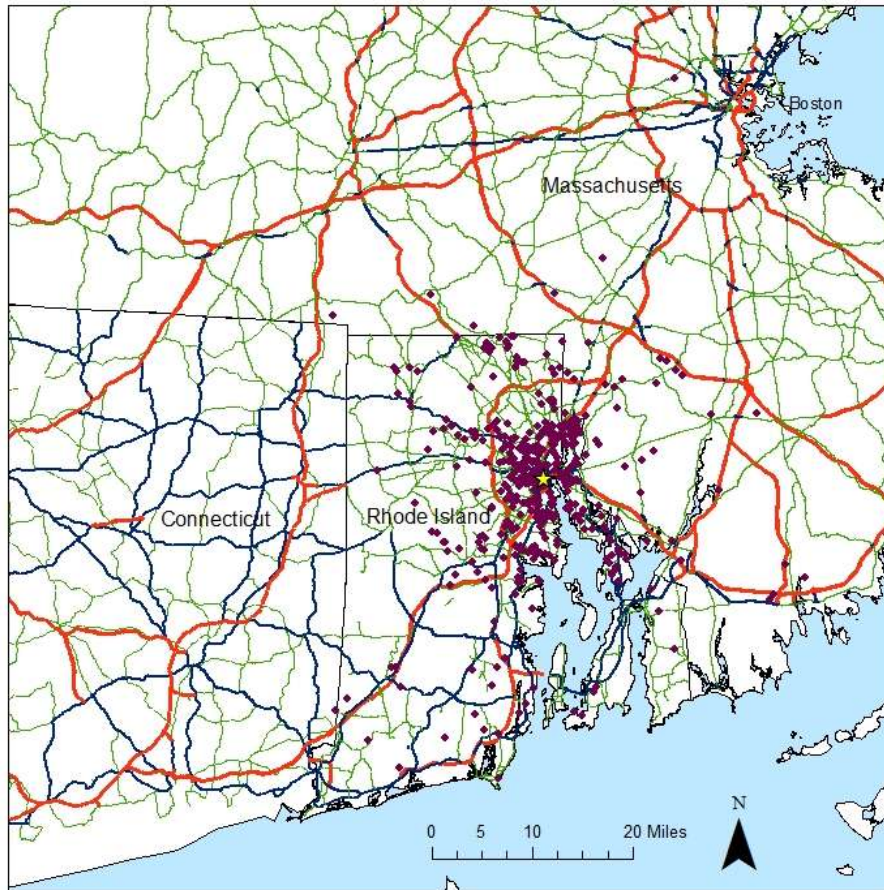


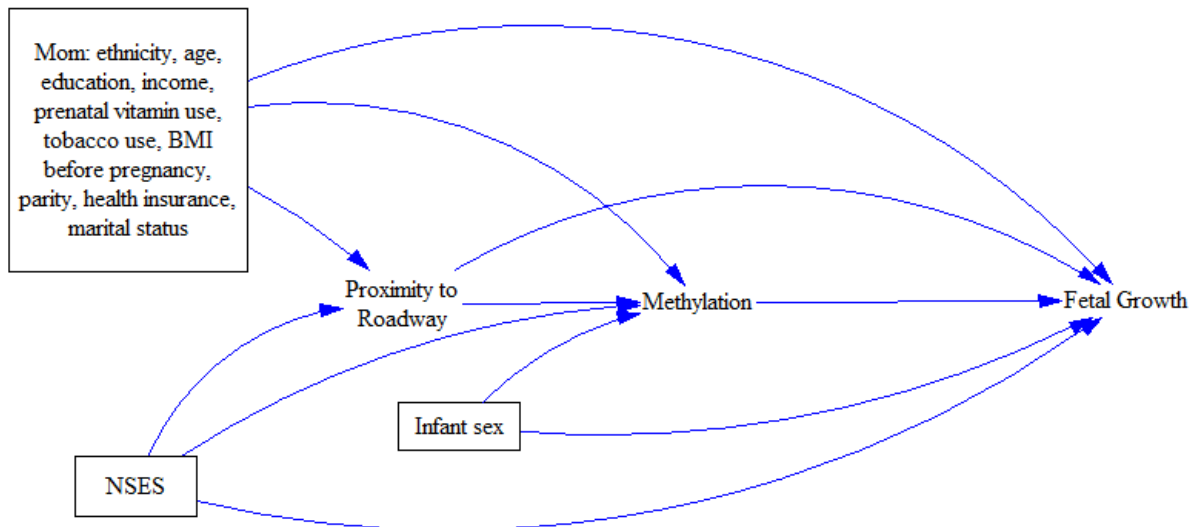
Participant Addresses and Hospital Location



Supplemental Figure 1: Participant addresses and hospital location

2.3 DNA Methylation Analysis

Within 2 hours of birth, full-thickness sections were taken from the maternal side of the placenta, 2 cm from the umbilical cord-insertion site and free of maternal decidua. These sections were immediately placed in RNAlater™ (Applied Biosystems, Inc., AM7020).



