

Epigenome-Wide DNA Methylation Analyses Identifies Novel Differentially Methylated Regions in Childhood Obesity

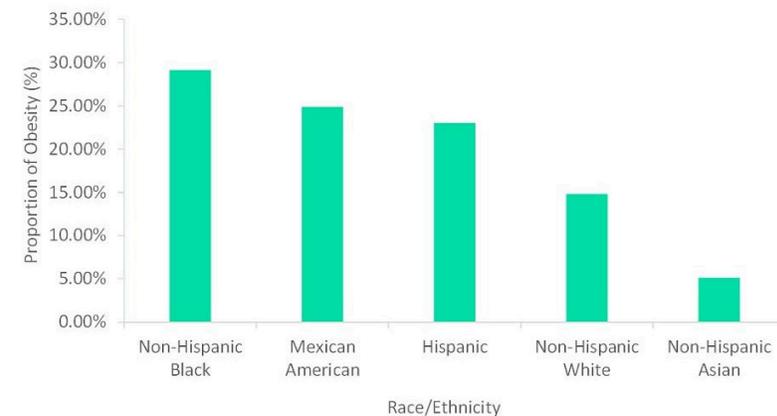
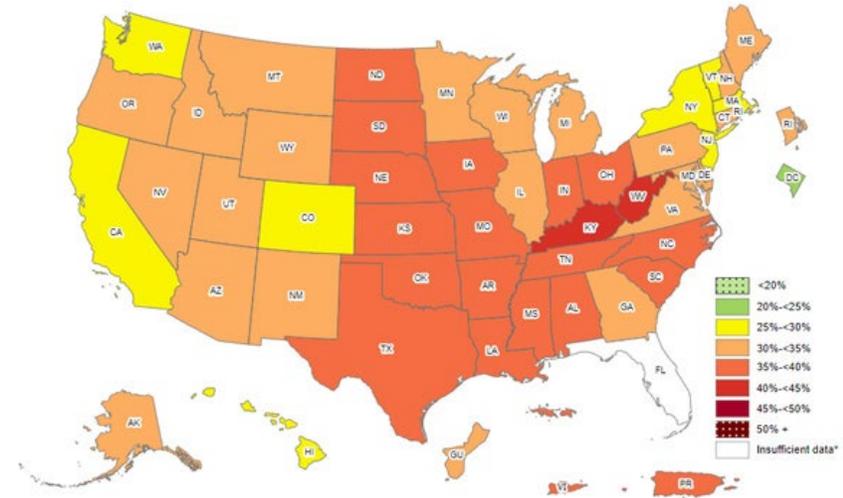
Priyadarshni Patel,
Major Professor: Dr. Geetha Thangiah
Department of Nutritional Sciences





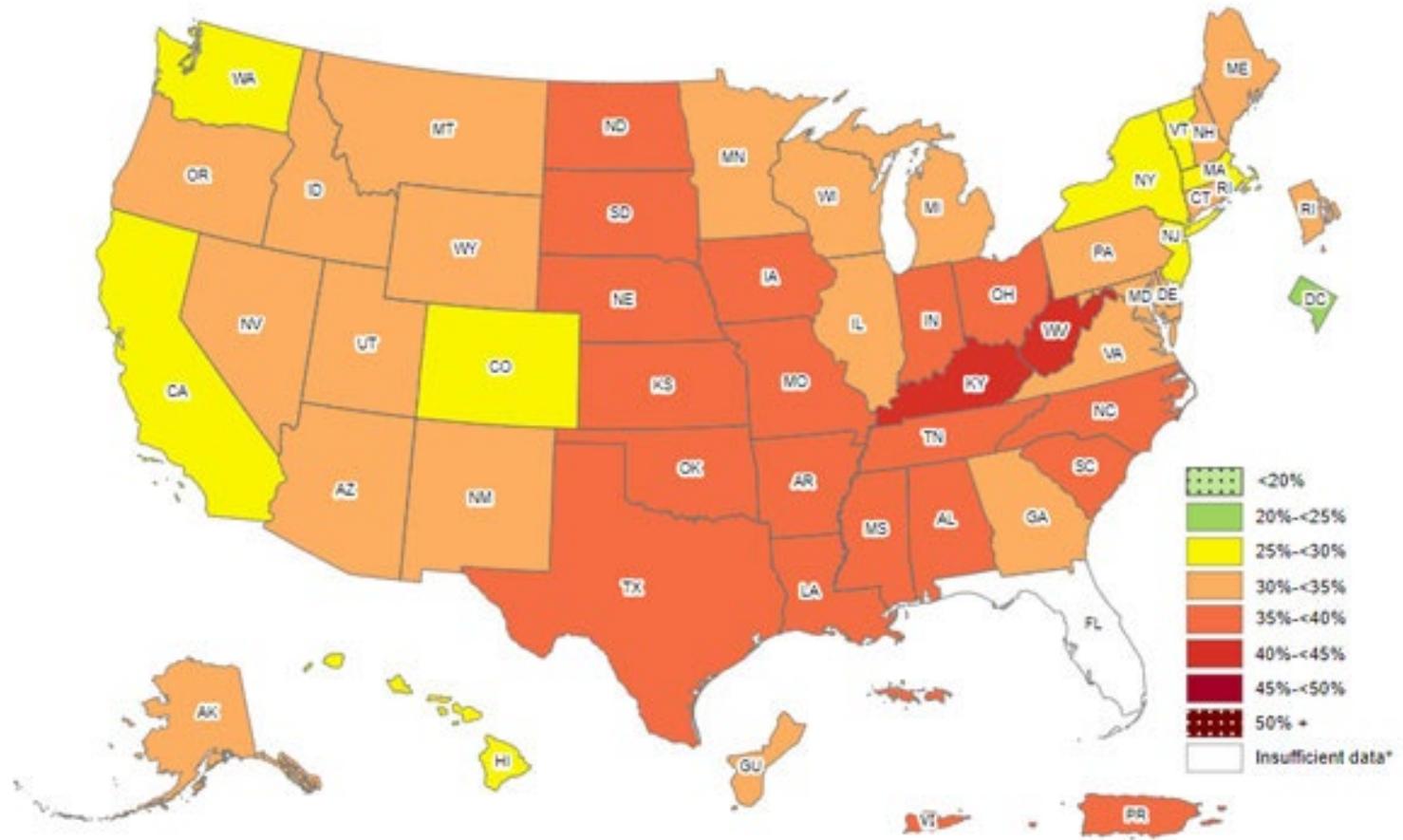
Childhood Obesity

- Childhood obesity remains an epidemic for the United States and the entire world, affecting the health of millions of children.
- In 2022, we see a historic high in the percent of obese children, which rose from 19% pre-pandemic to 22%.
- Alabama has the fifth-highest rate of obesity with a 21.8% childhood obesity rate.
- The prevalence of childhood obesity is significantly higher among racial and/or ethnic minority children in the United States.





