

Associations of long interspersed nuclear element-1 DNA methylation with preterm birth in a prospective cohort study

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Preterm birth affects over 12% of all infants born in the United States; yet the biology of early delivery remains unclear, including whether epigenetic mechanisms are involved. We examined associations of maternal and umbilical cord blood long interspersed nuclear element-1 (LINE-1) DNA methylation with length of gestation and odds of preterm birth in singleton pregnancies in Project Viva. In white blood cells from maternal blood during first trimester ($n = 914$) and second trimester ($n = 922$), and from venous cord blood at delivery ($n = 557$), we measured LINE-1 by pyrosequencing [expressed as %5 methyl cytosines within the LINE-1 region analyzed (%5mC)]. We ran linear regression models to analyze differences in gestation length, and logistic models for odds of preterm birth (<37 *v.* ≥ 37 weeks' gestation), across quartiles of LINE-1. Mean (s.d.) LINE-1 levels were 84.3 (0.6), 84.5 (0.4) and 84.6 (0.7) %5mC for first trimester, second trimester and cord blood, respectively. Mean (s.d.) gestational age was 39.5 (1.8) weeks, and 6.5% of infants were born preterm. After adjustment for maternal age, race/ethnicity, body mass index, education, smoking status and fetal sex, women with the highest *v.* lowest quartile of first trimester LINE-1 had longer gestations [0.45 weeks (95% CI 0.12, 0.78)] and lower odds of preterm birth [OR 0.40 (0.17, 0.94)], whereas associations with cord blood LINE-1 were in the opposite direction (−0.45 weeks, −0.83, −0.08) and [OR 4.55 (1.18, 17.5)]. In conclusion, higher early pregnancy LINE-1 predicts lower risk of preterm birth. In contrast, preterm birth is associated with lower LINE-1 in cord blood.

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Introduction

Despite much research and healthcare effort, preterm birth remains a major public health problem. In the United States over 12% of infants are born preterm (<37 weeks' gestation).¹ Preterm birth contributes to over a third of all US infant mortality cases,² and is linked to major long-term morbidities.^{3,4} Although social determinants such as poverty,⁵ African-American race/ethnicity⁶ and biological risk factors such as genital infections^{7–9} and tobacco exposure¹⁰ affect a woman's risk of delivering preterm, the mechanisms connecting risk factors to preterm birth remain unclear.¹¹

Gene–environment interactions may account for much of the variation in risk of delivering preterm and may work through epigenetic phenomena.¹² Epigenetics refers to heritable differences in gene expression in the absence of genetic sequence variation.¹³ DNA methylation of cytosine-guanine (CpG) dinucleotides represents one of several known epigenetic mechanisms. Typically, in eukaryotic cells, methylation of CpG sites within promoter regions of genes silences gene expression.¹⁴ One option of interrogating DNA methylation involves analyzing particular genes. Another approach is to analyze DNA repetitive sequences,^{13,15,16} as they comprise more than half of the human genome including over 500,000 heavily methylated long interspersed nuclear elements-1 (LINE-1).¹⁷ Typically LINE-1 is heavily methylated, but in states of cellular stress, repetitive elements can be hypomethylated.^{18,19}

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Although no human cohort studies report associations between epigenetic marks and the length of gestation or preterm birth, recent human studies raise the possibility that epigenetics may underlie differences in risk of preterm birth. Although DNA methylation of organ tissues specifically involved in preterm delivery, including the placenta and fetal tissues, may provide more information, the DNA methylation of circulating white blood cells has been associated with other non-hematologic disease processes.^{20,21} Blood DNA hypomethylation of LINE-1 has been associated with cardiovascular disease,²¹ and with risk factors for both cardiovascular disease and preterm birth^{22,23} including smoking²⁴ and folate deficiency.^{23,25} Cardiovascular disease and preterm delivery may share pathophysiologic mechanisms as women who have had a prior preterm delivery have been shown to have higher odds of developing cardiovascular disease.²⁶ Both of these states have been associated with inflammation, raising the question as to whether LINE-1 hypomethylation represents an aggregate measure of inflammation over time that could be associated with an increased risk of preterm birth.

