Online Supplementary Information

Neonatal Bloodspot DNA Methylation Patterns are Associated with Childhood Weight Status in the Healthy Families Project

Running Title: Bloodspot DNA Methylation and Obesity

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Variables	12-24 months (n=40)		3-5 years (n=40)		10-12 years (n=52)	
	Count (n)	(%)	Count (n)	(%)	Count (n)	(%)
Neonatal Bloodspots	40	-100	40	-100	52	-100
Childhood Blood Draws	19	-47.5	26	-65	20	-38.5
Child Gender						
Male	20	-50	22	-55	28	-54
Female	20	-50	18	-45	24	-46
Child Race*						
White	24	-60	31	-77.5	38	-73.1
Black	6	-15	7	-17.5	9	-17.3
American Indian or Alaska Native	0	0	0	0	1	0
Asian or Pacific Islander	8	-20	1	-2.5	1	-1.9
Biracial	2	-5	1	-2.5	3	-5.7
Other	0	0	0	0	0	0
Child Ethnicity						
Hispanic or Latino	4	-10	2	-5	2	-3.8
Not hispanic or latino	36	-90	38	-95	50	-96.2
Maternal education						
Did not complete High School	6	-15	1	-2.5	4	-7.7
Graduated High School	4	-10	2	-5	9	-17.3
Completed GED	1	-2.5	0	0	1	-1.9
Have some college courses	9	-22.5	10	-25	13	-25
Completed a 2-year degree	7	-17.5	6	-15	7	-13.4
Completed a 4-year degree	13	-32.5	21	-52.5	18	-34.6

*p<0.05

Table S1 - Healthy Families Demographics by Age Group. Based on consent, a subset of n=65 children provided later-life blood draw samples for longitudinal follow-up. By age group,

19 toddlers, 26 pre-schoolers, and 20 school-aged children consented to a childhood blood draw. For the child race/ethnicity and maternal education variables, a chi-squared test of equal proportions was used to check for significant deviations from the total population. Child race showed a significant change in proportion ($p=0.013$) in the toddler (12-24 months) age group compared to the total population. Ethnicity and maternal education variables did not significantly differ by age group.

Table S1 – Pyrosequencing primer sequences and PCR conditions.

Assay	LINE-1	*IGF2*[1]	*H19*[1]	*PPARA*	*LEP*[2]	*ESR1*	*SREBF1*[3]
Location (hg19)	Repetitive Element	chr11: 2169499; chr11: 2169515; chr11: 2169518	chr11: 2024254; chr11: 2024257; chr11: 2024259; chr11: 2024261	chr22: 46545064; chr22: 46545083	chr7: 127881127; chr7: 127881129; chr7:12788113	chr6: 152128834; chr6: 152128841; chr6: 152128845; chr6: 152128868	chr17: 17723203; chr17: 17723288; chr17: 17723297; chr17: 17723305
Forward PCR Primer	5'-TTGAGTTAGGTGTGGGATATAGTT-3'	5'-GGAGGGGGTTTATTTTTTTAGGAAG-3'	5'-TTTGTTGATTTTATTAAGGGAG-3'	5'-GGAGGTTTTTATGAGGATGTAGTT-3'	5'-GAGTTTTTGGAGGGATATTAAGGAT-3'	5'-GTTGGAGGTTAGGGAGTTTAGGA-3'	5'-TTTGTTTGGGTTTTGATGTAAATGTA-3'
Reverse PCR Primer	5'-[Biotin]-CAAAAAATCAAAAAATTCCCTTTCC-3'	5'-[Biotin]-AACCCCAACAAAAACCACTAAACAC-3'	5'-[Biotin]-CTATAAATAAACCCCAACCAAAC-3'	5'-[Biotin]-ACACATATTAACCAACAATAACTATCAT-3'	5'-[Biotin]-CAAAATTATATAAAACCCTATAACCTACCA-3'	5'-[Biotin]-CTAACCCCCACCCTACCCC-3'	5'-[Biotin]-ATTCAACTCCACCCCTATATTAAACTAC-3'
Sequencing Primer	5'-AGGTGTGGATATAGT-3'	5'-GGGGTTTATTTTTTAGGA-3'	5'-GTGTGGAATTAGAAGT-3'	5'-GGATGTGGTTGTTTG-3'	5'-GGGAGGTATTTAAGGG-3'; 5'-GGGAGGGGAGGGAGTTGG-3'	5'-GGTTAGGGAGTTTAGGAG-3'	5'-GTATTGGTTTTAAGTTAGGTT-3'
Sequence to Analyze	TTYGTGGTGYGTYGTTTTTTAAGTYGGTTTGAAAAG	AGTATAGTTAYGTYGTTTTTTATTGGTTTYGTTAAGTAGA	GGTYGYGYGGYGGTAGTGTAGGTTTATATA TTATAGTT	TATATTTTAYGAGATATGTAGGATATTAYGTGTATAGGTTATTTTATAAATTTTGAAATAA	TGYGYGYGTGGTTTTTGGHG	TTGGYGGAGGGYGTTYGTTTGGGATTGTATTTGTTTTYGT	TTTAGTGYGAGGTTGYGTTTATTTYGGTAATAAGTATATTAGGATTGTTAGTAATAGGGTATTTTAAGTAGTYGAGTGGAGTTTTAGTTTTTAAAGTTTG
Amplicon Length (bp)	~150 bp	93	145	198	383	119	293

