

INVESTIGATING THE ROLE OF MATERNAL MITOCHONDRIA IN PREGNANCY:
IMPLICATIONS FOR FETAL GROWTH AND GESTATIONAL LENGTH

By

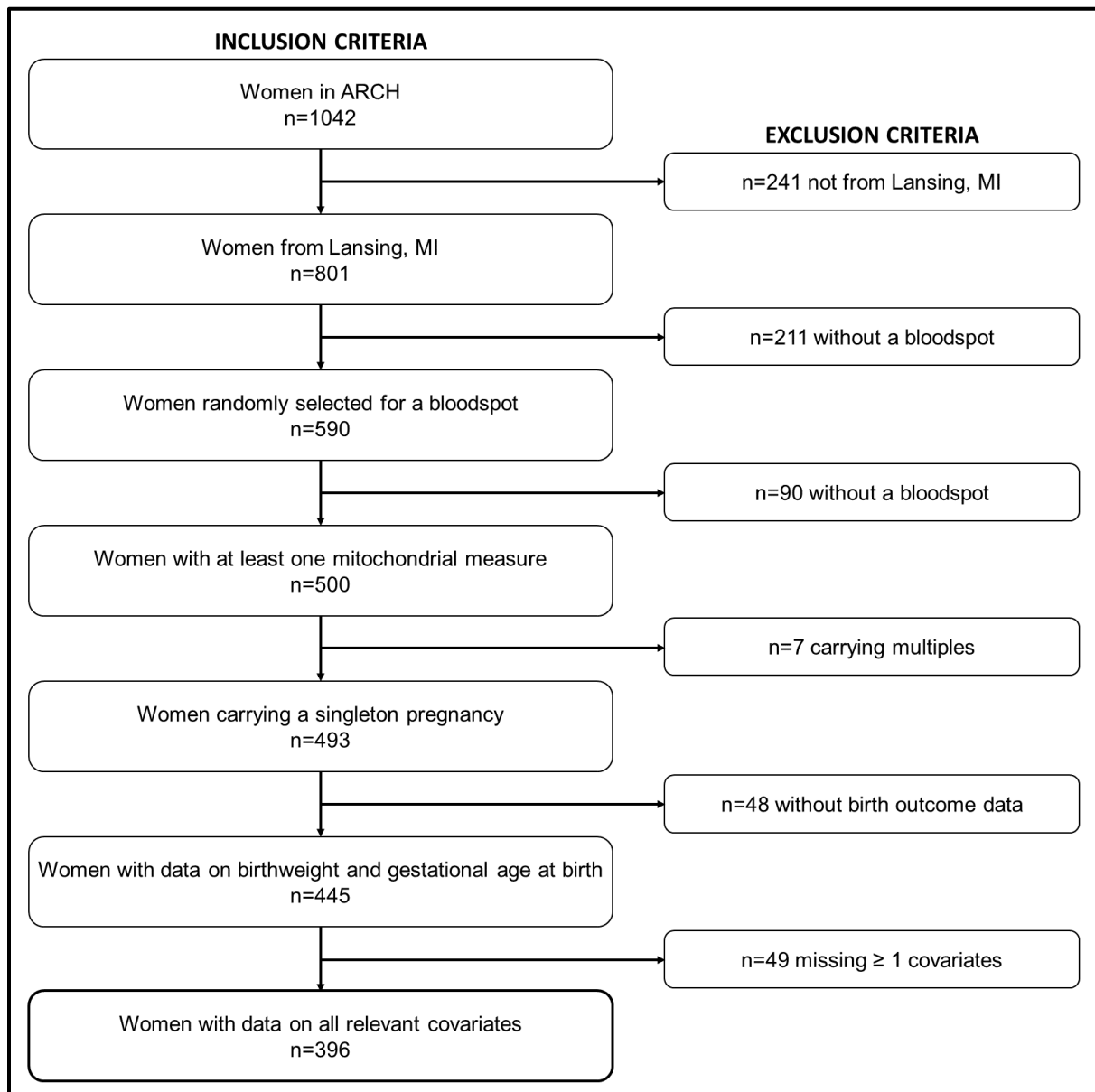
Maria E. Cinzori

A THESIS – Supplementary Materials

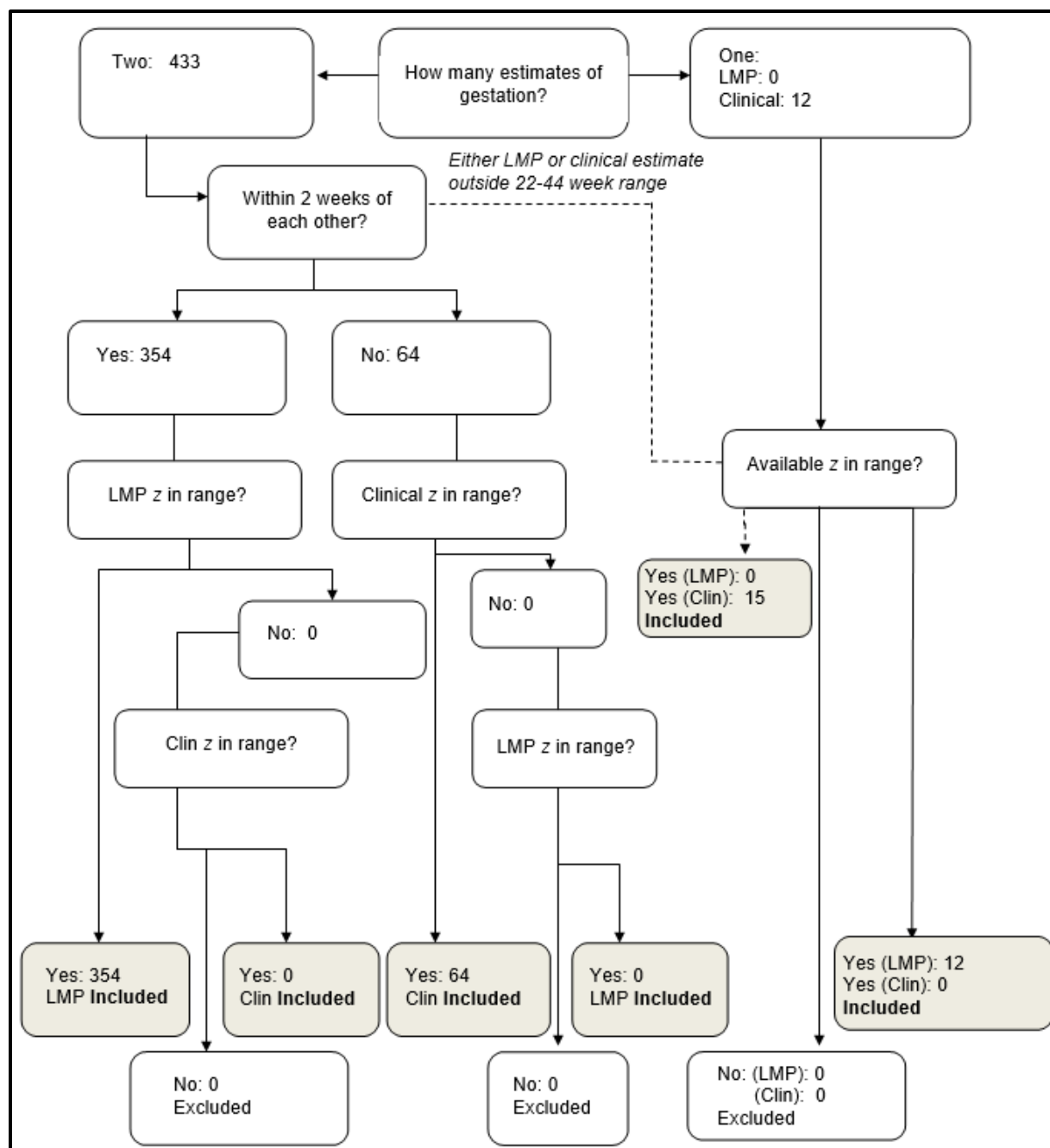
Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Epidemiology – Master of Science

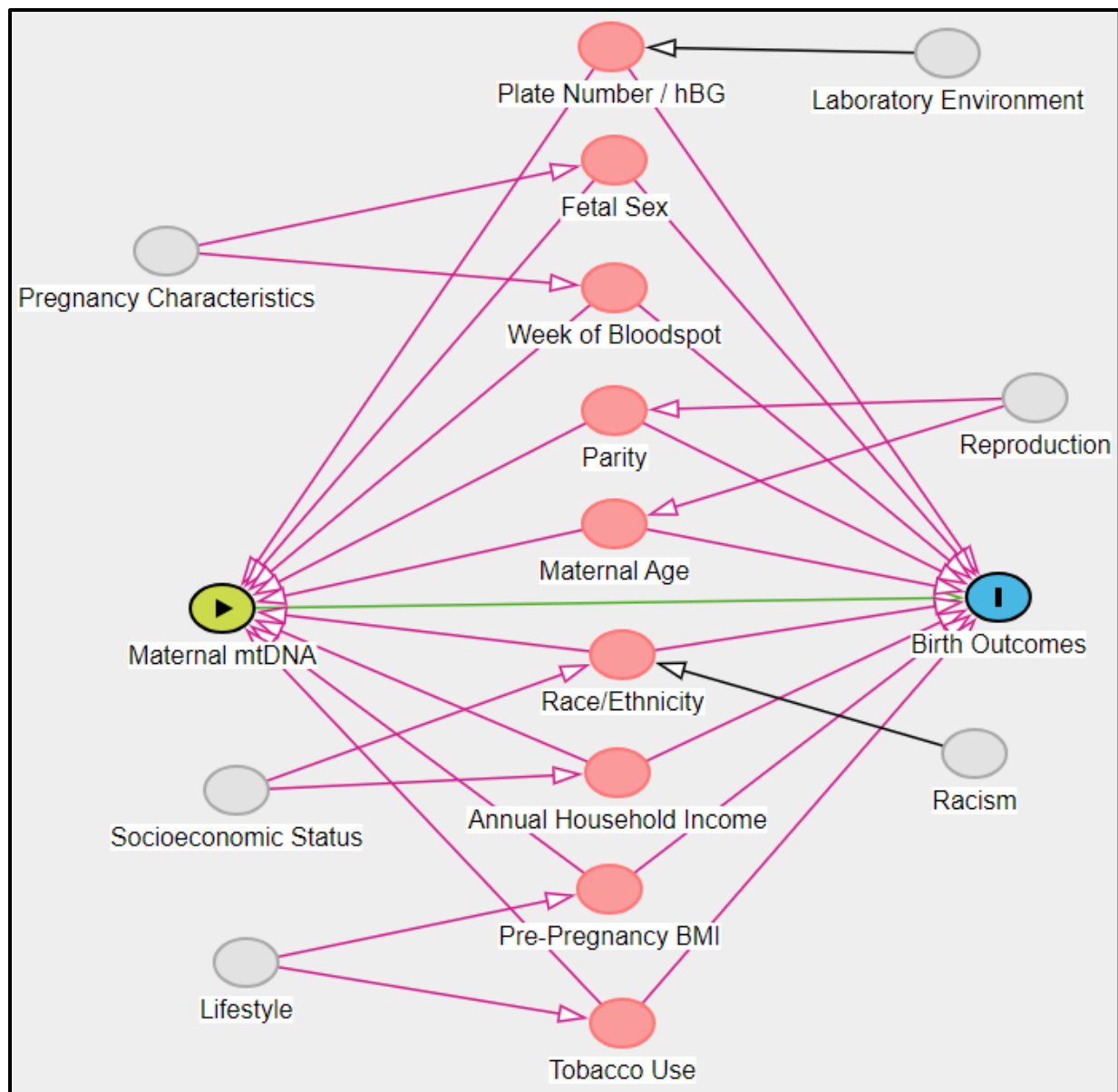
2024



Supplemental Figure 1. Derivation of the ARCH analytic sample (n=396). ARCH, Archives for Research on Child Health.