Other epidemiologic observations also suggest a role for epigenetic disruptions and an increased risk of preterm birth. Large meta-analyses have shown that pregnancies conceived via *in vitro* fertilization (IVF) have higher odds of preterm birth compared with non-IVF conceived pregnancies.^{27–29} During the process of IVF, manipulation of the cells occurs at a time when DNA is demethylated and remethylated,³⁰ which may be a window of particular epigenetic susceptibility, supported by studies linking higher risk of imprinting disorders and IVF.³¹ Whether epigenetic alterations caused by IVF lead to an increased risk of preterm birth or whether epigenetic factors contribute both to infertility and subsequent preterm birth remains unknown. However, the association between IVF and preterm birth suggests a potential role for epigenetic contributions to preterm birth.

The aim of this study was to examine the extent to which LINE-1 methylation in maternal peripheral blood during pregnancy, and in umbilical cord blood, is associated with length of gestation and risk of preterm birth. Because lower LINE-1 methylation has been associated with many states of poor health,^{20,21,32,33} we hypothesized that higher LINE-1 methylation during pregnancy would be associated with longer gestations and lower odds of preterm delivery.

Methods

Study subjects

We studied participants enrolled in Project Viva, a prebirth cohort study of mother–infant pairs recruited from obstetric offices of Harvard Vanguard Medical Associates, a multi-specialty group practice in eastern Massachusetts.³⁴ Eligibility to participate in the study included English fluency, singleton pregnancy and gestational age less than 22 weeks at the time of enrollment. Details of recruitment and retention procedures

are published elsewhere.^{34,35} Of the 2128 mothers with live births enrolled in the study, we analyzed data from the subset of 1160 participants with maternal blood DNA from the first trimester ($n = 914$), the second trimester ($n = 922$) and/or umbilical cord blood DNA at delivery ($n = 577$). Not all participants had samples available at each time point. However, there was substantial overlap; 729 women had both first and second trimester samples available and for the 557 infants with cord blood samples there were 428 maternal first trimester and 427 maternal second trimester samples. In comparison with the 1160 participants in this analysis, 968 excluded participants showed a higher proportion of maternal white race (74% *v.* 58%) and college or graduate education (70% *v.* 59%) and a slightly lower proportion of infants born <37 weeks' gestation (6% *v.* 8%). Mothers provided written, informed consent to participate in the study and to have their DNA analyzed. The institutional review boards of the participating institutions, including Harvard Pilgrim Health Care, Brigham and Women's Hospital and Beth Israel Deaconess Medical Center, approved the study.

Gestational age

We calculated gestational age in weeks by subtracting the date of the last menstrual period (LMP) from the date of delivery. Eighty-six percent of the mothers had ultrasound data available at 16–20 weeks. For 12% of subjects, ultrasound pregnancy dating estimates differed by more than 10 days from the LMP, and for them we used the dating obtained from the ultrasound to determine gestational age at birth. We categorized infants as preterm if the gestational age at birth was less than 37 completed weeks of gestation.

Covariate ascertainment

Using a combination of interviews, study questionnaires and medical record reviews, we collected information on maternal age, self-designated race/ethnicity, parity, smoking habits, education, marital status, household income and infant sex. We calculated prepregnancy body mass index (BMI, kg/m²) based on self-reported prepregnancy height and weight. We categorized deliveries as 'spontaneous' if labor preceded either a vaginal birth or a cesarean section, or as 'medically indicated' if cesarean or induced vaginal birth occurred in the absence of spontaneous labor.

LINE-1 DNA methylation analysis

We collected venous whole blood samples at the end of the first and second trimesters of pregnancy during in-person study visits and from the umbilical cord at delivery. We immediately refrigerated samples and transferred them to laboratory. There, they were spun and blood components were separated into aliquots for storage at -80°C . We extracted high-molecular-weight genomic DNA from the

buffy coat with commercially available PureGene Kits (Gentra Systems, Minneapolis, MN, USA) to prepare the samples for pyrosequencing.

As previously described,^{21,36,37} we quantified DNA methylation using bisulfite-PCR and pyrosequencing to analyze the methylation at four CpG sites repeated throughout the genome. We used primers designed toward a consensus LINE-1 sequence that repeats with no variation in the majority of the genomic LINE-1 repeated elements. To verify bisulfite conversion, we used non-CpG cytosines as built-in controls. We measured methylation as a percentage of 5-methyl cytosines (%5mC), within the regions as studied, in two replicates (runs) and combined measures from two runs and four sites as described below. As part of a pilot, we had previously analyzed a subset ($n = 48$) of the cord blood samples at just three CpG sites.