Supplemental Figure 2: Causal diagram depicting hypothesized causal mechanism between residential proximity to major roadways and markers of fetal growth

Note: Markers of fetal growth are 1) birthweight adjusted for gestational age and 2) infant growth status, which includes small for gestational age, average for gestational age, and large for gestational age. A box around a variable or set of variables indicates adjustment in a regression model.

Abbreviations: BMI is body mass index, NSES is neighborhood socioeconomic status

We performed secondary analyses to better understand potential mediation effects. We examined the association between proximity to major roadway and birth weight additionally adjusted for mean LINE-1 methylation levels and mean AluYb8 methylation levels to test the hypothesis that the effects of traffic pollution on fetal growth are mediated at least in part through changes in global methylation levels as assessed by LINE-1 and AluYb8. If some or all of the effect on birth weight is mediated through placental methylation, under certain unverifiable assumptions (Cole and Hernan 2002; VanderWeele and Vansteelandt 2009, 2010)

Supplemental Table 1: LINE-1 and AluYb8 methylation levels by population characteristics(group mean \pm standard deviation)

Characteristic	LINE-1 (n =469)	p-value ^a	AluYb8 (n =467)	p-value ^a
Maternal				
Education		0.78		0.06
\leq High school	41.5 \pm 3.2		71.1 \pm 4.4	
Some college	41.9 \pm 3.4		70.4 \pm 3.7	
College or more	41.7 \pm 3.0		70.2 \pm 3.5	
Tobacco use during pregnancy		0.90		0.08
Yes	41.6 \pm 3.0		69.1 \pm 2.8	
No	41.7 \pm 3.1		70.6 \pm 3.8	
Alcohol use during pregnancy		0.98		0.15
Yes	41.7 \pm 3.6		73.2 \pm 9.2	
No	41.7 \pm 3.1		70.5 \pm 3.7	
Prenatal care during pregnancy		0.48		0.62
Yes	41.7 \pm 3.1		70.5 \pm 3.8	
No	40.7 \pm 3.2		71.4 \pm 2.1	
Prenatal vitamin use		0.91		0.21
Yes	41.7 \pm 3.1		70.5 \pm 3.8	
No	41.8 \pm 3.9		69.6 \pm 2.5	
Annual household income		0.071		0.19
<\$25,000	41.3 \pm 3.2		70.6 \pm 4.2	
\$25,000-49,999	41.4 \pm 4.1		70.6 \pm 4.1	
\$50,000-79,999	41.7 \pm 2.9		70.4 \pm 3.9	

\$80,000-99,999	41.9 ± 3.0		71.6 ± 4.4	
≥\$100,000	42.0 ± 2.7		69.7 ± 3.0	
Marital status		0.49		0.92
Married	41.80 ± 3.1		70.5 ± 3.8	
Not married	41.6 ± 3.2		70.5 ± 3.8	
Health insurance		0.29		0.87
None/self-pay	41.9 ± 3.8		71.4 ± 3.7	
Private	41.9 ± 2.9		70.4 ± 3.6	
Public/other	41.5 ± 3.5		70.4 ± 3.7	
Race/ethnicity		0.63		0.65
Non-Caucasian	41.9 ± 3.3		70.3 ± 3.6	
Caucasian	41.7 ± 3.1		70.5 ± 3.8	
Parity		0.19		0.77
1	41.4 ± 3.0		70.4 ± 3.4	
2	41.7 ± 3.0		70.5 ± 4.1	
≥3	41.9 ± 3.2		70.5 ± 3.7	
Newborn				
Gestational age (weeks)		0.93		0.14
37	41.3 ± 3.2		69.8 ± 2.5	
38	41.8 ± 3.3		69.7 ± 2.8	
39	41.8 ± 3.1		70.7 ± 4.2	
40	42.6 ± 2.8		70.7 ± 3.3	
41	40.00 ± 3.1		70.5 ± 3.5	
Growth status		0.21		0.29
SGA	41.8 ± 3.8		70.2 ± 3.1	

AGA	41.5 ± 3.0		70.4 ± 3.8
LGA	42.2 ± 2.9		70.8 ± 4.0
Infant sex		0.53	<0.001
Male	41.7 ± 3.2		71.3 ± 3.7
Female	41.8 ± 3.0		69.6 ± 3.6

Abbreviations: SGA is small for gestational age, AGA is average for gestational age, and LGA is large for gestational age.