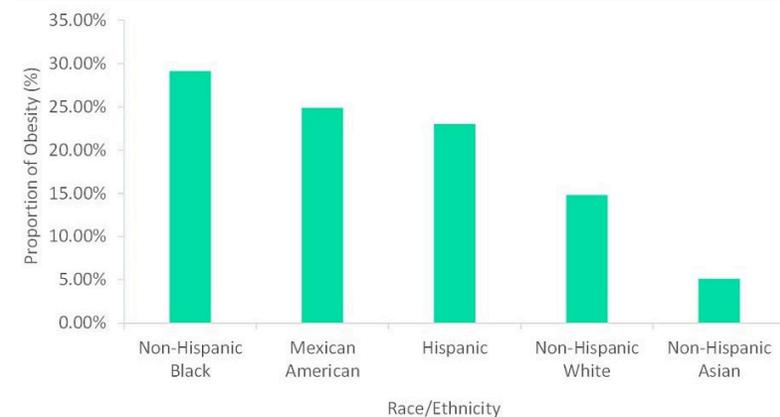
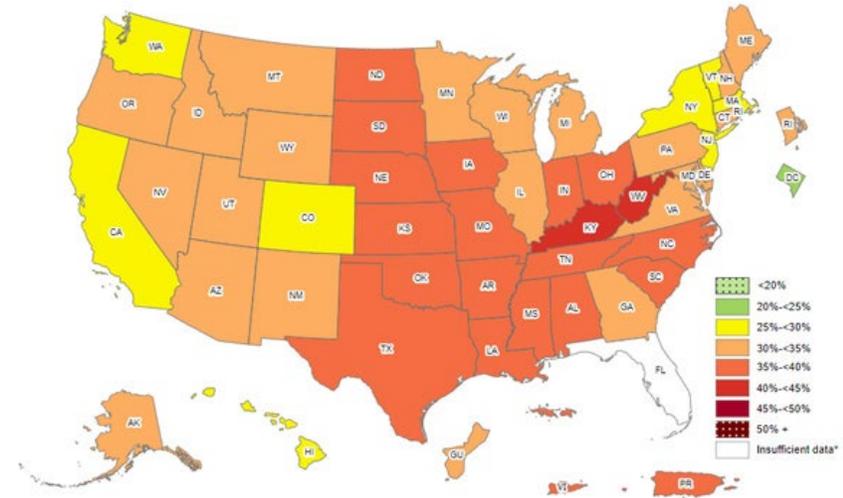
Childhood Obesity