Statistical analysis

To estimate LINE-1 methylation, we fit a mixed effects model³⁸ to the direct LINE-1 measures at the four CpG sites allowing a different mean level at each site in each run, for eight modeled means all together. For example, site 1 in run 1 had a different modeled mean from site 2 in run 1 and from site 1 in run 2. This approach was necessary because assuming a common constant mean across runs and sites was untenable as between-run, within-site Pearson correlation coefficients were as low as 0.23 (median 0.39, maximum 0.73). Between-run correlations of mean LINE-1 were 0.6 for each of the three time points. We allowed a separate set of 6 additional means for the 48 subjects for whom we measured LINE-1 at only three sites. We used this approach because we observed differences between the samples from these 48 subjects and the other subjects. We fit a random intercept and a general covariance structure allowing different variances for each site and different correlations between sites within run, and different correlations between and within site across runs. We used the predicted random intercepts (the empirical Bayes' estimates) as the underlying LINE-1 methylation level for the analysis.

We examined LINE-1 as a continuous variable as well as in quartiles. For cord blood LINE-1 we used sex-specific quartiles because males had higher levels. We ran unadjusted and multivariable-adjusted linear regression models to analyze differences in gestation length, and logistic models for odds of preterm birth (<37 *v.* ≥ 37 weeks' gestation). We adjusted multivariable models for maternal age, race/ethnicity, pre-pregnancy BMI, education, smoking during pregnancy and fetal sex. To explore possible effect modification by sex on the relationship between cord blood LINE-1 and gestational age, we introduced infant sex interaction terms to multivariable regression models. We also ran stratified logistic multivariable regression models to determine whether the relationship between preterm and LINE-1 differed by spontaneous *v.* medically indicated deliveries. We performed all analyses using SAS version 9.2 (SAS Institute, Cary, NC, USA).

Results

Mean (s.d.) LINE-1 %5mC were 84.3 (0.6), 84.5 (0.7) and 84.6 (0.7) in first trimester, second trimester and cord blood, respectively. Correlations among the three time points were very low (Pearson $r = 0.02$ for first and second trimester LINE-1, 0.01 for second trimester and cord LINE-1, and -0.04 for first trimester and cord). Women who were white, multiparous or married/cohabitating had slightly higher first trimester LINE-1 than their counterparts (Supplementary Table S1). Second trimester LINE-1 did not vary by covariates. Cord blood LINE-1 was higher among male *v.* female infants (84.8 *v.* 84.4, $P < 0.001$). Mean (s.d.) gestational ages were 39.5 (1.7), 39.5 (1.7) and 39.7 (1.5) weeks among infants included in first trimester, second trimester and cord blood analyses, respectively. The percent of preterm infants (born <37 weeks' gestation) was 6.3, 6.6 and 4.9 in the first trimester, second trimester and cord LINE-1 analyses, respectively (Table 1).

Maternal blood LINE-1

First trimester LINE-1 was positively associated with gestational age in unadjusted and adjusted models. Analysis of LINE-1 as a continuous variable revealed that each %5mC increase in LINE-1 predicted 0.20 (95% CI 0.01, 0.39) weeks longer gestation. Covariate adjustment did not change this estimate. Analyzing LINE-1 in quartiles, we found that mothers with the highest *v.* the lowest quartile of first trimester LINE-1 predicted longer gestations by an average of 0.45 weeks (95% CI 0.12, 0.78; Fig. 1). They also had substantially lower odds of preterm birth (OR 0.40, 95% CI 0.17, 0.93; Table 3). Covariate adjustment did not materially alter this estimate. The odds ratios of preterm delivery associated with the lowest (*v.* highest) quartile of LINE-1 did not differ by spontaneous (0.41, 95% CI 0.12, 1.34) and medically indicated (0.35, 95% CI 0.10, 1.29) deliveries.

