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Epigenetic Patterns in Successful Weight Loss Maintainers: A Pilot Study

Yen-Tsung Huang, MD, MPH, SM, ScD[#1], **Jennifer Z.J. Maccani, PhD**[#2], **Nicola L. Hawley, PhD**[3,4,5], **Rena R. Wing, PhD**[3,4], **Karl T. Kelsey, MD, MOH**[1,6], and **Jeanne M. McCaffery, PhD**[3,4]

[1]Department of Epidemiology, School of Public Health, Brown University, Providence, Rhode Island, USA

[2]Tobacco Center of Regulatory Science, Department of Public Health Sciences, Pennsylvania State University College of Medicine, Hershey, Pennsylvania, USA

[3]Department of Psychiatry and Human Behavior, Alpert Medical School of Brown University, Providence, Rhode Island, USA

[4]The Miriam Hospital Weight Control and Diabetes Research Center, Providence, Rhode Island, USA

[5]Department of Chronic Disease Epidemiology, Yale School of Public Health, New Haven, Connecticut, USA

[6]Department of Pathology and Laboratory Medicine, Brown University, Providence, Rhode Island, USA

[#] These authors contributed equally to this work.

Abstract

DNA methylation changes occur in animal models of calorie restriction, simulating human dieting, and in human subjects undergoing behavioral weight loss interventions. This suggests that obese individuals may possess unique epigenetic patterns that may vary with weight loss. Here, we examine whether methylation patterns in leukocytes differ in individuals who lost sufficient weight to go from obese to normal weight (successful weight loss maintainers; SWLM) vs currently obese (OB) or normal weight (NW) individuals.

This study examined peripheral blood mononuclear cell (PBMC) methylation patterns in NW (n=16, current/lifetime BMI 18.5-24.9) and OB individuals (n=16, current BMI≥30), and SWLM (n=16, current BMI 18.5-24.9, lifetime maximum BMI ≥30, average weight loss 57.4 lbs) using an Illumina Infinium HumanMethylation450 BeadArray. No leukocyte population-adjusted

CORRESPONDING AUTHOR: Jeanne McCaffery, PhD Psychiatry and Human Behavior (Research), The Miriam Hospital Weight Control and Diabetes Research Center, 196 Richmond Street, Providence, RI 02904, United States. Tel.: +1 (401) 793 8010; fax: +1 (401) 793 8944. JMcCaffery@lifespan.org, Jeanne_McCaffery@brown.edu..

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epigenome-wide analyses were significant; however, potentially differentially methylated loci across groups were observed in *RYR1* (p=1.54E-6), *MPZL3* (p=4.70E-6), and *TUBA3C* (p=4.78E-6). In 32 obesity-related candidate genes, differential methylation patterns were found in *BDNF* (gene-wide p=0.00018). In *RYR1*, *TUBA3C* and *BDNF*, SWLM differed from OB but not NW.

In this preliminary investigation, leukocyte SWLM DNA methylation patterns more closely resembled NW than OB individuals in three gene regions. These results suggest that PBMC methylation is associated with weight status.

Keywords

epigenetics; methylation; obesity; weight loss; weight maintenance

INTRODUCTION

Epigenetic mechanisms (1) have been increasingly implicated in the development of obesity, and animal and human research suggests that DNA methylation is associated with weight loss (2). In this pilot study, we contribute to this literature by determining whether individuals from the National Weight Control Registry (NWCR) (3), who have been highly successful in weight loss and maintenance, resemble currently obese or normal weight individuals in their methylation patterns. We consider methylation in peripheral blood mononuclear cells (PMBCs) and control for possible confounding by differences in cellular composition (4).

SUBJECTS AND METHODS

Subjects

Participants were 48 men and women (ages 25 – 55) in three groups differing by weight history but matched on age, race, and gender. Full eligibility requirements and recruitment procedures are described by Phelan et al. (5). Briefly, eligibility criteria for successful weight loss maintainers (SWLM; n=16) included history of obesity (BMI ≥ 30) and current normal weight (BMI 18.5-24.9; maintained for ≥ 3 years). SWLM had lost on average 57.4 lbs and maintained this loss for an average of 9 years. Normal weight (NW) participants had BMIs of 18.5-25 and no history of being overweight/obese (BMI ≥25). Obese (OB) participants had BMIs≥30. All participants were weight stable for ≥2 years. The Miriam Hospital Institutional Review Board approved the study (Providence, Rhode Island).

