004)	−0.23
Childhood passive smoke exposure	0.15 (−0.19, 0.48)	0.25 (−0.08, 0.58)	0.19
Adult			
Current BMI (≥25:<25 kg/m^2)	−0.19 (−0.46, 0.07)	−0.21 (−0.48, 0.05)	−0.19
Smoking status			
Former-never	−0.02 (−0.30, 0.27)	−0.17 (−0.46, 0.12)	−0.14
Current-never	0.15 (−0.20, 0.50)	0.17 (−0.17, 0.52)	0.12
Parity (nulliparous-parous)		−0.30 (−0.57, −0.02)	−0.25
Age at first birth		−0.03 (−0.06, −0.01)	−0.35

(23, 24); however, in this study, we used single batches of fresh [^{3}H] *S*-adenosylmethionine and enzyme. These measurement issues in exposures and outcome, however, would likely result in nondifferential misclassification toward the null, and thus, the true associations are likely to be even larger. Overall, we observed that selected exposures throughout the life course were correlated with DNA methylation levels. Replicating these associations in larger samples and examining within-individual differences in DNA methylation over time are necessary next steps.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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