We did not detect any association between continuous second trimester LINE-1 and gestational age (-0.05 weeks, 95% CI -0.32 , 0.22), or quartiles of second trimester LINE-1 and gestational age (Table 2). Nor did we detect associations between second trimester LINE-1 %5mC and preterm birth (OR for highest *v.* lowest quartile 1.16, 95% CI 0.56, 2.40; Table 3).

Cord blood LINE-1

Cord blood LINE-1 was inversely associated with gestational age (Table 4). For each %5mC increase in cord blood LINE-1, gestational age was 0.19 weeks shorter (95% CI 0.01, 0.38) in unadjusted models and the results did not change appreciably with adjustment for covariates (0.18 weeks shorter, 95% CI -0.03 , 0.38). Infants with the highest quartile of cord blood LINE-1 were born 0.45 weeks (95% CI 0.08, 0.83) earlier than infants with the lowest quartile of LINE-1 (Table 2). Cord blood LINE-1 was inversely associated with

Table 1. Characteristics of Project Viva participants by blood sample in which LINE-1 was analyzed

	1st trimester blood, <i>n</i> = 914; <i>n</i> (%)	2nd trimester blood, <i>n</i> = 922; <i>n</i> (%)	Cord blood, <i>n</i> = 557; <i>n</i> (%)
Maternal age (years)			
15 to <25	72 (7.9)	57 (6.2)	52 (9.3)
25 to <30	180 (19.7)	189 (20.5)	114 (20.5)
30 to <35	370 (40.5)	390 (42.3)	231 (41.5)
35 to <45	292 (31.9)	286 (31.0)	160 (28.7)
Race/ethnicity			
White	683 (74.9)	703 (76.5)	407 (73.2)
Black	97 (10.6)	97 (10.6)	58 (10.4)
Hispanic	62 (6.8)	50 (5.4)	43 (7.7)
Other	70 (7.7)	69 (7.5)	48 (8.6)
Prepregnancy BMI (kg/m ²)			
<25	569 (62.5)	590 (64.2)	349 (62.9)
25 to <30	209 (22.9)	204 (22.2)	124 (22.3)
≥30	133 (14.6)	125 (13.6)	82 (14.8)
Education			
Less than college graduate	273 (29.9)	266 (28.9)	175 (31.5)
College graduate	639 (70.1)	653 (71.1)	381 (68.5)
Smoking during pregnancy			
Never	595 (67.0)	620 (69.0)	373 (69.5)
Former	192 (21.6)	190 (21.1)	111 (20.7)
During pregnancy	101 (11.4)	89 (9.9)	53 (9.9)
Infant sex			
Male	481 (52.6)	470 (51.0)	294 (52.8)
Female	433 (47.4)	452 (49.0)	263 (47.2)
Gestational age at delivery (weeks)			
<34	15 (1.6)	12 (1.3)	6 (1.1)
34 to <37	43 (4.7)	49 (5.3)	21 (3.8)
≥37	856 (93.7)	861 (93.4)	530 (95.2)

LINE-1, long interspersed nuclear element-1; %5mC, %5-methylated cytosines; BMI, body mass index. Values may not add up to total *n* because of missing data.

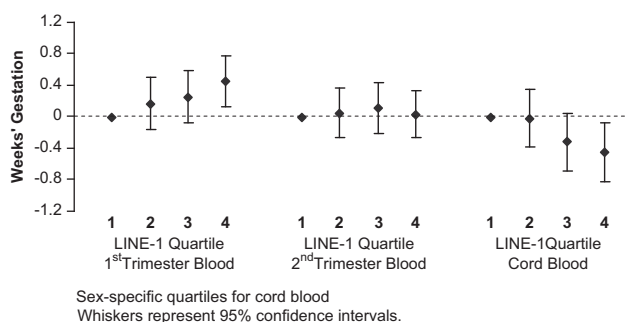


Fig. 1. Multivariable-adjusted associations of long interspersed nuclear element-1 (LINE-1) DNA methylation with gestational age at birth, Project Viva.

preterm delivery. Infants in the highest sex-specific quartile of LINE-1 *v.* the lowest quartile had higher odds of having been born preterm (OR 3.87, 95% CI 1.05, 14.2). Covariate adjustment only strengthened this association (OR 4.55, 95%

CI 1.18, 17.5; Table 3). We found no differences in associations with mean gestational age (interaction *P* = 0.97) or preterm birth (*P* = 0.84) according to infant sex.