Supplemental Figure 2. Identification of eligible ARCH singleton live births. The derivation of eligible participants was limited to ARCH women with singleton deliveries, non-missing birthweight, and gestation length between 22 and 44 weeks.



Supplemental Figure 3. Directed acyclic graph for associations of mtDNA biomarkers with birth outcomes. mtDNA biomarkers are the exposure (green/black circle) and the birth outcomes are the outcome (blue/black circle). Red circles indicate variables associated with both the exposure and outcome, and gray circles represent latent constructs. BMI, body mass index; hBG, human β -globin, mtDNA, mitochondrial DNA.

Supplemental Table 1. Gene names and reference primers.

Gene Name	MTTF	DLOOP	CYTB	LDLR
Reference	Novielli et al. 2017	Vos et al 2020	Owa et al.	Vos et al 2020
Forward Primer	TAAAGTAATATA TTGAAAATGTTT AGA	TGGAAAGTGGTT GTGTAGATATTT AA	TGTTAGGATGAG GATGGATAGTA AT	TTTTTAGTGTATTG TTTTGAGGAGGTA AGT
Reverse Primer	biotin- TACTTAATACTTA TCCCTTTTAATC	biotin- CTTTAATTCCTAC CTCATCCTATTAT TT	biotin- CTTCTCTTCCTT CTCTCCTTAATA ACA	biotin- CACTCCCATACTA CTAATCTCATCA
Sequencing Primer	TATTGAAAATGT TTAGA (seq 1) GATTATATATGT AAGTATTT (seq 2)	AATTAATTAATA TATTTTAGTAAG	ATGGATAGTAAT AGGGTAAG	TTTTTGGGGTTTG GT
Sequence Analyzed	YGGGTTTATATT (seq 1) TYGTTTTAGTGA GTTTATTTTTTAA ATTATTAYGA (seq 2)	TATGTTYGTT TGTAATATTG AAYGTAGGTG YGATAAATAA TAGGATGAGG TAGGAAT	GAYGTTTTTTTAG TTTGTTAGGGAY GGATYGGAGAA TTGTGTAGGYGA ATAGGAAATATT ATTYGGGTTG	TGGTTYGGGG TATGGGGTTA GTAGYGGTGT GTGTGTGTTG GGTAGGATGG GYGGGGGTTG TATTGATGAG ATTAGTAGT
PCR Conditions	In PCR reaction: Taq, water, 0.45 μ M forward primer, 0.3 μ M reverse biotinylated primer, 2 μ L bisulfite converted DNA. PCR conditions: 15 min at 95C then [50 cycles X (95C for 30 sec, 54C for 30 sec, 72C for 60 sec)]; 72C for 5 min, 4C hold	In PCR reaction: 0.375 μ M forward primer, 0.25 μ M reverse biotinylated primer; 2 μ L bisulfite converted DNA. PCR conditions: 15 min at 95C then [50 cycles X (95C for 30 sec, 54C for 30 sec, 72C for 60 sec)]; 72C for 5 min, 4C hold	In PCR reaction: 0.3 μ M forward primer, 0.2 μ M reverse biotinylated primer; 2 μ L bisulfite converted DNA. PCR conditions: 15 min at 95C then [50 cycles X (95C for 30 sec, 52C for 30 sec, 72C for 60 sec)]; 72C for 5 min, 4C hold	In PCR reaction: 0.3 μ M forward primer, 0.2 μ M reverse biotinylated primer; 2 μ L bisulfite converted DNA. PCR conditions: 15 min at 95C then [50 cycles X (95C for 30 sec, 57C for 30 sec, 72C for 60 sec)]; 72C for 5 min, 4C hold
Amplicon Length	168 bp	139 bp	240 bp	153 bp
# CpG Sites	1 (seq 1) 2 (seq 2)	3	4	3
Amplicon Location	597 – 765	chrM:132-270	chrM: 15443:15652	amplicon: chrM: 463- 615

Supplemental Table 2. Sensitivity analyses evaluating associations between maternal mtDNA_{Acn} and mtDNA methylation with gestation length, using Cox Proportional Hazards models.