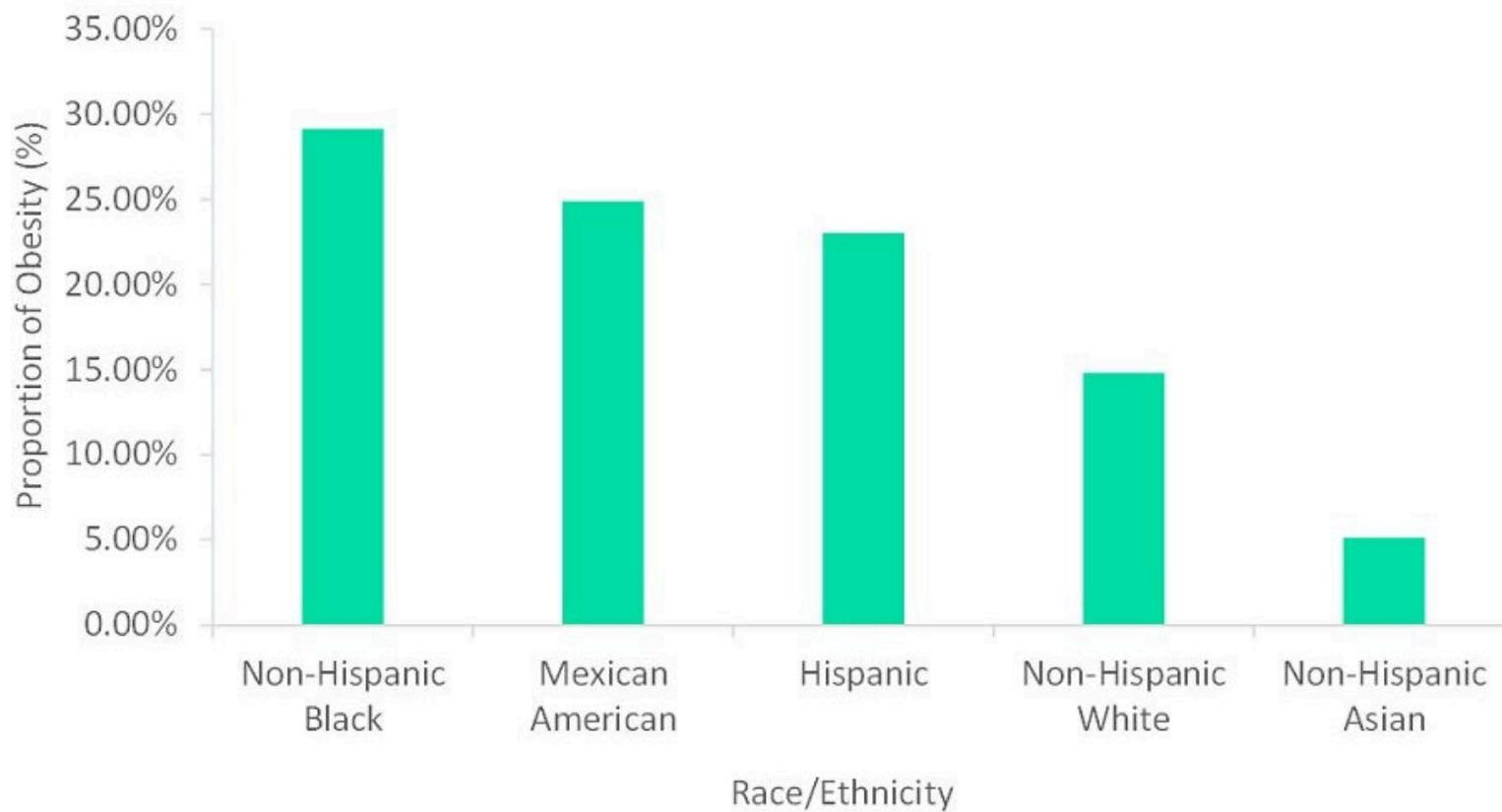
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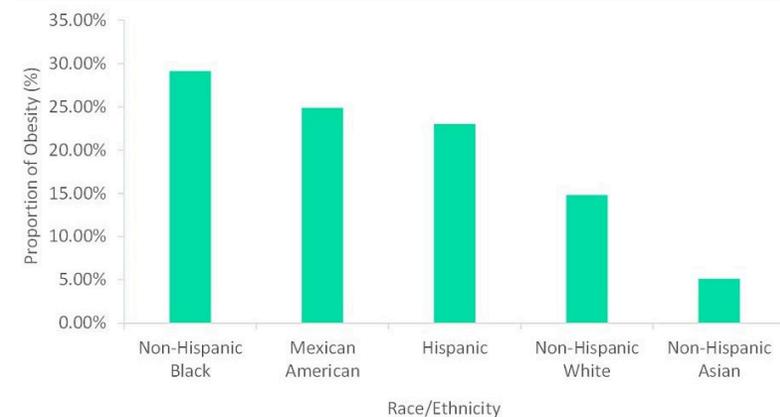
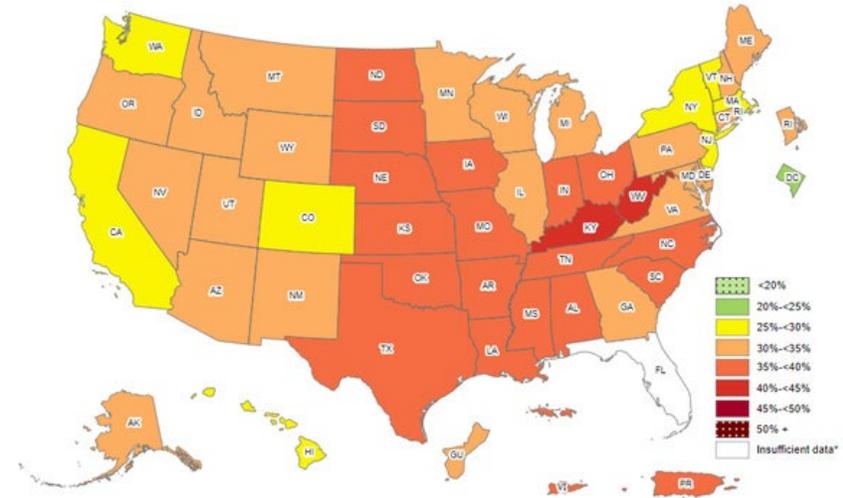
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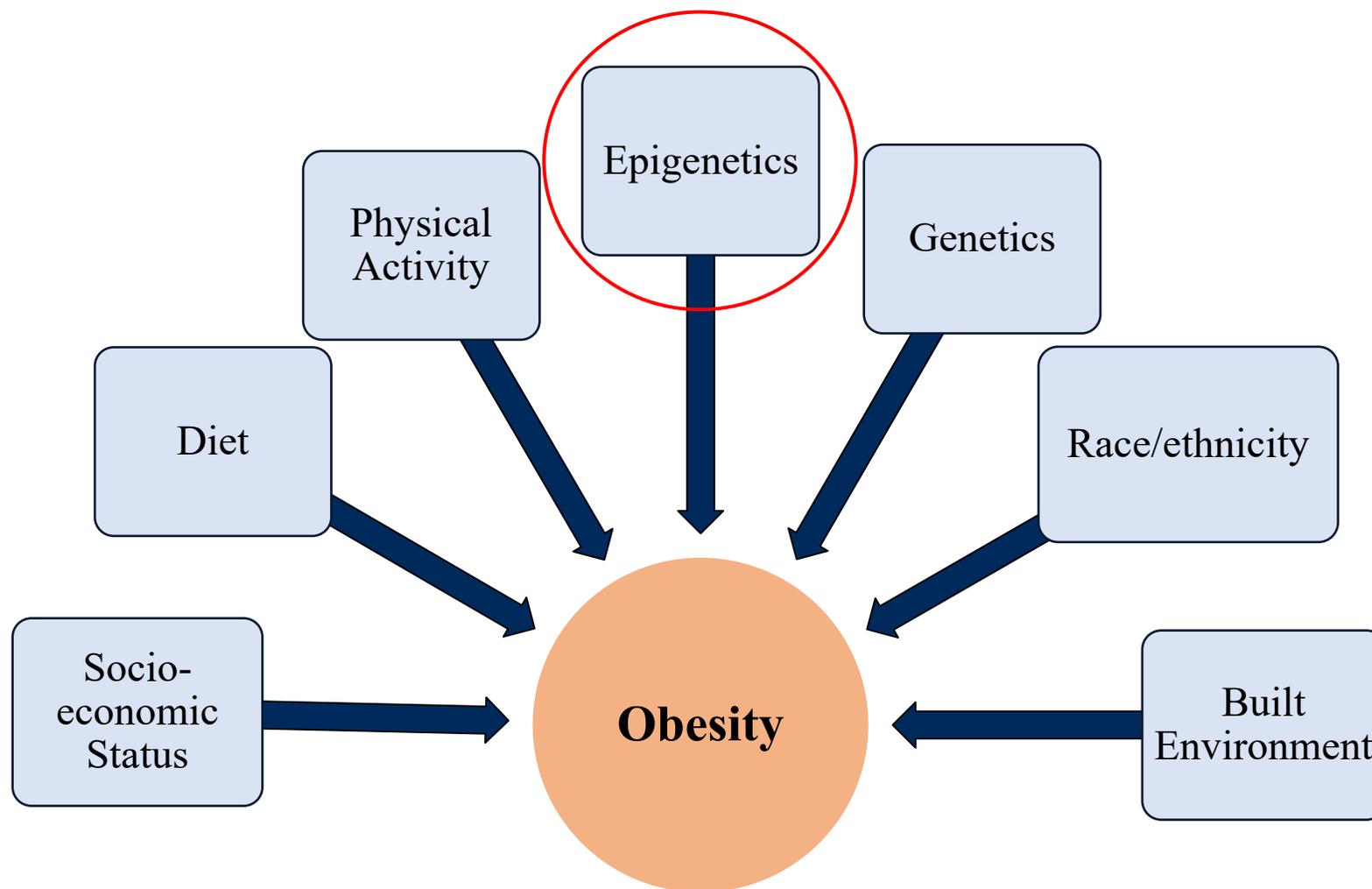
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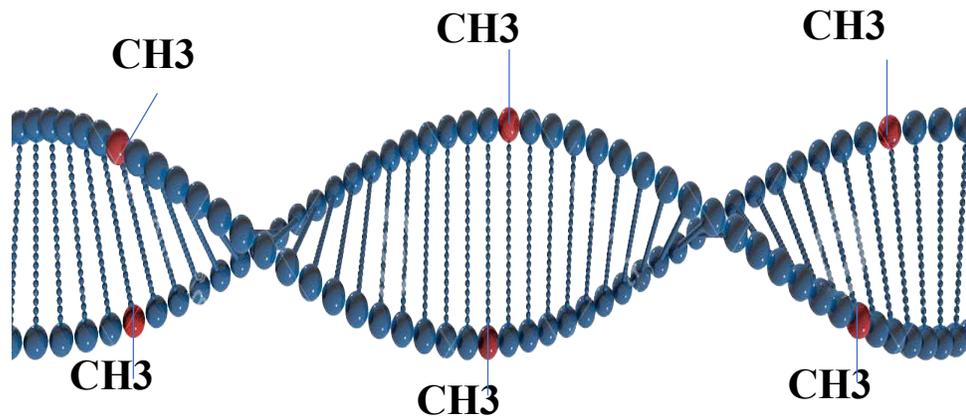
Causes of Obesity