Discussion

In this cohort study, higher LINE-1 DNA methylation of maternal peripheral blood leukocytes during the first trimester predicted longer gestation and decreased odds of delivering preterm, as hypothesized. Conversely, we found cord blood LINE-1 DNA methylation to be inversely correlated with the length of gestation. These associations persisted after adjustment for many known risk factors for preterm birth. We observed similar associations of first trimester LINE-1 with preterm birth irrespective of whether the birth was spontaneous or medically induced. Medically indicated preterm birth may occur when a fetus has been showing signs of distress or poor growth, or if the mother has developed preeclampsia or other illness prompting delivery. Preeclampsia,

Table 2. Gestation length differences according to quartile of LINE-1 methylation in leukocytes among participants enrolled in Project Viva^a

	LINE-1%5mC			Mean difference in gestation length weeks (95% CI)	
	Mean (S.D.)	Min	Max	Unadjusted	Adjusted ^b
First trimester maternal blood					
1st quartile	83.5 (0.3)	80.9	83.8	0.00 (reference)	0.00 (reference)
2nd quartile	84.1 (0.1)	83.8	84.3	0.16 (−0.16, 0.48)	0.17 (−0.17, 0.50)
3rd quartile	84.5 (0.1)	84.3	84.7	0.22 (−0.10, 0.54)	0.25 (−0.08, 0.58)
4th quartile	85.0 (0.2)	84.7	85.7	0.45 (0.12, 0.77)	0.45 (0.12, 0.78)
Second trimester maternal blood					
1st quartile	84.0 (0.2)	83.0	84.3	0.00 (reference)	0.00 (reference)
2nd quartile	84.4 (0.1)	84.3	84.5	0.06 (−0.25, 0.37)	0.05 (−0.26, 0.37)
3rd quartile	84.7 (0.1)	84.5	84.8	0.09 (−0.22, 0.40)	0.11 (−0.21, 0.43)
4th quartile	85.1 (0.2)	84.8	85.8	0.02 (−0.29, 0.33)	0.02 (−0.30, 0.34)
Venous umbilical cord blood ^c					
1st quartile	83.9 (0.6)	79.5	84.5	0.00 (reference)	0.00 (reference)
2nd quartile	84.5 (0.2)	84.1	84.8	−0.02 (−0.38, 0.34)	−0.02 (−0.39, 0.35)
3rd quartile	84.8 (0.2)	84.4	85.2	−0.33 (−0.69, 0.03)	−0.32 (−0.69, 0.05)
4th quartile	85.4 (0.4)	84.8	86.5	−0.45 (−0.82, −0.09)	−0.45 (−0.83, −0.08)

LINE-1, long interspersed nuclear element-1; %5mC, %5-methylated cytosines; BMI, body mass index.

^a First trimester $n = 914$ (unadjusted) and $n = 884$ (adjusted); second trimester $n = 922$ (unadjusted) and $n = 893$ (adjusted); cord blood $n = 557$ (unadjusted) and $n = 534$ (adjusted) because of missing covariate data.

^b Adjusted for maternal age, prepregnancy BMI, smoking status, education and infant sex in multivariable linear regression models.

^c Sex-specific quartiles are responsible for overlapping ranges.

Table 3. Odds of preterm birth by quartile of LINE-1 DNA methylation among 1160^a participants enrolled in Project Viva

	Preterm cases, n (%)	OR (95% CI)	
		Unadjusted	Adjusted ^b
First trimester			
1st quartile	19 (8.3)	1.0 (reference)	1.0 (reference)
2nd quartile	17 (7.4)	0.88 (0.45, 1.74)	0.91 (0.45, 1.81)
3rd quartile	14 (6.1)	0.72 (0.35, 1.47)	0.72 (0.35, 1.49)
4th quartile	8 (3.5)	0.40 (0.17, 0.93)	0.40 (0.17, 0.94)
Second trimester			
1st quartile	15 (6.5)	1.0 (reference)	1.0 (reference)
2nd quartile	12 (5.2)	0.79 (0.36, 1.72)	0.79 (0.36, 1.75)
3rd quartile	17 (7.4)	1.14 (0.55, 2.34)	1.14 (0.55, 2.37)
4th quartile	17 (7.4)	1.14 (0.56, 2.35)	1.16 (0.56, 2.40)
Cord blood (sex-specific quartiles)			
1st quartile	3 (2.2)	1.0 (reference)	1.0 (reference)
2nd quartile	6 (4.3)	2.01 (0.49, 8.22)	2.14 (0.50, 9.08)
3rd quartile	7 (5.0)	2.37 (0.60, 9.35)	2.29 (0.55, 9.48)
4th quartile	11 (7.9)	3.87 (1.05, 14.2)	4.55 (1.18, 17.5)