Model	Unadjusted n=196-396	Preterm birth n=196-396	No hypertension n=175-358	No GDM n=184-369
Gene/site	HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
<i>NDI</i> ¹	0.9 (0.7, 1.1)	0.9 (0.5, 1.7)	0.8 (0.6, 1.2)	0.9 (0.6, 1.3)
<i>LDLR</i> CpG1 ¹	1.1 (0.9, 1.4)	1.0 (0.6, 1.7)	1.0 (0.8, 1.4)	1.1 (0.8, 1.5)
<i>LDLR</i> CpG2 ¹	1.2 (0.9, 1.6)	1.3 (0.8, 2.2)	1.2 (0.9, 1.7)	1.2 (0.9, 1.6)
<i>LDLR</i> CpG3 ¹	1.0 (0.8, 1.3)	0.9 (0.5, 1.6)	1.0 (0.8, 1.5)	1.0 (0.8, 1.4)
<i>LDLR</i> Average ¹	1.1 (0.8, 1.5)	0.9 (0.5, 1.7)	1.0 (0.7, 1.5)	1.1 (0.8, 1.5)
<i>DLOOP</i> CpG1 ¹	0.9 (0.7, 1.2)	0.7 (0.4, 1.3)	0.8 (0.5, 1.1) #	0.9 (0.6, 1.2)
<i>DLOOP</i> CpG2 ¹	1.1 (0.8, 1.5)	1.1 (0.5, 2.4)	1.0 (0.7, 1.5)	1.0 (0.7, 1.5)
<i>DLOOP</i> CpG3 ¹	1.0 (0.7, 1.5)	1.1 (0.5, 2.3)	0.9 (0.6, 1.4)	1.0 (0.7, 1.5)
<i>DLOOP</i> Average ¹	1.0 (0.7, 1.4)	0.7 (0.3, 1.5)	0.8 (0.5, 1.2)	0.9 (0.7, 1.4)
<i>CYTB</i> CpG1 ¹	1.0 (0.8, 1.2)	0.5 (0.2, 1.0) *	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)
<i>CYTB</i> CpG2 ¹	1.1 (0.9, 1.3)	0.8 (0.5, 1.2)	1.1 (0.9, 1.4)	1.1 (0.9, 1.3)
<i>CYTB</i> CpG3 ¹	1.0 (0.8, 1.3)	0.8 (0.4, 1.6)	1.0 (0.8, 1.3)	1.0 (0.8, 1.3)
<i>CYTB</i> Average ¹	1.1 (0.9, 1.4)	0.8 (0.4, 1.4)	1.1 (0.8, 1.4)	1.1 (0.8, 1.4)
<i>MTTF</i> S1 CpG1 ¹	0.8 (0.6, 1.1)	0.7 (0.3, 1.3)	0.8 (0.6, 1.1)	0.9 (0.6, 1.2)
<i>MTTF</i> S2 CpG1 ²	1.1 (0.9, 1.2)	1.0 (0.8, 1.2)	1.0 (0.9, 1.1)	1.0 (0.9, 1.1)
<i>MTTF</i> S2 CpG2 ²	1.1 (1.0, 1.2) #	1.0 (0.8, 1.3)	1.0 (0.9, 1.2)	1.1 (0.9, 1.2)
<i>MTTF</i> S2 Average ²	1.1 (1.0, 1.2) #	1.1 (0.9, 1.3)	1.0 (0.9, 1.2)	1.1 (0.9, 1.2)
All adjusted Cox PHMs accounted for plate number or hBG, race/ethnicity, income, age, tobacco use, pre-pregnancy BMI, week of bloodspot collection, parity, and fetal sex. Bolded values are considered meaningful. *p ≤ 0.05; #p ≤ 0.10.				
¹ Gene sites are ln-transformed and hazard ratios (HRs) (95% CIs) were back-transformed using the following equation: $e^{\log(\text{HR}) \cdot \log(2)}$. Results interpreted as the percent change in the HR for each two-fold increase in mtDNA methylation.				
² Gene sites not transformed and HRs (95% CIs) were not adjusted. Results interpreted as the change in the HR for each one-unit increase in mtDNA methylation.				
BMI, body mass index; CYTB, cytochrome b; <i>DLOOP</i> , D-loop promoter region, heavy strand; hBG, human β-globin; HR, hazard ratio; <i>LDLR</i> , D-loop promoter region, light strand; mtDNA, mitochondrial DNA; MTTF, mitochondrially encoded tRNA phenylalanine; <i>NDI</i> , NADH-ubiquinone oxidoreductase chain 1; PHM, proportional hazards model; S1, location 1; S2, location 2.				

Supplemental Table 3. Sensitivity analyses evaluating associations between maternal mtDNA_{Acn} and mtDNA methylation with birthweight using linear regression.

Model	Unadjusted n=196-396	No hypertension n=175-358	No GDM n=184-369
Gene/site	β (95% CI)	β (95% CI)	β (95% CI)
<i>NDI</i> ¹	67.9 (-13.5, 149.3)[#]	85.1 (-28.5, 198.6)	51.6 (-61.9, 165.1)
<i>LDLR</i> CpG1 ¹	9.786 (-64.9, 84.4)	57.4 (-21.7, 136.5)	35.2 (-44.8, 115.2)
<i>LDLR</i> CpG2 ¹	9.9 (-86.3, 106.0)	63.6 (-40.8, 169.0)	36.6 (-67.0, 140.2)
<i>LDLR</i> CpG3 ¹	26.8 (-55.6, 109.3)	54.9 (-30.4, 140.2)	52.5 (-33.7, 138.8)
<i>LDLR</i> Average ¹	13.4 (-75.6, 102.4)	78.5 (-19.9, 177.0)	61.0 (-38.4, 160.3)
<i>DLOOP</i> CpG1 ¹	16.4 (-65.1, 97.9)	-22.6 (-106.6, 61.4)	6.0 (-75.5, 87.5)
<i>DLOOP</i> CpG2 ¹	3.4 (-105.6, 112.4)	-2.7 (-112.0, 106.6)	3.5 (-107.5, 114.5)
<i>DLOOP</i> CpG3 ¹	-7.8 (-114.1, 98.6)	6.8 (-114.4, 128.0)	0.6 (-113.5, 114.6)
<i>DLOOP</i> Average ¹	17.7 (-85.8, 121.2)	-7.7 (-113.9, 98.5)	12.17 (-91.4, 115.7)
<i>CYTB</i> CpG1 ¹	20.8 (-44.4, 86.0)	0.7 (-66.1, 67.4)	-1.6 (-69.5, 66.4)
<i>CYTB</i> CpG2 ¹	17.5 (-39.5, 74.5)	18.7 (-36.6, 74.1)	17.0 (-40.1, 74.1)
<i>CYTB</i> CpG3 ¹	60.3 (-15.5, 136.1)	73.3 (-2.1, 148.6)[#]	54.4 (-20.5, 129.4)
<i>CYTB</i> Average ¹	28.1 (-49.0, 105.2)	22.8 (-53.7, 99.3)	13.9 (-64.1, 92.0)
<i>MTTF</i> S1 CpG1 ¹	66.2 (-19.4, 151.8)	54.1 (-30.4, 138.6)	34.8 (-49.2, 118.9)
<i>MTTF</i> S2 CpG1 ²	8.2 (-23.9, 40.3)	17.8 (-16.3, 51.9)	17.0 (-17.1, 51.1)
<i>MTTF</i> S2 CpG2 ²	-4.5 (-28.2, 19.1)	16.2 (-18.92, 51.4)	10.7 (-22.8, 44.1)
<i>MTTF</i> S2 Average ²	1.1 (-27.8, 29.9)	17.7 (-16.7, 52.0)	14.0 (-20.3, 48.2)
Covariate-adjusted linear regression models accounted for plate number or hBG, race/ethnicity, income, age, tobacco use, pre-pregnancy BMI, week of bloodspot collection, parity, and fetal sex. Bolded values are considered meaningful. *p ≤ 0.05; [#] p ≤ 0.10.			
¹ Gene sites were ln-transformed and β (95% CIs) were back-transformed using the following equation: $\beta \cdot \ln(2)$. Results interpreted as the percent change in birthweight for each two-fold increase in mtDNA methylation.			
² Gene sites not transformed and β (95% CIs) were not adjusted. Results interpreted as the change in birthweight for each one-unit increase in mtDNA methylation.			
BMI, body mass index; CYTB, cytochrome b; <i>DLOOP</i> , D-loop promoter region, heavy strand; GDM, gestational diabetes mellitus; hBG, human β -globin; <i>LDLR</i> , D-loop promoter region, light strand; MTTF, mitochondrially encoded tRNA phenylalanine; <i>NDI</i> , NADH-ubiquinone oxidoreductase chain 1; S1, location 1; S2, location 2.			