Epigenetics

Epigenetics is the study of changes in organisms caused by modification of gene expression rather than alteration of the genetic code itself. There are different types of epigenetic changes such as histone modification and DNA methylation.



DNA Methylation



A light blue circle containing a list of gene names: NRF1, FTO, LEPR, PPARG, and MC4R, stacked vertically.

Obesity-related genes

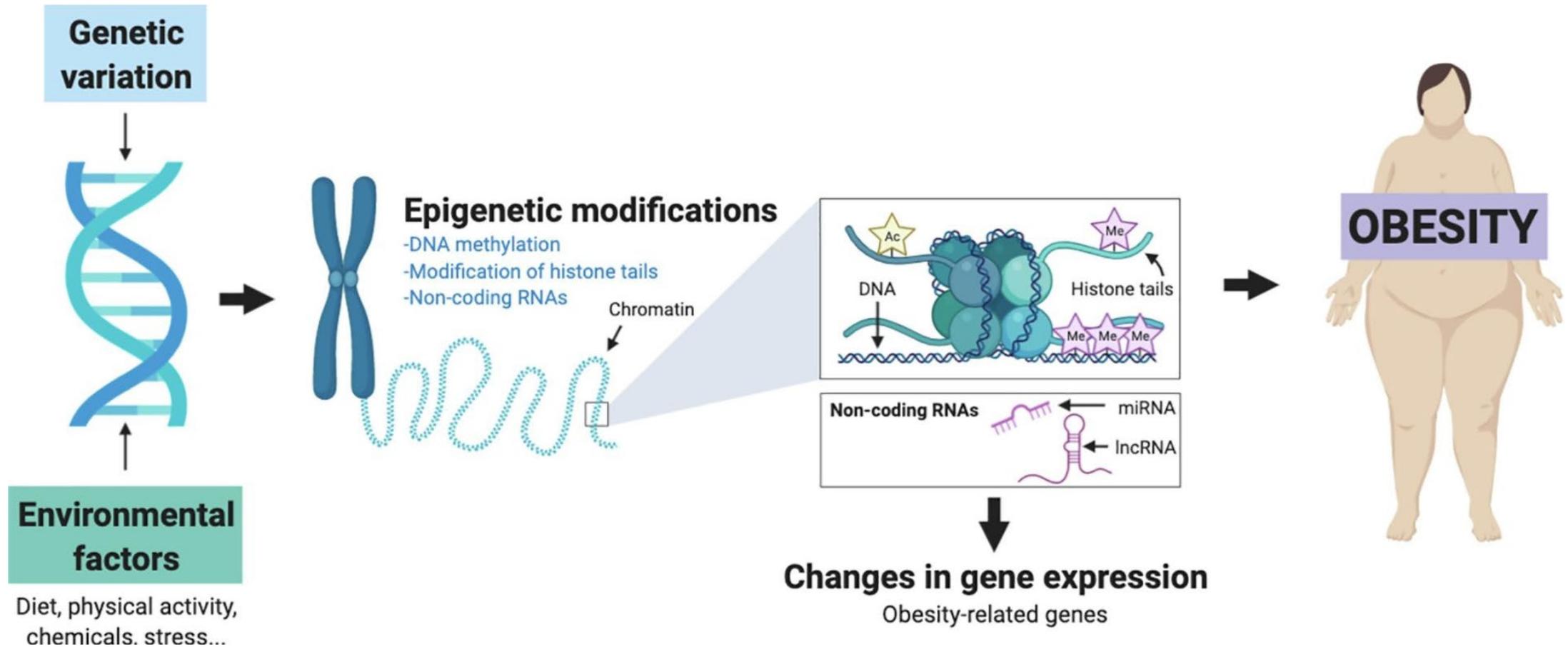


Up-regulation

Down-regulation



Role of Epigenetics in Obesity





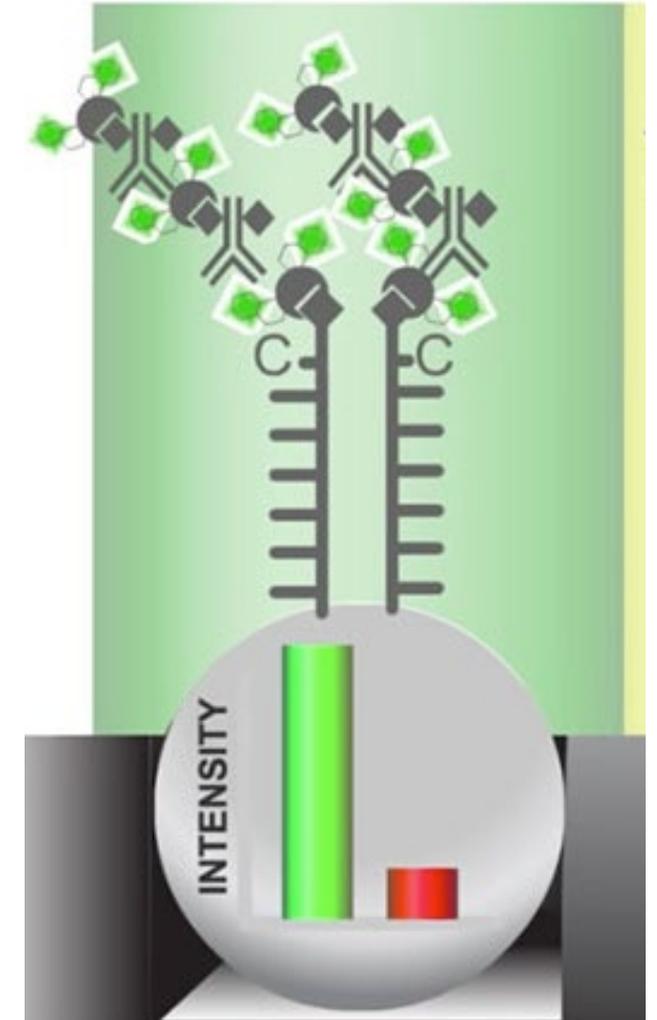
Study Aim

The main Aim of the study was to identify **differentially methylated regions (DMRs)** using IlluminaEpic Methylation BeadChip Array (EWAS) between **normal weight (NW)** and **overweight (OW)/obese (OB)** children and between the races **European American (EA)** and **African American (AA)**.