DNA extraction/bisulfite modification

Genomic DNA was isolated from 20-190 μl buffy coat samples using the QIAmp DNA Blood Mini Kit (Qiagen, Inc., Alameda, CA, USA, 51104). Samples with low concentration were concentrated using the Genomic DNA Clean & Concentrator™ Kit (Zymo Research, Irvine, CA, USA, D4010). Samples were bisulfite converted via EZ DNA Methylation™ Kit (Zymo Research, D5001).

Methylation profiling

Bisulfite-converted DNA was processed at the University of California San Francisco Institute for Genetics Genomics Core using the Infinium HumanMethylation450 BeadArray (6). Illumina BeadChips were scanned with iScan (Illumina, San Diego, CA, USA). Methylation β values were calculated from intensity of methylated (M) and unmethylated (U) alleles, where the ratio of fluorescent signals β = Max(M,0)/[Max(M,0) + Max(U,0) + 100] and β ranged from 0 (no methylation) to 1 (complete methylation). Poor-performing loci, X- and Y-linked loci and loci <100 base pairs (bp) of a known single-nucleotide polymorphism (SNP) were removed, resulting in data for 384 477 autosomal, non-SNP-associated loci.

Leukocyte adjustment

As leukocyte subpopulations possess distinct methylation patterns (4), differences in PBMC leukocyte populations are potential confounders in methylation analyses. Relative proportion of granulocytes, CD8- and CD4-T cell, monocytes, B-cells, and natural killer (NK)-cells were characterized in each sample using cell-type-specific differentially methylated regions (DMRs) (4).Subsequent analyses adjusted for these leukocyte subpopulations

Statistical analysis

Methylation array data were normalized using ComBat (7). Potential logit-transformed methylation associations across groups (NW, OB, SWLM) were investigated in locus-by-locus analyses with permutation-based omnibus tests for epigenome-wide significance, which fit individual regression models to each of 384 477 loci and calculated regression coefficients for associations between methylation and group. Type I error was controlled for by an assessment of false discovery rate (8). Methylation analyses of loci in candidate gene regions previously associated with obesity were also performed (9) via gene-set analysis methodology (10).

RESULTS

By design, groups differed significantly in current and lifetime maximum BMI. Average current BMI for NW was 21.9 kg/m^2 (sd=1.8); OB, 34.5 (sd=3.7); SWLM, 23.8 (sd=1.6) ($p<0.001$; NW vs OB, $p=3.07\times10^{-13}$; NW vs SWLM, $p=0.0090$; OB vs SWLM, $p=4.63\times10^{-12}$). Lifetime maximum BMI for NW was 23.1 (sd=2.0); OB, 35.6 (3.6); SWLM, 33. (3.1) ($p<0.001$; NW vs OB, $p=9.35\times10^{-13}$; NW vs SWLM, $p=5.54\times10^{-12}$; OB vs SWLM, $p=0.039$). Age, race and sex did not differ across groups (see **Supplemental Table S1**).

Methylation at leukocyte-associated loci did not differ significantly across groups (granulocyte p = 0.99, CD8-T cell p = 0.20, CD4-T cell p = 0.23, monocyte p = 0.62, B-cell p = 0.37 and NK-cell p = 0.37; **Supplemental Figure S1**. **Figure 1** presents a Manhattan plot illustrating probability of differences in leukocyte-adjusted methylation across groups at 384 477 loci. Although the lowest q-value following false discovery rate adjustment was

0.54, the three top loci by p-value are located within plausible biological candidate genes *RYR1*, *MPZL3* and *TUBA3C* (highlighted in **Figure 1**).

Supplemental Table S2 shows the top 20 loci by leukocyte-adjusted p-value. **Supplemental Figures S2, S3** and **S4** show –log(p)-values and percent methylation by group for *RYR1*, *MPZL3* and *TUBA3C*, respectively. Three gene-body *RYR1* loci were differentially methylated in OB individuals (**Figure 2A-C**) compared to NW and SWLM, who did not differ from one another. At one *MPZL3* promoter locus <1500 bp from the transcription start site (TSS) (cg19069882, p=4.70E-06), distinct methylation patterns between groups were also observed (**Figure 2D**). Additionally, one *TUBA3C* locus <1500 bp from the TSS (cg22274414, p=4.78E-06; **Supplemental Figure S4** and **Figure 2E)** differed in OB individuals from other groups.