Supplemental Table 4. Sensitivity analyses evaluating associations between maternal mtDNAcn and mtDNA methylation with birthweight z-score using linear regression.

Model	Unadjusted n=196-396		No hypertension n=175-358		No GDM n=184-369	
Gene/site	β (95% CI)	p	β (95% CI)	p	β (95% CI)	p
<i>NDI</i> ¹	0.09 (-0.06, 0.25)	0.22	0.08 (-0.14, 0.30)	0.46	0.01 (-0.20, 0.23)	0.90
<i>LDLR</i> CpG1 ¹	0.03 (-0.11, 0.17)	0.65	0.11 (-0.04, 0.26)	0.16	0.11 (-0.05, 0.26)	0.17
<i>LDLR</i> CpG2 ¹	0.11 (-0.06, 0.28)	0.21	0.16 (-0.04, 0.36)	0.11	0.14 (-0.06, 0.33)	0.17
<i>LDLR</i> CpG3 ¹	0.08 (-0.07, 0.23)	0.30	0.11 (-0.06, 0.28)	0.20	0.12 (-0.04, 0.29)	0.14
<i>LDLR</i> Average ¹	0.04 (-0.12, 0.20)	0.63	0.14 (-0.05, 0.33)	0.15	0.15 (-0.04, 0.34)	0.12
<i>DLOOP</i> CpG1 ¹	-0.01 (-0.17, 0.14)	0.85	-0.09 (-0.26, 0.07)	0.26	-0.03 (-0.18, 0.13)	0.73
<i>DLOOP</i> CpG2 ¹	-0.04 (-0.24, 0.17)	0.72	-0.01 (-0.23, 0.21)	0.92	-0.01 (-0.23, 0.21)	0.95
<i>DLOOP</i> CpG3 ¹	-0.04 (-0.25, 0.17)	0.70	-0.03 (-0.27, 0.22)	0.83	-0.01 (-0.24, 0.21)	0.90
<i>DLOOP</i> Average ¹	-0.01 (-0.20, 0.18)	0.92	-0.06 (-0.26, 0.15)	0.59	0.00 (-0.20, 0.20)	0.99
<i>CYTB</i> CpG1 ¹	0.00 (-0.12, 0.12)	0.97	-0.02 (-0.14, 0.11)	0.81	-0.03 (-0.16, 0.10)	0.63
<i>CYTB</i> CpG2 ¹	0.00 (-0.11, 0.10)	0.95	0.04 (-0.07, 0.14)	0.49	0.03 (-0.08, 0.13)	0.62
<i>CYTB</i> CpG3 ¹	0.08 (-0.06, 0.22)	0.26	0.12 (-0.02, 0.27) [#]	0.09	0.09 (-0.05, 0.23)	0.19
<i>CYTB</i> Average ¹	0.02 (-0.12, 0.16)	0.82	0.05 (-0.09, 0.20)	0.48	0.03 (-0.12, 0.18)	0.70
<i>MTTF</i> S1 CpG1 ¹	0.07 (-0.08, 0.23)	0.34	0.04 (-0.12, 0.20)	0.60	0.03 (-0.13, 0.18)	0.74
<i>MTTF</i> S2 CpG1 ²	0.04 (-0.02, 0.10)	0.21	0.04 (-0.02, 0.11)	0.22	0.04 (-0.02, 0.11)	0.19
<i>MTTF</i> S2 CpG2 ²	0.02 (-0.02, 0.07)	0.28	0.04 (-0.02, 0.11)	0.21	0.04 (-0.03, 0.10)	0.26
<i>MTTF</i> S2 Average ²	0.04 (-0.01, 0.09)	0.13	0.05 (-0.01, 0.12)	0.12	0.05 (-0.01, 0.12)	0.11

Covariate-adjusted linear regression models accounted for plate number or hBG, race/ethnicity, income, age, tobacco use, pre-pregnancy BMI, week of bloodspot collection, parity, and fetal sex. **Bolded values are considered meaningful.** *p ≤ 0.05; #p ≤ 0.10.

¹Gene sites were ln-transformed and β (95% CIs) were back-transformed using the following equation: $\beta * \ln(2)$. Results interpreted as the percent change in birthweight z-score for each two-fold increase in mtDNA methylation.