Background

- Epigenome-wide association studies (EWAS) have been widely applied to identify methylation CpG sites associated with human disease.
- **Infinium MethylationEPIC array** is commonly used for high-throughput DNA methylation profiling.
- The array utilizes Illumina's bead chip technology that does not require polymerase chain reaction (PCR) but is subject to dye intensity biases between the two platforms.
- This array can detect up to 870,000 CpGs across the epigenome.





Methodology

- Inclusion criteria
 - children between ages 6 to 10 years
 - self-identify as European American or African American ethnicity
- Exclusion criteria
 - with any medical condition such as diabetes or cardiovascular disease
 - taking any medications and antibiotics during the study
- Anthropometric measurements- body weight, and height
- Saliva was collected.

DNA Isolation



Samples sent to University of Minnesota for beadchip analysis



Statistical Analysis

Methylation β values were derived as the ratio of methylation probe intensity to overall intensity

Q-value that measures the FDR (False Discovery Rate) was calculated that measures the proportion of false positive when a particular test is significant (t-test)

Hypermethylated and Hypomethylated target IDs

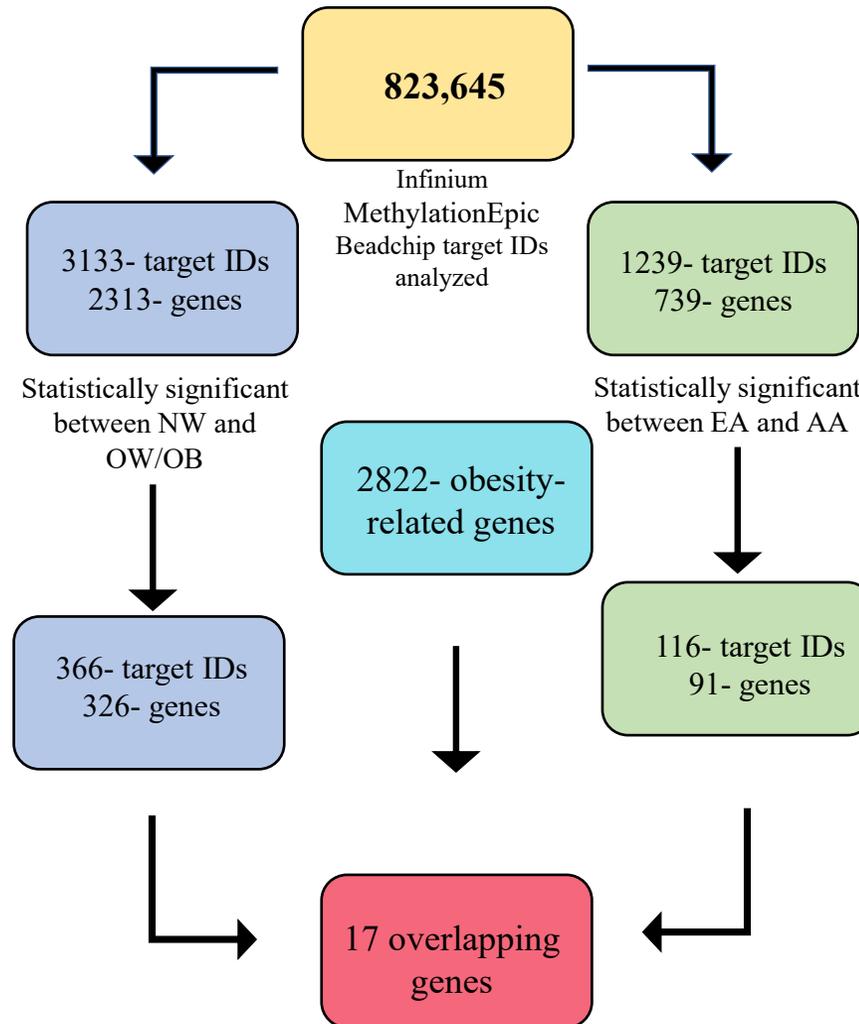


General Characteristics of the Study Population

Parameter	Total	NW	OW/OB	<i>p</i> value	EA	AA	<i>p</i> value
Number of participants	31	11	20	-	16	15	-
Sex (male/female)	17/14	9/3	8/11	-	(10/6)	(7/8)	-
Age (years)	8.56±1.34	8.21±0.42	8.75±0.29	-	8.12±0.339	9.03±0.311	-
Height (cm)	134.35±12.39	130.38±3.55	136.53±2.78	0.190	130.53±2.50	138.43±3.53	0.076
Weight (kg)	36.04±12.63	27.16±1.89	40.33±12.72	0.002	32.48±2.49	39.92±3.68	0.098
BMI (kg/m ²)	19.42±3.57	15.80±0.40	21.41±0.61	0.000	18.06±0.82	20.29±0.97	0.194
BMI z-score	1.27±1.21	0.09 ±0.31	2.03±0.10	0.000	1.05±0.32	1.50±0.28	0.312