In leukocyte-adjusted analyses of loci in 32 obesity-related candidate genes (9), significant associations were found between *BDNF* gene-wide methylation and group (**Supplemental Figure S5**; p=5.3E-5, significance passing Bonferroni threshold of α=0.05/32, or p<0.0016, accounting for 32 genes). *BDNF* hypomethylation was observed in OB versus NW and SWLM individuals (epigenome-wide, False Discovery Rate (FDR)-adjusted q-value=0.84) (**Figure 2F**).

DISCUSSION

We observed differential methylation patterns in PBMCs across NW, OB, and SWLM groups in several genes, suggesting that current BMI may be more strongly associated with methylation than obesity history. NW individuals and SWLM displayed similar PBMC methylation patterns relative to OB individuals in *RYR1*, *TUBA3C* and *BDNF*. Distinct methylation patterns were observed for all groups in *MPZL3*. These results contribute to literature suggesting that methylation may be associated with weight status (2).

The ryanodine receptor-1, or *RYR1*, gene encodes a calcium release channel protein found in the sarcoplasmic reticulum expressed in skeletal muscle, and is associated with exercise and caloric restriction in obese rats (11). OB individuals showed reduced methylation at three loci.

The alpha 3c tubulin, or *TUBA3C*, gene is a tubulin superfamily member, which encodes a major protein component of microtubules in many cell types; a *TUBA3C* BMISNP interaction is associated with insulin resistance (12). Here, OB individuals showed lower methylation at cg22274414 in the promoter region of this gene, suggesting a potential role in gene expression.

Brain-derived neurotrophic factor, or *BDNF*, encodes an abundant neurotrophin (9). *BDNF* SNPs are associated with BMI (9); exercise is associated with *BDNF* epigenetic changes in rats (13). We observed *BDNF* hypomethylation in OB versus NW and SWLM individuals. Loci with greatest p-value differences across groups were associated with the TSS of *BDNF's* transcript variants (variants 3, 2/7/8, 9, 5, 10/11/17/18, 12, 4/13/14, and 6), suggesting a potential role in *BDNF* transcription and/or expression.

The *MPZL3*, or myelin protein zero-like 3, gene encodes a predicted type I transmembrane cell-cell adhesion protein linked, in mice, to body weight, energy expenditure, and insulin sensitivity (14). Here, NW individuals had the highest levels of *MPZL3* methylation, OB individuals were hypomethylated, and SWLM individuals were most hypomethylated.

In an important innovation, we controlled for potential confounding by major PBMC leukocyte subpopulations. Though we accounted for several major immune cell types, methods have not yet been developed to quantify the relative proportion of activated immune cells, macrophages and dendritic cells, which may relate to obesity. Additional strengths of this study include use of NWCR subjects and matching of subjects on age, race, gender and categories of current and lifetime weight. Our study is limited by small sample size and tissue specificity. Future studies will benefit from confirmation of these results in larger sample sizes, analyses of potential gene expression changes, determination of cross-tissue specificity or correspondence to adipose and skeletal muscle, and investigation of immune implications, as innate immunity is the primary function of PBMCs, and *RYR1* is associated with dendritic and T cell activation (15), *TUBA3C* with lymphocyte migration (16), and *BDNF* with mitogen-activation in lymphocytes (17).

In summary, we observed potential differences in PBMC methylation between SWLM, NW and OB individuals at *RYR1*, *MPZL3*, *TUBA3C* and *BDNF* loci. These findings suggest a relationship between obesity, weight and methylation. Future studies should investigate within-subject, weight-loss-associated methylation changes and examine cross-tissue correspondence and immunologic relevance of these results.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS

SNP	single nucleotide polymorphism
PBMCs	peripheral blood mononuclear cells
BMI	body mass index
EWAS	epigenome-wide association study
TSS	transcription start site
NK	natural killer
NW	cell normal weight
NWCR	National Weight Control Registry