LINE-1, long interspersed nuclear element-1; %5mC, %5-methylated cytosines; BMI, body mass index.

^a First trimester $n = 914$ (unadjusted) and $n = 884$ (adjusted); second trimester $n = 922$ (unadjusted) and $n = 893$ (adjusted); cord blood $n = 557$ (unadjusted) and $n = 534$ (adjusted) because of missing data.

^b Adjusted for maternal age, BMI, race/ethnicity, education, smoking and infant sex in multivariable logistic regression models.

characterized by hypertension and proteinuria, accounts for ~20% of extremely preterm (<28 weeks' gestation) deliveries.³⁹ A broader category of ischemic placental disease is

responsible for over half of medically indicated preterm deliveries.⁴⁰ Higher LINE-1 could be a marker of less ischemic placental disease or preeclampsia. We previously showed that

Table 4. Venous umbilical cord blood LINE-1 DNA methylation in leukocytes (%5mC) by gestational age category among 557 mother–infant pairs enrolled in Project Viva

	Overall				Female		Male	
	<i>n</i>	Mean (s.d.)	Min.	Max.	<i>n</i>	Mean	<i>n</i>	Mean
Gestational age (weeks)								
Preterm								
<34	6	85.1 (0.6)	84.3	85.7	1	84.3	5	85.3
34 to <37	21	84.8 (0.7)	82.8	86.0	9	84.5	12	85.1
Full term								
37 to <39	109	84.6 (0.6)	82.6	86.3	49	84.5	60	84.8
39 to <41	307	84.7 (0.7)	79.5	86.5	148	84.4	159	84.9
≥41	114	84.5 (0.6)	82.8	86.1	56	84.3	58	84.7

LINE-1, long interspersed nuclear element-1; %5mC, %5-methylated cytosines.

higher LINE-1 expression is associated with lower risk of ischemic heart disease in non-pregnant adults.⁴¹ As preeclampsia may have roots early in placental development secondary to implantation abnormalities, poor perfusion and subsequent endothelial damage,⁴² first trimester LINE-1 may be hypomethylated in preeclampsia as it is in ischemic heart disease. Mean LINE-1 was lower in the first trimester among women who developed preeclampsia (84.1 %5mC) compared with women who did not (84.3 %5mC, $P = 0.15$). Preliminary analysis suggests a relationship between increasing first trimester LINE-1 and decreased multivariable-adjusted odds of subsequent preeclampsia (OR 0.65, 95% CI 0.35, 1.19), but wide confidence intervals prevent conclusions about this association. Further work should be done to evaluate potential associations between maternal DNA methylation early in pregnancy and the development of preeclampsia.

Plausible mechanisms also support associations with spontaneous preterm birth, which can result from preterm premature rupture of membranes secondary to cervical insufficiency, or from preterm labor resulting from infection, uterine stretch from uterine fibroids or trauma. Inflammation is the hallmark of spontaneous preterm labor.⁴³ In a nested case–control study within Project Viva, we have shown that women with higher early pregnancy serum C-reactive protein had higher odds of preterm delivery.⁴⁴ We have also demonstrated LINE-1 hypomethylation to be associated with elevation vascular cell adhesion molecule-1.⁴⁵ Furthermore, interleukin-6 can induce LINE-1 hypomethylation in oral cancer cells studied *in vitro*.⁴⁶ Others have proposed that LINE-1 hypomethylation itself could contribute to inflammation and the development of autoimmune disease through triggering the innate immunity pathway.⁴⁷ LINE-1 hypomethylation may affect cellular function through encouraging transcription of sequences activated during conditions of cellular stress or inflammation.^{18,19,21}

The lack of an association between second trimester LINE-1 methylation and preterm birth remains somewhat puzzling, given the inverse association of first trimester LINE-1 with

odds of preterm birth. We speculate that LINE-1 status during the first trimester may reflect the inflammatory state of the mother closer to the timing of implantation, the success of which appears to be a key step for avoiding for preeclampsia and placental insufficiency more broadly.⁴⁸ Furthermore, the low correlation between first and second trimester LINE-1 suggests that LINE-1 at these time points reflect different processes (Pearson $r = 0.02$).