^ap-values obtained from t-tests for dichotomous variables and one-way ANOVA tests for variables with more than two categories

To examine the association between proximity to nearest major roadway and birth weight not mediated through placental DNA methylation, we additionally adjusted for mean LINE-1 and AluYb8 methylation levels and found that living near a major roadway was associated with a 172.0 (95% CI: -316.7, -27.3; p=0.02) and 179.8 (95% CI: -324.1, -35.6; p=0.015) lower birth weight, respectively (Table 2), which is essentially unchanged from the 175.9 g difference found in the main analyses not adjusted for LINE-1 or AluYb8.

versus further away (Table 3 and Figure 2). Three of these CpG sites were mapped to genes PTPRN2, TMEM125, and VPS4A, but the other 4 sites map to non-genic regions. Figure 3 plots the coefficient for each CpG site versus its p-value. Loci on the PTPRN2 gene, highlighted in red, appear many times in the top 1000 sites. It also has the most significant p-value, as shown in Table 3.

Supplemental Table 2: Population characteristics for total population and sub-population

	Total (n = 471)	Subset (n = 215)	p-value^a
Maternal characteristics			
Age, years, mean +- SD	30.0 ± 5.6	30.3 ± 5.4	0.58
BMI before pregnancy, kg/m ²	26.9 ± 7.1	26.6 ± 6.5	0.49
Tobacco use during pregnancy	23 (4.9%)	10 (4.7%)	0.99
Alcohol use during pregnancy	4 (0.8%)	2 (0.9%)	0.99
Prenatal care during pregnancy	458 (97.2%)	213 (99.1%)	0.22
Prenatal vitamin use	434 (92.1%)	202 (94.0%)	0.49
Annual household income			0.96
<\$25,000	101 (21.4%)	51 (23.7%)	
\$25,000-49,999	71 (15.1%)	32 (14.9%)	
\$50,000-79,999	82 (17.4%)	45 (20.9%)	
\$80,000-99,999	55 (11.7%)	31 (14.4%)	
≥\$100,000	117 (24.8%)	56 (26.0%)	
Married	301 (63.9%)	145 (67.4%)	0.42
Race, Caucasian	361 (76.6%)	174 (80.9%)	0.25
Education			0.65

< High school	102 (21.7%)	41 (19.1%)	
Some college	115 (24.4%)	52 (24.2%)	
College or more	249 (52.9%)	122 (56.7%)	
Health insurance			0.35
None/self-pay	8 (1.7%)	5 (2.3%)	
Private	282 (59.9%)	141 (65.6%)	
Public/other	175 (37.2%)	69 (32.1%)	
Parity			0.98
1	123 (26.1%)	58 (27.0%)	
2	164 (34.8%)	74 (34.4%)	
≥3	183 (38.9%)	83 (38.6%)	
Newborn characteristics			
Gestational age (weeks), mean ± SD	39.0 ± 0.9	39.0 ± 1.0	0.94
Infant sex, Male	244 (51.8%)	116 (54.0%)	0.66
Neighborhood SES, z-score	0.0 ± 5.3	0.3 ± 5.4	0.50
Outcomes			
Birth weight (g), mean ± SD	3568.3 ± 664.2	3573.1 ± 706.8	0.93
Growth status			0.79
SGA	77 (16.3%)	36 (16.7%)	
AGA	260 (55.2%)	113 (52.6%)	
LGA	134 (28.5%)	66 (30.7%)	

Note: Near major roadway defined as ≤150 m of A1/A2 or ≤50 m of A3 and far from major roadways defined as >150 m of A1/A2 or >50 m of A3. Not all N values equal 476 because of missing values. Neighborhood SES z-sum is the sum of the z-scores for median household income, percent of households with interests, dividends, or rent income, percent of residents with high school diploma, percent with college degree, percent with professional occupation, and median value of owner-occupied housing units.

Abbreviations: SD is standard deviation, BMI is body mass index, SGA is small for gestational age, AGA is average for gestational age, LGA is large for gestational age, and SES is socioeconomic status.

^ap-values obtained from chi square tests for categorical variables and one-way ANOVA tests for continuous variables