OB	obese
SWLM	successful weight loss maintainer

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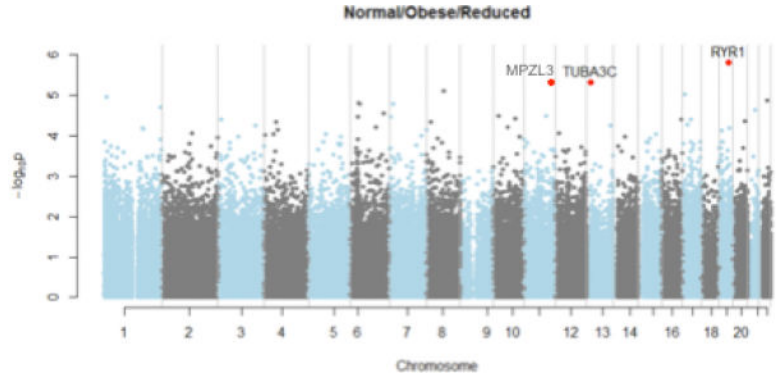

Figure 1.

Manhattan plot for differences in methylation status across groups of NW, OB, and SWLM participants at 384 477 autosomal, non-single-nucleotide polymorphism (SNP)-associated CpG loci. Red dots indicate the top three CpG loci by p-value, which were located in the *RYR1* region on chromosome 19, in the *MPZL3* region on chromosome 11, and in the *TUBA3C* region on chromosome 13.

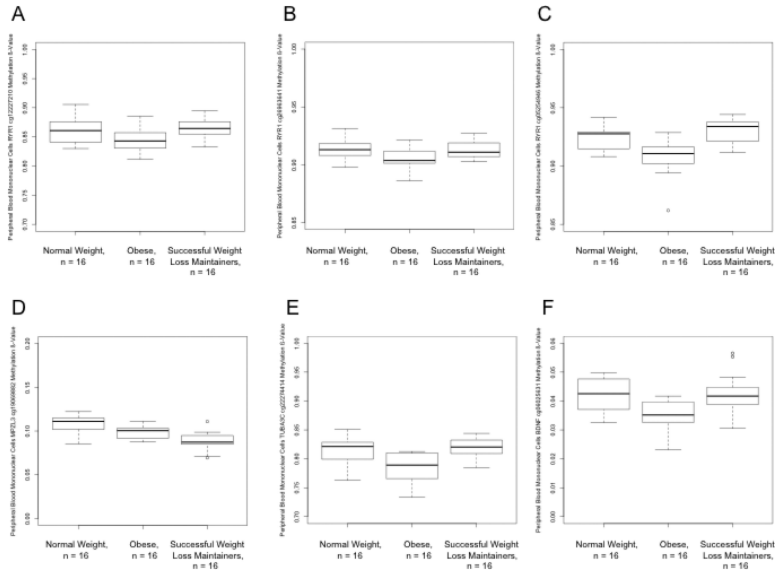

Figure 2.

Differences in PBMC methylation status by group (NW, OB, and SWLM) at A) *RYR1* cg12227210 (p=0.0075; for OB vs SWLM, $p=9.61\times10^{-4}$; NW vs SWLM, p=0.44; NW vs OB, p=0.026); B) *RYR1* cg26963641 (p=0.012; for OB vs SWLM, p=0.079; NW vs SWLM, p=0.86; NW vs OB, p=0.016); C) *RYR1* cg05254946 ($p=1.54\times10^{-6}$; for OB vs SWLM, $p=8.76\times10^{-6}$; NW vs SWLM, p=0.11; NW vs OB, $p=3.30\times10^{-5}$); D) *MPZL3* cg19069882 ($p=4.70\times10^{-6}$; for OB vs SWLM, $p=5.58\times10^{-3}$; NW vs SWLM, $p=2.75\times10^{-5}$; NW vs OB, p=0.03); E) *TUBA3C* cg22274414 ($p=4.78\times10^{-6}$; for OB vs SWLM, $p=1.79\times10^{-5}$; NW vs SWLM, p=0.30; NW vs OB, $p=1.32\times10^{-4}$); and F) *BDNF* cg06025631 ($p=6.11\times10^{-4}$; for OB vs SWLM, p=0.0029; NW vs SWLM, p=0.47; NW vs OB, p=0.0015).