Annealing Temperature (°C)	55	55	51	54.5	52	58/56*	56
Number of Cycles	48	50	50	48	50	15/29*	44
Number of CpG Sites	4	3	4	2	3	4	4
Location	Promoter	DMR	DMR	Promoter	Promoter	Promoter	Gene body

Table S2 - Pyrosequencing primer sequences and PCR conditions. Pyrosequencing primer sequences and PCR conditions. Information for each assay, including genomic location (individual CpGs analyzed), primer sequences (5'-3'), sequence to analyze, amplicon length, annealing temperature, number of cycles, and number of CpG sites measured. Touchdown PCR was used for *ESR1* assay, as indicated by the multiple temperatures and cycle numbers. Location refers to regulatory region where CpG sites are located for each assay. [1] = Assay adapted from Hoyo *et al.,* 2011 and Murphy *et al.,* 2012; [2] = Assay adapted from Lesseur *et al.,* 2013; [3] = Assay adapted from Adaikalakoteswari *et al.,* 2016.

References

1. Lesseur C, Armstrong DA, Paquette AG, Koestler DC, Padbury JF, Marsit CJ. Tissue-specific Leptin promoter DNA methylation is associated with maternal and infant perinatal factors. *Mol Cell Endocrinol.* 2013; 381:160–7.

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3. Hoyo, C., Murtha, A. P., Schildkraut, J. M., Jirtle, R., Demark-Wahnefried, W., Forman, M. R., Iversen, E.S., Kurtzberg, J., Overcash, F., Huang, Z., and Murphy, S. K. Methylation variation at IGF2 differentially methylated regions and maternal folic acid use before and during pregnancy. *Epigenetics* 2011; 6: 928–936.

4. Murphy, S. K., Huang, Z., & Hoyo, C. Differentially Methylated Regions of Imprinted Genes in Prenatal, Perinatal and Postnatal Human Tissues. *PLoS ONE* 2012; 7: e40924.