²Gene sites not transformed and β (95% CIs) were not adjusted. Results interpreted as the change in birthweight z-score for each one-unit increase in mtDNA methylation.

BMI, body mass index; CYTB, cytochrome b; *DLOOP*, D-loop promoter region, heavy strand; GDM, gestational diabetes mellitus; hBG, human β -globin; *LDLR*, D-loop promoter region, light strand; MTTF, mitochondrially encoded tRNA phenylalanine; *NDI*, NADH-ubiquinone oxidoreductase chain 1; S1, location 1; S2, location 2.

Supplemental Table 5. Associations of maternal mtDNAcn and mtDNA methylation with gestation length overall, using quantile regression, where gestational age at birth is imputed for n=89 women whose obstetric estimate was used in main analyses.

Quantile	5 th Percentile	25 th Percentile	50 th Percentile	75 th Percentile	95 th Percentile
Gene/site	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
<i>NDI</i> ¹	4.8 (-2.0, 11.6)	1.1 (-3.0, 5.2)	-0.6 (-3.0, 1.7)	1.1 (-0.8, 3.0)	-2.4 (-5.2, 0.4) #
<i>LDLR</i> CpG1 ¹	-0.7 (-3.9, 2.5)	-0.5 (-3.1, 2.2)	1.0 (-0.7, 2.7)	-0.1 (-1.5, 1.3)	-0.2 (-2.1, 1.8)
<i>LDLR</i> CpG2 ¹	-0.2 (-5.6, 5.2)	-1.0 (-4.4, 2.5)	-0.9 (-3.2, 1.4)	0.0 (-1.8, 1.8)	1.1 (-0.9, 3.2)
<i>LDLR</i> CpG3 ¹	-2.4 (-6.6, 1.7)	0.4 (-2.3, 3.1)	0.6 (-1.6, 2.8)	-0.2 (-1.5, 1.1)	2.5 (0.7, 4.2) *
<i>LDLR</i> Average ¹	0.0 (-4.9, 4.8)	-0.9 (-4.1, 2.4)	1.0 (-1.1, 3.2)	-0.3 (-2.0, 1.4)	0.2 (-2.1, 2.6)
<i>DLOOP</i> CpG1 ¹	3.4 (-0.5, 7.3) #	2.3 (-0.5, 5.1) #	1.4 (-0.4, 3.1)	0.6 (-0.7, 2.0)	-1.3 (-2.7, 0.2) #
<i>DLOOP</i> CpG2 ¹	3.9 (-2.8, 10.6)	1.6 (-2.2, 5.4)	1.0 (-1.4, 3.5)	0.5 (-1.4, 2.3)	-2.6 (-5.1, -0.1) *
<i>DLOOP</i> CpG3 ¹	-4.7 (-7.9, -1.4) *	0.2 (-3.4, 3.8)	0.8 (-1.9, 3.5)	0.8 (-1.1, 2.8)	0.2 (-2.1, 2.5)
<i>DLOOP</i> Average ¹	5.5 (-0.7, 11.6) #	2.6 (-0.8, 6.0)	1.9 (-0.5, 4.2)	1.6 (-0.3, 3.5) #	-2.0 (-4.0, -0.1) *
<i>CYTB</i> CpG1 ¹	2.7 (0.0, 5.4) *	0.9 (-1.6, 3.3)	1.4 (-0.2, 2.9) #	0.9 (-0.1, 1.8) #	1.0 (0.0, 2.0) *
<i>CYTB</i> CpG2 ¹	2.5 (0.4, 4.7) *	-0.5 (-2.5, 1.5)	-0.5 (-1.6, 0.6)	0.5 (-0.4, 1.5)	0.0 (-1.0, 1.1)
<i>CYTB</i> CpG3 ¹	2.1 (-0.7, 4.9)	0.0 (-2.7, 2.7)	0.4 (-1.2, 1.9)	1.0 (-0.3, 2.2)	0.7 (-0.3, 1.7)
<i>CYTB</i> Average ¹	3.3 (1.1, 5.6) *	0.1 (-2.7, 2.9)	0.5 (-1.2, 2.2)	1.1 (-0.1, 2.4) #	1.5 (0.2, 2.9) *
<i>MTTF</i> S1 CpG1 ¹	7.7 (3.2, 12.1) *	2.4 (-0.1, 4.9) #	1.1 (-1.0, 3.2)	0.5 (-0.9, 1.9)	-0.8 (-2.3, 0.8)
<i>MTTF</i> S2 CpG1 ²	-0.2 (-1.3, 0.9)	-0.2 (-1.6, 1.1)	-0.1 (-0.9, 0.7)	0.3 (-0.2, 0.8)	0.5 (-0.3, 1.3)
<i>MTTF</i> S2 CpG2 ²	0.2 (-1.1, 1.4)	-0.5 (-1.7, 0.7)	-0.1 (-0.9, 0.6)	-0.2 (-0.7, 0.3)	0.2 (-0.8, 1.1)
<i>MTTF</i> S2 Average ²	-0.5 (-1.8, 0.9)	-0.9 (-2.1, 0.4)	-0.2 (-0.9, 0.6)	0.2 (-0.3, 0.8)	0.4 (-0.6, 1.4)

Covariate-adjusted quantile regression models accounted for plate number or hBG, race/ethnicity, income, age, tobacco use, pre-pregnancy BMI, week of bloodspot collection, parity, and fetal sex. **Bolded values are considered meaningful.** *p ≤ 0.05; #p ≤ 0.10.

¹Gene sites were ln-transformed and β (95% CIs) were back-transformed using the following equation: $\beta * \ln(2)$. Results interpreted as the percent change in gestation length for each two-fold increase in mtDNAcn or mtDNA methylation.

²Gene sites not transformed and β (95% CIs) were not adjusted. Results interpreted as the change in gestation length (in days) for each one-unit increase in mtDNA methylation.