Workflow of Infinium MethylationEpic Beadchip Target IDs Analyzes



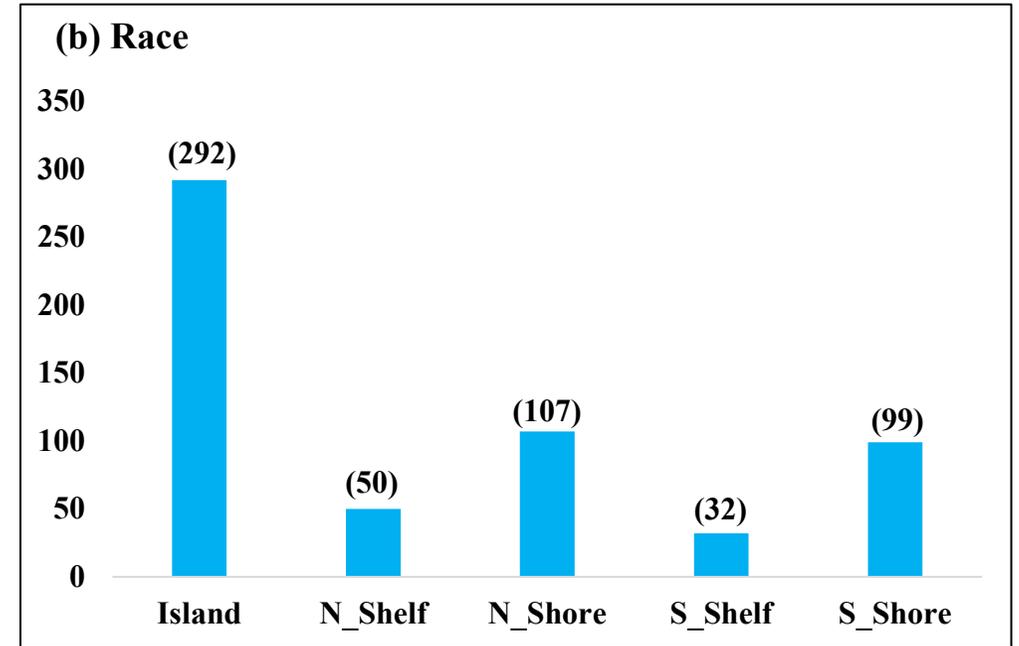
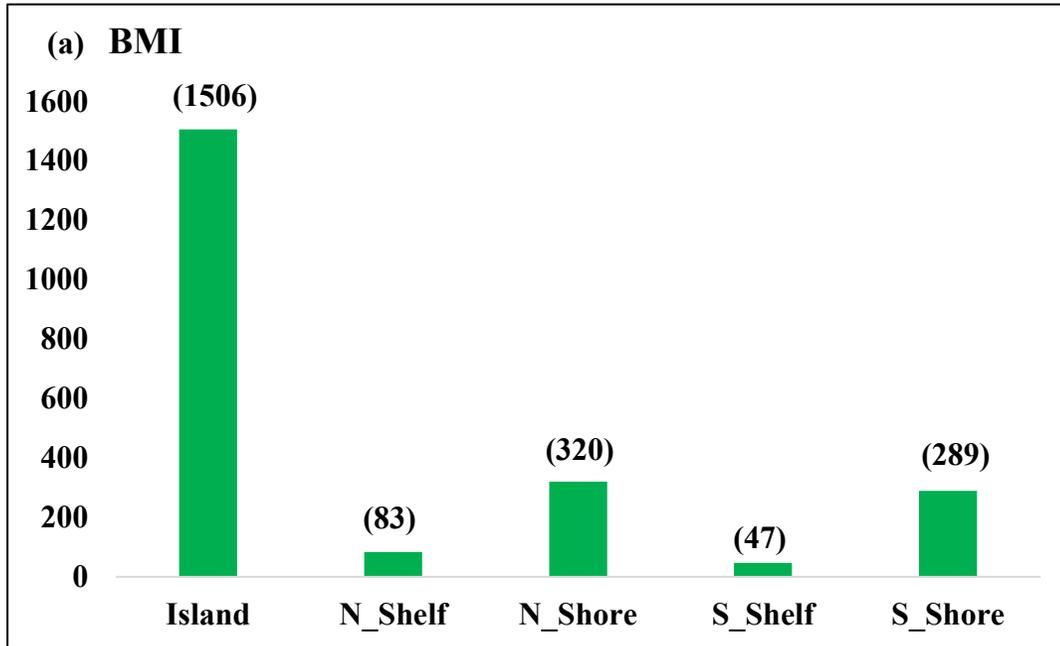


Significant Target IDs

	A	B	C	D	E	F	G	H
1	Table S1- DMRs between NW and OW/OB children							
2	Target	gene name	NW mean± SEM	OW/OB mean± SEM	p.value	Qvalue	chr no	cpg location
3	HYPERMETHYLATED							
4	cg06271387	A2BP1	0.725±0.01	0.774±0.006	0.000138295	0.02441964	16	0
5	cg02591554	AACS	0.826±0.011	0.849±0.015	8.56E-05	0.01571889	12	S_Shelf
6	cg24357302	ABCA8	0.748±0.004	0.774±0.006	1.00E-05	0.001946748	17	0
7	cg25921170	ABL2	0.87±0.005	0.886±0.004	1.00E-05	0.001946748	1	0
8	cg08015290	ACSL5	0.876±0.004	0.903±0.003	1.90E-05	0.003673811	10	0
9	cg19681266	ADARB2	0.62±0.011	0.66±0.007	1.00E-05	0.001946748	10	0
10	cg26218737	ADARB2	0.64±0.005	0.675±0.008	1.00E-05	0.001946748	10	N_Shelf
11	cg27332063	ADAT2	0.89±0.003	0.914±0.004	1.00E-05	0.001946748	6	0
12	cg24438644	ADRB3	0.105±0.006	0.131±0.005	1.00E-05	0.001946748	8	Island
13	cg20906621	AGTR1	0.507±0.014	0.568±0.011	1.00E-05	0.001946748	3	S_Shore
14	cg23058731	AKR1C3	0.88±0.004	0.896±0.004	1.00E-05	0.001946748	10	0
15	cg26225646	AKT1S1	0.01±0.002	0.021±0.001	1.00E-05	0.001946748	19	Island
16	cg19743721	ALKBH4	0.53±0.017	0.58±0.009	1.00E-05	0.001946748	7	S_Shelf
17	cg27569858	ALX3	0.76±0.009	0.796±0.007	1.00E-05	0.001946748	1	S_Shore
18	cg24039723	ANK3	0.863±0.006	0.886±0.004	1.00E-05	0.001946748	10	0
19	cg26460887	ANO4	0.81±0.007	0.838±0.004	1.00E-05	0.001946748	12	0
20	cg19847577	APBA2	0.43±0.02	0.5±0.014	1.00E-05	0.001946748	15	0
21	cg20101237	ARHGEF11	0.8±0.007	0.83±0.005	1.00E-05	0.001946748	1	0
22	cg06200818	ARHGEF3	0.697±0.007	0.733±0.005	0.000305588	0.04982335	3	0
23	cg06025541	ARHGEF33	0.864±0.004	0.892±0.004	8.77E-05	0.0160644	2	N_Shore
24	cg26879013	ARID1B	0.86±0.004	0.881±0.004	1.00E-05	0.001946748	6	0
25	cg21105827	ASAP3	0.752±0.007	0.796±0.009	1.00E-05	0.001946748	1	Island
26	cg24580199	ASAP3	0.714±0.012	0.778±0.011	1.00E-05	0.001946748	1	N_Shore
27	cg17995857	ASIC2	0.7±0.01	0.75±0.01	1.00E-05	0.001946748	17	0
28	cg26052783	ASIC2	0.74±0.009	0.777±0.005	1.00E-05	0.001946748	17	0
29	cg21161625	ATG9A	0.841±0.004	0.858±0.003	1.00E-05	0.001946748	2	0
30	cg06188566	ATOX1	0.892±0.004	0.911±0.002	0.000240109	0.04040175	5	0
31	cg13976657	ATP11A	0.88±0.003	0.903±0.003	1.00E-05	0.001946748	13	Island
32	cg26185920	ATP11A	0.76±0.005	0.776±0.002	1.00E-05	0.001946748	13	S_Shore
33	cg27026368	ATP2B4	0.9±0.004	0.912±0.003	1.00E-05	0.001946748	1	0
34	cg27410534	ATP9B	0.68±0.004	0.719±0.006	1.00E-05	0.001946748	18	0
35	cg24750156	B4GALNT1	0.607±0.008	0.663±0.009	1.00E-05	0.001946748	12	N_Shore