In contrast to our findings with first trimester LINE-1, we found that infants with higher cord blood LINE-1 had shorter gestations and higher odds of preterm birth. The cross-sectional nature of these observations prevents conclusions about causality. Although Tabano *et al.* found that adult peripheral blood leukocytes had higher LINE-1 methylation (67.3%) than umbilical cord blood (60.1%), most studies of adults³² and across the lifespan⁴⁹ suggest that global methylation decreases with age. We hypothesize that LINE-1 may become less methylated as cells divide and thus decreases with age in fetal life as it does in adult life. Furthermore, there are both maternal and fetal contributions to preterm birth. For example, infants with multiple congenital anomalies are more likely to be born preterm regardless of maternal health state.⁵⁰ Thus, if indeed there is a causal relationship between DNA methylation and preterm birth, it is possible that the impacts on the length of gestation of maternal and fetal DNA methylation differ from one another.

A recent study comparing the global DNA methylation of placentas from preeclamptic pregnancies and normotensive pregnancies revealed that methylation levels were higher in preeclamptic placentas.⁵¹ Although initially this may seem counter to our findings, another study suggests discordance between placental and fetal tissue methylation with placental tissue being relatively hypomethylated compared with the fetus.⁴⁹ Tabano *et al.*⁵² also found that the placenta ($n = 46$) displayed relative hypomethylation of LINE-1 methylation (41.8%, range 34.0–52.3) compared with umbilical cord blood samples ($n = 10$; 60.1%, range 57.5–77.7). Despite using

the same technique,⁵³ we found our mean cord blood methylation value of 84.6 %mC (range 71.5–86.5) is much higher. It is possible that simply by chance, their small sample size obtained estimates on the lower end of what is expected. However, it also highlights possible lack of comparability of DNA methylation values across studies.

Our findings should be considered in the context of inherent limitations. First, DNA methylation of peripheral blood leukocytes may or may not reflect the methylation of the tissues responsible for the conditions leading to preterm birth. Tissues such as the myometrium may be more appropriate for DNA methylation studies of preterm labor risk. Second, methylation of different leukocyte subtypes varies and we did not have the ability to adjust for the leukocyte differential, although adjustment has not affected the results of other cohort studies using LINE-1 methylation as an exposure.^{21,45} Third, DNA methylation is one of several epigenetic mechanisms that work in conjunction with one another to regulate gene expression, and further work should be done to understand how histone modifications or other mechanisms may affect the risk of preterm birth. Fourth, gene-specific or genome-wide DNA methylation analyses may provide more insight into mechanisms underlying the processes prompting preterm birth, and although such technologies exist, they continue to be costly for cohort studies with large sample sizes. Fifth, the relatively high socio-economic status of our cohort may limit the generalizability of our findings, but our findings should be internally valid. Lastly, our preterm birth rate (6.5%) seems low compared with national estimates of 12% overall, and 10–11% among singleton births in the last two decades.⁵⁴ However, a more appropriate comparison for our singleton-only, Massachusetts-based cohort may be the Massachusetts preterm birth rate of ~8%,⁵⁵ which includes multiples, who are at higher risk of preterm birth, suggesting that our rate of 6.5% among singletons may be representative of the region's overall singleton rate.

In summary, we found that higher LINE-1 DNA methylation in maternal peripheral blood DNA during the first trimester was associated with longer gestations and decreased odds of preterm birth and that higher cord blood LINE-1 was associated with shorter gestations. Whether LINE-1 methylation simply serves as a potential biomarker for preterm birth or affects a woman's risk of delivering preterm by altering gene expression and subsequent cellular function awaits further study.

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Supplementary material

For supplementary material referred to in this article, please visit <http://dx.doi.org/10.1017/S2040174412000104>.

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