BMI, body mass index; *CYTB*, cytochrome b; *DLOOP*, D-loop promoter region, heavy strand; hBG, human β -globin; *LDLR*, D-loop promoter region, light strand; *MTTF*, mitochondrially encoded tRNA phenylalanine; *NDI*, NADH-ubiquinone oxidoreductase chain 1; S1, location 1; S2, location 2.

Supplemental Table 6. Associations of maternal mtDNAcn and mtDNA methylation with gestation length in females, using quantile regression, where gestational age at birth is imputed for n=42 women whose obstetric estimate was used in main analyses.

Quantile	5 th Percentile	25 th Percentile	50 th Percentile	75 th Percentile	95 th Percentile
Gene/site	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
<i>NDI</i> ¹	7.7 (-4.6, 20.0)	-1.4 (-6.0, 3.3)	-2.7 (-5.8, 0.4)	-0.2 (-4.3, 3.8)	-3.5 (-7, -0.1) *
<i>LDLR</i> CpG1 ¹	2.1 (-0.6, 4.9)	-0.9 (-4.2, 2.5)	-0.6 (-2.9, 1.8)	-0.6 (-2.6, 1.3)	1.7 (-0.5, 3.9)
<i>LDLR</i> CpG2 ¹	4.2 (1.6, 6.8) *	-2.4 (-6.7, 2.0)	-0.4 (-3.4, 2.6)	-1.6 (-4.7, 1.4)	3.3 (1.4, 5.1) *
<i>LDLR</i> CpG3 ¹	3.5 (-33.8, 40.7)	0.0 (-3.6, 3.7)	0.6 (-2.0, 3.2)	-1.0 (-3.4, 1.4)	1.3 (-0.1, 2.8) #
<i>LDLR</i> Average ¹	2.7 (-1.6, 7.1)	-1.9 (-6.1, 2.3)	-0.1 (-2.9, 2.7)	-1.6 (-4.4, 1.1)	3.0 (1.0, 4.9) *
<i>DLOOP</i> CpG1 ¹	2.8 (-7.0, 12.7)	4.0 (1.0, 7.0) *	2.9 (0.2, 5.6) *	2.4 (0.0, 4.9) *	1.8 (-1.0, 4.6)
<i>DLOOP</i> CpG2 ¹	1.0 (-16.3, 18.3)	0.8 (-3.7, 5.2)	2.0 (-1.6, 5.5)	0.2 (-3.3, 3.7)	3.0 (-0.6, 6.6) #
<i>DLOOP</i> CpG3 ¹	1.0 (-7.0, 9.0)	2.7 (-1.2, 6.5)	1.9 (-2.1, 5.9)	0.0 (-3.6, 3.5)	0.6 (-6.4, 7.6)
<i>DLOOP</i> Average ¹	3.8 (-8.7, 16.3)	4.1 (0.3, 7.8) *	2.8 (-0.8, 6.4)	2.9 (-0.6, 6.3) #	1.8 (-2.3, 5.9)
<i>CYTB</i> CpG1 ¹	0.2 (-12.3, 12.7)	-0.2 (-2.5, 2.1)	1.4 (-0.6, 3.5)	1.0 (-0.7, 2.7)	1.0 (-2.0, 4.0)
<i>CYTB</i> CpG2 ¹	3.1 (-8.4, 14.5)	-1.1 (-3.3, 1.1)	-1.3 (-3.0, 0.4)	0.9 (-0.4, 2.2)	0.8 (0.1, 1.4) *
<i>CYTB</i> CpG3 ¹	2.2 (-10.3, 14.6)	-0.9 (-3.6, 1.8)	3.1 (0.5, 5.6) *	2.1 (0.6, 3.7) *	1.6 (-0.5, 3.7)
<i>CYTB</i> Average ¹	3.3 (-10.2, 16.7)	-0.9 (-3.7, 1.9)	1.4 (-0.8, 3.7)	1.9 (0.0, 3.7) *	3.2 (0.5, 5.9) *
<i>MTTF</i> S1 CpG1 ¹	10.2 (5.2, 15.2) *	2.5 (-0.9, 5.9)	2.4 (-0.6, 5.3)	1.6 (-0.8, 3.9)	0.0 (-3.4, 3.3)
<i>MTTF</i> S2 CpG1 ²	-0.1 (-5.0, 4.8)	-0.5 (-1.8, 0.9)	0.0 (-1.1, 1.2)	0.2 (-0.8, 1.1)	1.1 (1.0, 1.3) *
<i>MTTF</i> S2 CpG2 ²	0.3 (-12.1, 12.7)	-0.8 (-1.9, 0.3)	0.0 (-1.2, 1.2)	-0.4 (-1.3, 0.5)	0.8 (0.5, 1.0) *
<i>MTTF</i> S2 Average ²	0.5 (-4.5, 5.5)	-0.9 (-2.3, 0.6)	0.0 (-1.1, 1.2)	0.1 (-0.9, 1.1)	0.9 (0.1, 1.7) *

Covariate-adjusted quantile regression models accounted for plate number or hBG, race/ethnicity, income, age, tobacco use, pre-pregnancy BMI, week of bloodspot collection, parity, and fetal sex. **Bolded values are considered meaningful.** *p ≤ 0.05; #p ≤ 0.10.

¹Gene sites were ln-transformed and β (95% CIs) were back-transformed using the following equation: $\beta \cdot \ln(2)$. Results interpreted as the percent change in gestation length for each two-fold increase in mtDNAcn or mtDNA methylation.

²Gene sites not transformed and β (95% CIs) were not adjusted. Results interpreted as the change in gestation length (in days) for each one-unit increase in mtDNA methylation.

BMI, body mass index; *CYTB*, cytochrome b; *DLOOP*, D-loop promoter region, heavy strand; hBG, human β -globin; *LDLR*, D-loop promoter region, light strand; *MTTF*, mitochondrially encoded tRNA phenylalanine; *NDI*, NADH-ubiquinone oxidoreductase chain 1; S1, location 1; S2, location 2.