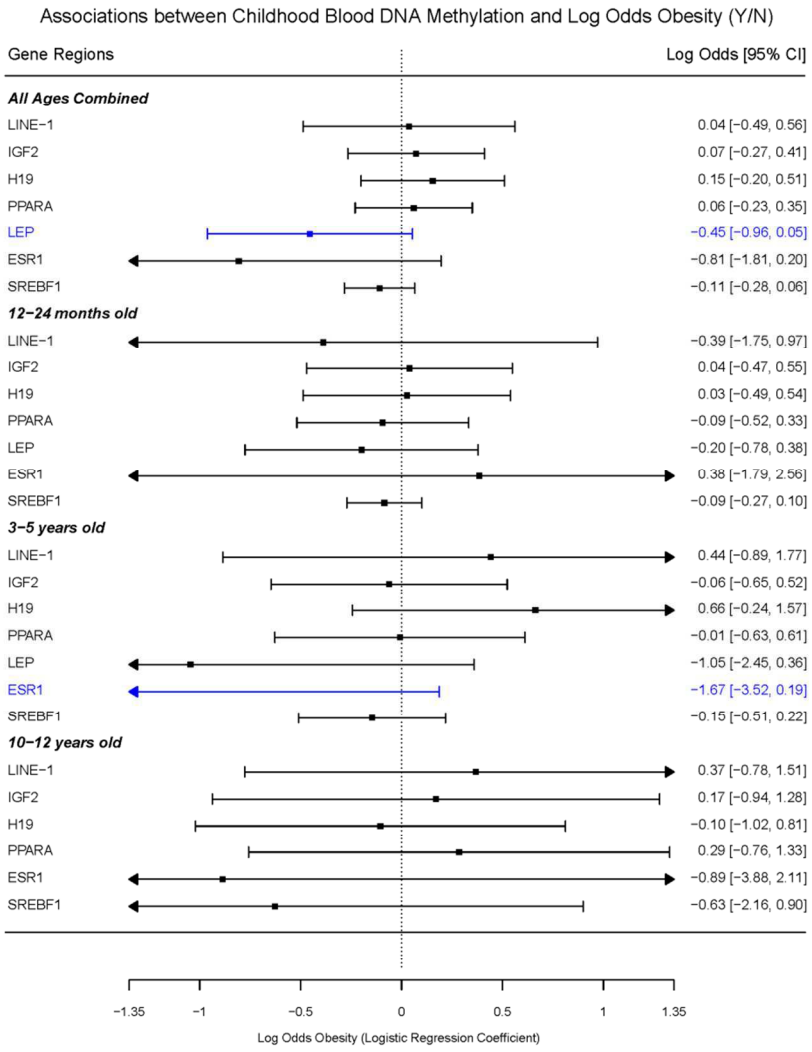

Figure S1 - Associations between Childhood Blood DNA Methylation and Log Odds Obesity (Y/N). Forest plot of obesity likelihood by childhood blood draw DNA methylation at investigated target genes. Logistic regression coefficients are represented as log odds of obesity in the right column. The left column shows how the obesity likelihood outcome was grouped by age in the regression models. It also lists all of the target gene methylation predictors included in bloodspot logistic regression models. For the 10-12 year old age group, no *LEP* methylation data was available for obese individuals. Trends that approach significance ($p<0.10$) are indicated in blue.

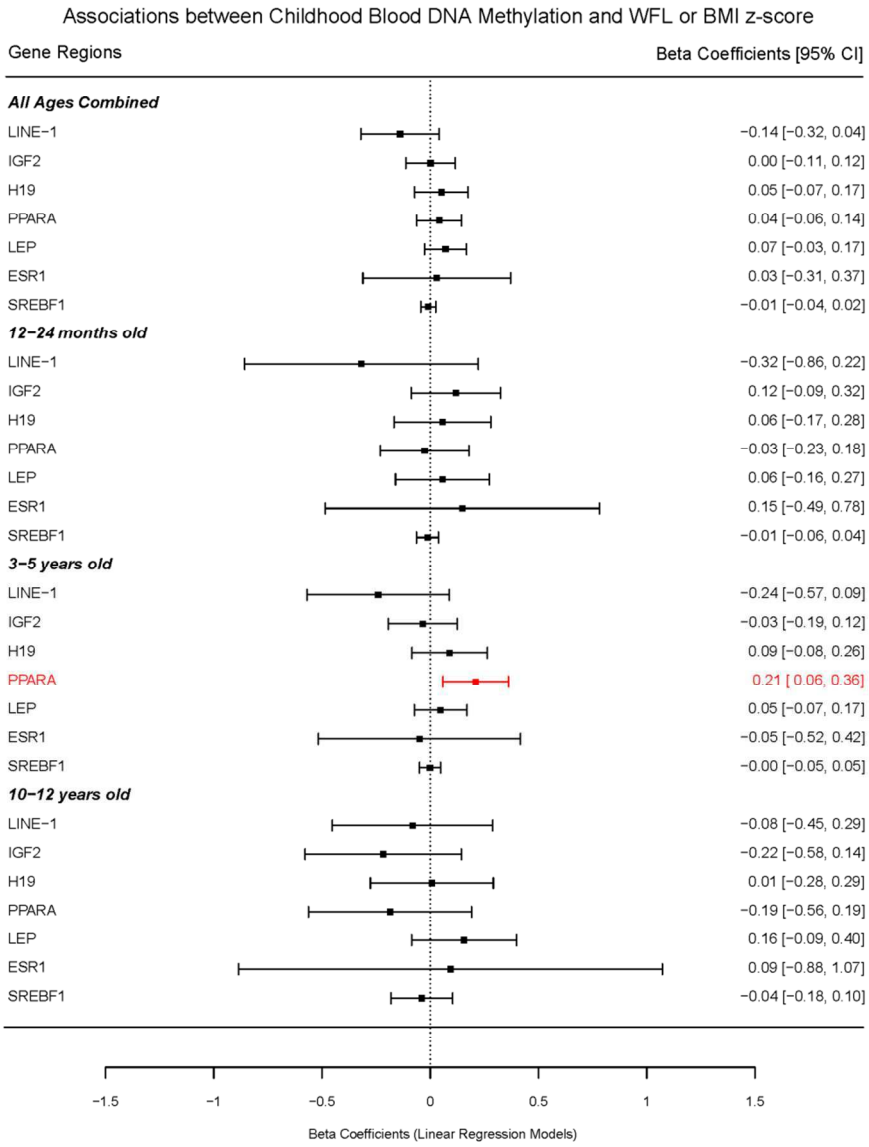

Figure S2 - Associations between Childhood Blood DNA Methylation and WFL or BMI z-score. Forest plot of continuous WFL or BMI z-score by childhood blood draw DNA methylation at investigated target genes. Linear regression coefficients are shown in the right column. The left column shows how the regression models were grouped by age. It also lists all of the target gene methylation predictors included in blood logistic regression models. Significant positive association between *PPARA* blood DNA methylation and WFL or BMI z-score ($p<0.05$) is indicated in red.