Genomic Location of Significant Target IDs



- CpG Island - Cluster of cytosine and guanine from 200-1000 base pairs.
- Shore – 2kb next to CpG island.
- Shelf – 2kb adjacent to shore.



A cluster of target IDs significantly differentially methylated between NW and OW/OB children

No	Gene	No of significant Target IDs
1	PCDHGA4	52
2	BRD2	18
3	PTPRN2	15
4	DIP2C	13
5	TAPBP	13
6	TGIF1	13
7	PITX2	12
8	COL9A3	12
9	PAX6	11
10	FDFT1	11
11	NKX6-2	11
12	SMC4	11
13	PCDHB16	11
14	GFI1	10
15	TRIM27	10
16	BAHCC1	10
17	HS3ST3B1	10
18	KIAA1949	10
19	MEIS2	10
20	NFIX	10



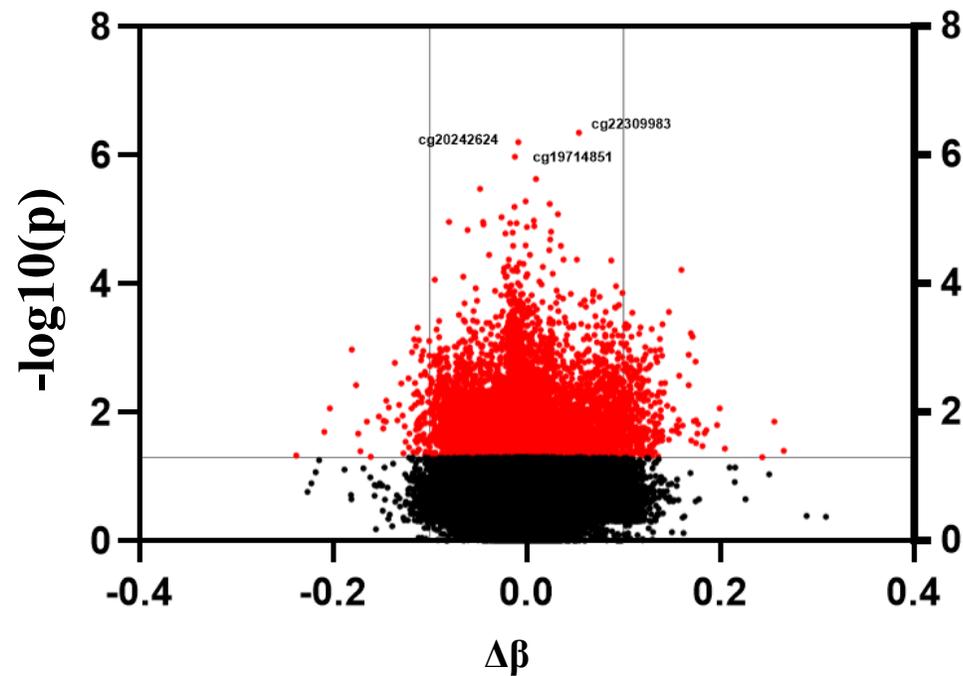
A cluster of target IDs significantly differentially methylated between EA and AA children

Sr. No	Gene	No of significant Target IDs
1	PTPRN2	10
2	TNXB	9
3	MDLL1	8
4	PRDMI6	7
5	COC23A1	6
6	CSMD1	6
7	TRIM31	6
8	TTC7B	6
9	BAT5	5
10	EFR3B	5
11	LIMCH1	5
12	LRRC14B	5
13	NRG2	5
14	PALLD	5
15	RNF39	5
16	RPH3AL	5
17	RPS6KA2	5
18	WDR90	5
19	LHX5	5
20	PM20D1	5

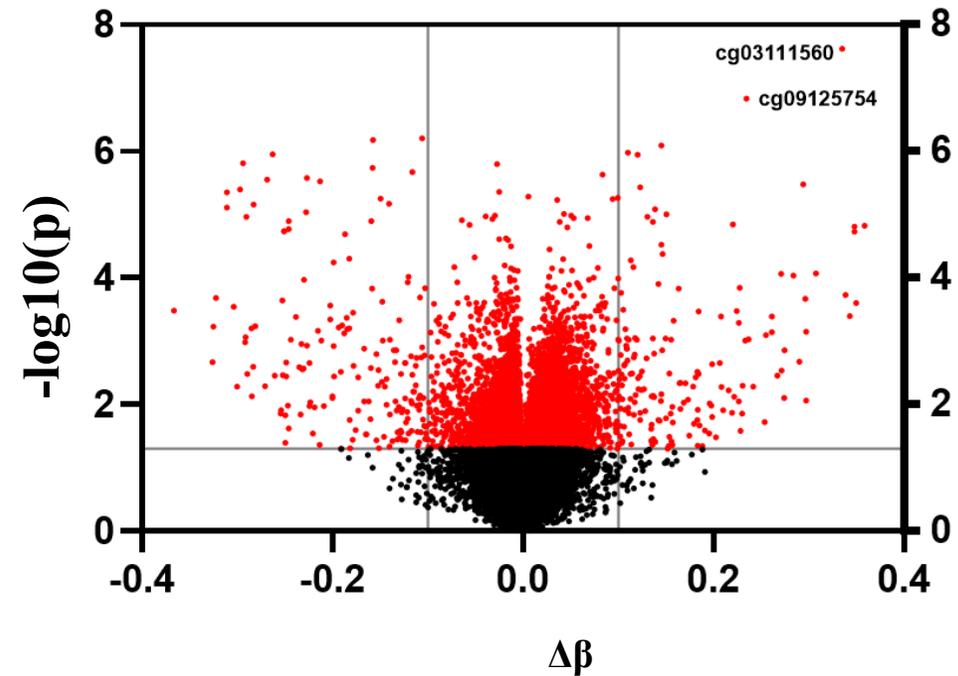


Volcano Plot of Target IDs Associated with Obesity-Related Genes

(a) BMI



(b) Race



$-\log_{10}(p)$ of 1.3 and above indicates $p < 0.05$
Delta beta of -0.1 to 0.1 indicates more than 10% difference