Supplemental Table 7. Associations of maternal mtDNAcn and mtDNA methylation with gestation length in males, using quantile regression, where gestational age at birth is imputed for n=47 women whose obstetric estimate was used in main analyses.

Quantile	5 th Percentile	25 th Percentile	50 th Percentile	75 th Percentile	95 th Percentile
Gene/site	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)	β (95%CI)
<i>NDI</i> ¹	3.7 (0.0, 7.3) *	4.1 (-3.7, 11.9)	1.2 (-2.4, 4.8)	0.9 (-2.3, 4.0)	1.9 (-4.3, 8.0)
<i>LDLR</i> CpG1 ¹	-1.1 (-5.0, 2.9)	0.2 (-4.1, 4.4)	1.5 (-0.9, 4.0)	1.2 (-0.7, 3.1)	-0.1 (-3.8, 3.6)
<i>LDLR</i> CpG2 ¹	-1.2 (-1.7, -0.8) *	0.2 (-5.5, 5.9)	-1.1 (-3.9, 1.6)	0.6 (-1.5, 2.6)	-2.9 (-9.7, 3.9)
<i>LDLR</i> CpG3 ¹	2.5 (2.3, 2.7) *	-0.4 (-5.0, 4.2)	-0.8 (-3.7, 2.1)	-0.2 (-2.8, 2.4)	-0.4 (-7.0, 6.3)
<i>LDLR</i> Average ¹	-1.7 (-5.9, 2.5)	1.5 (-3.9, 7.0)	1.0 (-2.2, 4.2)	1.3 (-0.9, 3.5)	-2.2 (-6.7, 2.2)
<i>DLOOP</i> CpG1 ¹	5.9 (3.3, 8.6) *	0.4 (-3.9, 4.6)	-0.2 (-2.4, 2.0)	-1.1 (-2.8, 0.6)	-3.1 (-6.2, 0.0) *
<i>DLOOP</i> CpG2 ¹	-0.4 (-6.7, 5.8)	3.5 (-2.6, 9.7)	0.5 (-2.8, 3.7)	1.6 (-1.2, 4.4)	-2.8 (-8.2, 2.6)
<i>DLOOP</i> CpG3 ¹	-0.9 (-7.4, 5.6)	-1.6 (-7.3, 4.1)	0.6 (-3.2, 4.3)	1.3 (-1.3, 3.9)	0.0 (-2.4, 2.4)
<i>DLOOP</i> Average ¹	7.2 (4.6, 9.9) *	0.6 (-4.9, 6.1)	-0.2 (-3.1, 2.7)	-0.7 (-3.2, 1.9)	-2.9 (-6.3, 0.4) #
<i>CYTB</i> CpG1 ¹	9.0 (5.0, 12.9) *	3.5 (-0.4, 7.3) #	1.3 (-0.9, 3.4)	0.9 (-0.6, 2.5)	-0.7 (-1.5, 0.1) #
<i>CYTB</i> CpG2 ¹	0.1 (-1.5, 1.8)	1.4 (-1.8, 4.7)	-0.2 (-1.8, 1.5)	0.4 (-0.7, 1.6)	-1.0 (-1.6, -0.4) *
<i>CYTB</i> CpG3 ¹	2.3 (-0.4, 5.0) #	2.3 (-1.5, 6.2)	0.3 (-1.8, 2.5)	0.3 (-1.3, 1.8)	-1.1 (-1.5, -0.7) *
<i>CYTB</i> Average ¹	0.2 (-2.0, 2.4)	1.9 (-2.4, 6.1)	0.7 (-1.6, 3.0)	0.3 (-1.4, 2.0)	-0.9 (-1.7, -0.1) *
<i>MTTF</i> S1 CpG1 ¹	5.0 (2.0, 8.0) *	1.8 (-3.8, 7.5)	-0.3 (-3.0, 2.5)	-0.9 (-3.1, 1.2)	-1.6 (-4.8, 1.6)
<i>MTTF</i> S2 CpG1 ²	-0.4 (-1.7, 1.0)	-0.3 (-2.3, 1.7)	-0.1 (-1.1, 0.9)	0.2 (-0.5, 0.9)	-1.0 (-1.4, -0.6) *
<i>MTTF</i> S2 CpG2 ²	-0.3 (-0.8, 0.2)	-0.6 (-2.8, 1.5)	-0.7 (-1.7, 0.3)	-0.2 (-0.9, 0.5)	-0.7 (-2.1, 0.7)
<i>MTTF</i> S2 Average ²	-1.2 (-2.7, 0.2) #	-1.2 (-2.9, 0.6)	-0.2 (-1.2, 0.7)	-0.2 (-0.8, 0.5)	-1.1 (-1.5, -0.6) *

Covariate-adjusted quantile regression models accounted for plate number or hBG, race/ethnicity, income, age, tobacco use, pre-pregnancy BMI, week of bloodspot collection, parity, and fetal sex. **Bolded values are considered meaningful.** *p ≤ 0.05; #p ≤ 0.10.

¹Gene sites were ln-transformed and β (95% CIs) were back-transformed using the following equation: $\beta * \ln(2)$. Results interpreted as the percent change in gestation length for each two-fold increase in mtDNAcn or mtDNA methylation.

²Gene sites not transformed and β (95% CIs) were not adjusted. Results interpreted as the change in gestation length (in days) for each one-unit increase in mtDNA methylation.

BMI, body mass index; *CYTB*, cytochrome b; *DLOOP*, D-loop promoter region, heavy strand; hBG, human β -globin; *LDLR*, D-loop promoter region, light strand; *MTTF*, mitochondrially encoded tRNA phenylalanine; *NDI*, NADH-ubiquinone oxidoreductase chain 1; S1, location 1; S2, location 2.