Overlapping Obesity-Related Genes Between NW-OW/OB and EA/AA Children

NW- OW/OB

EA-AA

AACS, ABCG2, ACHE, ACP1, ACP5, ACSL1, ACSL5, ACTB, ADA, ADGRV1, ADRA2A, ADRB3, AGPAT2, AGTR1, AHI1, AIRE, AKR1C3, ALDH2, ALOXE3, ALPL, AMD1, ANK2, APC2, ARL15, ARL2BP, ARL6, ARVCF, ASAP1, ASCL1, ATF3, ATF4, ATIC, ATM, ATP10A, ATP10D, ATRN, BACE1, BAD, BCAT1, BDKRB2, BECN1, BLK, BLVRA, BMP2, BMP2K, BRCA1, BUB1B, C1QTNF1, CADM1, CADM2, CALR, CAPN2, CASC15, CAT, CBS, CD14, CD274, CDK5, CDK8, CDKN2A, CEBPA, CES1, CHRM2, CILP2, CNNM2, CNTN4, COL12A1, COL25A1, COL9A3, COPA, COX7C, CREBBP, CRK, CTSB, CTSS, CX3CR1, CXCR6, CYB5A, CYP1B1, CYP26B1, CYP4F3, DAPK2, DCTN6, DDIT3, DOCK5, DPYSL4, EGLN1, ELAVL2, ELOVL5, EML6, ETFA, F11R, F2RL1, FAM161A, FAS, FAT1, FBXO3, FDF1, FGF19, FGGY, FGL1, FNDC5, FST, GCH1, GGT1, GJA3, GJC1, GLDC, GLI2, GLS2, GNA12, GNAQ, GNAS, GNRHR, GORASP2, GOT2, GPR39, GPS2, GRB14, GRIN1, GRK5, GRN, GSK3B, GTF2H1, GTF3A, H19, H6PD, HADH, HDAC4, HEXA, HIF3A, HK1, HK2, HMGB2, HSF1, HSPA2, HSPA4, IDE, IFT140, IFT74, IL17RD, IMPACT, INSIG1, IRF4, ITPR3, JAK2, KAT5, KCNH2, KCNK3, KCNMA1, KCP, KISS1R, KITLG, KLF7, KRT20, LARP6, LGR4, LSR, LTBP3, LZTFL1, MACROD2, MAD2L2, MAP9, MARK1, MARK4, MFN2, MGAT3, MIR152, MIR425, MMP2, MOGS, MSRA, MST1R, MT2A, MTIF3, MTOR, MTR, MYC, MYLIP, NBEAL1, NCOA3, NHLH2, NMU, NNT, NOB1, NOG, NR3C2, NRIP1, NRP1, NUCKS1, OLFM1, OPRD1, P4HB, PAX5, PAX6, PCDH9, PCSK7, PDCD4, PDE3A, PDX1, PDXK, PFKP, PGR, PHACTR1, PHIP, PIK3CA, PKM, PLA2G4A, PLA2G7, PLAG1, PMAIP1, PNPLA6, PON3, PPARG, PPARGC1A, PPARGC1B, PPP1R3G, PRDM2, PRDM4, PRDM6, PREPL, PRKAG2, PRKAR1A, PRKCZ, PROP1, PRPF6, PSENEN, PTEN, PTPRB, PYY, QPCTL, RAPIB, RBM6, REEP5, RERE, RFC2, RFX7, RGMA, RIPK3, RORC, RPRGIP1L, RSRC1, RUNX1T1, RXFP3, SCR2, SDC2, SEC16A, SEMA4D, SENP2, SESN2, SFPQ, SGIP1, SGMS1, SGSM2, SIN3A, SLC12A9, SLC25A19, SLC33A1, SLC4A4, SLIT2, SMAD4, SMARCA2, SMUG1, SOCS1, SOCS2, SOD1, SOX6, SPHK1, SPPL3, SPTBN1, SREBF2, ST6GAL1, SUCLG2, TAS2R38, TBK1, TCOF1, THBD, TM7SF2, TMED7, TMT1, TNFRSF1A, TOMM40, TREM2, TRIM13, TRPC1, TSHR, TTC28, TWIST1, TYRP1, UCN, UCP2, ULK4, UQCRC2, UTRN, VASH1, VAV2, VGF, VPS13B, WDTC1, WFS1, WNT3A, WNT4, WNT5A, WNT5B, WTAP, WWOX, XXYL1, ZNF385B, ZNF423, ZNF608

SLC24A3
 PLOD2
 RPTOR
 GH2
 RELN
 LRRN1
 VKORC1L1
 YAP1
 GDF15
 PDE11A
 PLSCR3
 LRRFIP1
 NEDD4L
 ROCK1
 CASZ1
 DRD4
 TP73

POTEF, TRPV1, IKBKB, VEGFC, SNED1, PM20D1, SLC19A1, PLAT, PKHD1, FYN, SLC2A9, CAST, CPS1, LCT, FSHR, CD81, KSR2, FLT1, ASPG, XBP1, CERK, SYNE1, AHRR, PLIN5, HLA-DRB1, ALOX5, OSBPL11, PRG4, NCAM2, TRPS1, TSPO, ADCY8, ELOVL6, DOC2B, MYH9, AHS1, ACOT11, IKBKE, C1QTNF7, TNFRSF17, TNC, NRXN1, C3, NDRG4, ADM2, STAT4, IL6ST, HMGA2, TAGLN2, GATA4, ITIH5, NPFFR2, BBS7, SEMA4A, APOA1, CPT1B, AQP7, GRK4, SLC30A10, SLN, TCN2, PADI4, LY75-CD302, HOXB3, PEX11A, IFI30, SELPLG, RUNX1, KCNQ1, LIPA, PTBP2, PDGFB7



Summary

- Overall, 2313 genes were differentially methylated between NW and OW/OB children, while 739 genes were between EA and AA.
- Total of 17 obesity-related overlapping genes were found between NW-OW/OB and EA/AA that were differentially methylated.
- The results of this study can be used to identify different obesity and non-obesity-related genes with methylation difference and further validation studies can be carried out.
- Additionally, we want to establish that salivary sample can be used to determine the DNA methylation as genome-wide DNA methylation profiles of saliva in adults and adolescents are more than 90% similar to those in blood.



QUESTIONS