INSIG2 rs7566605 single nucleotide variant and global DNA methylation index levels are associated with weight loss in a personalized weight reduction program

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Received July 26, 2017; Accepted August 17, 2017

DOI: 10.3892/mmr.2017.8039

Abstract. Single nucleotide polymorphisms associated with lipid metabolism and energy balance are implicated in the weight loss response caused by nutritional interventions. Diet-induced weight loss is also associated with differential global DNA methylation. DNA methylation has been proposed as a predictive biomarker for weight loss response. Personalized biomarkers for successful weight loss may inform clinical decisions when deciding between behavioral and surgical weight loss interventions. The aim of the present study was to investigate the association between global DNA methylation, genetic variants associated with energy balance and lipid metabolism, and weight loss following a non-surgical weight loss regimen. The present study included 105 obese participants that were enrolled in a personalized weight loss program based on their allelic composition of the following five energy balance and lipid metabolism-associated loci: Near insulin-induced gene 2 (*INSIG2*); melanocortin 4 receptor; adrenoceptor β2; apolipoprotein A5; and G-protein subunit β3. The present study investigated the association between a global DNA methylation index (GDMI), the allelic composition of the five energy balance and lipid metabolism-associated loci, and weight loss during a 12 month program, after controlling for age, sex and body mass index (BMI). The results demonstrated a significant association between the GDMI and near *INSIG2* locus, after adjusting for BMI and weight loss, and significant trends were observed when stratifying by gender. In conclusion, a combination of genetic and epigenetic biomarkers may be used to design personalized weight loss interventions, enabling adherence and ensuring improved outcomes for obesity treatment programs. Precision weight loss programs designed based on molecular information may enable the creation of personalized interventions for patients, that use genomic biomarkers for treatment design and for treatment adherence monitoring, thus improving response to treatment.

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Key words: near insulin-induced gene 2, melanocortin 4 receptor, adrenoceptor β2, apolipoprotein A5, G-protein subunit β3, global DNA methylation index, energy balance, single nucleotide variant, obesity, lipid metabolism, personalized weight reduction program

Introduction

Obesity is a major public health concern, and contributes to morbidity and mortality rates via associations with chronic diseases (1,2), including type 2 diabetes mellitus, cardiovascular diseases, osteoarthritis and certain types of cancer (3). Although weight loss through dietary regimes and exercise is commonly prescribed, there is minimal insight into the molecular basis of weight loss, particularly concerning disparities in the extent of weight loss among individuals (4,5). The observed differences in weight loss among individuals is an important issue for clinicians and patients, and these differences are predominantly observed between males and females undergoing the same treatment courses (6).

The current understanding of the etiology of obesity and weight loss involves environmental, genetic and epigenetic factors (4,7,8). External factors in the pathogenesis of obesity include diet, physical exercise and stress. Previous studies

indicate that single nucleotide polymorphisms (SNPs) are also important determinants of weight loss (8,9). It is accepted that genetic factors partially determine individual susceptibility to weight gain and obesity, however, the established genetic variants only partially explain the variation observed. As a result, interest in understanding the potential role of epigenetics as a mediator of gene-environment interactions in obesity development has increased (10). Previous studies have investigated gene-environment interactions associated with the development of obesity (4,11-13). The initial studies focused on the associations between obesity and global DNA methylation (14-16). Global DNA methylation refers to the overall level of methylcytosine in the genome as a percentage of total cytosine, while gene-specific methylation refers to the methylation status of a specific site. While numerous studies have reported a complex association between global DNA methylation and body mass index (BMI), there is no consistent evidence for an association between global DNA methylation and obesity, which may be due to the lack of a gold standard method of measuring global DNA methylation (14,17-22). Genome-wide arrays have demonstrated a concurrent loss of methylation in the non-coding areas of the genome and gain of methylation in CpG islands located on promoter regions of obese patients with a high BMI, compared with patients with a low BMI. The studies also identified multiple obesity-associated differentially methylated sites, primarily in blood cells (23-26).

Cytosine methylation in SNPs (allele-specific methylation) has previously been successfully investigated in weight loss (9). Accumulating evidence indicates that the tendency towards adult obesity has early developmental origins, which are associated with a 'nutritional memory response' that can take form in epigenetic modifications during a lifetime. Associations between methylation marks at birth and later life obesity were reported (14,27-36). However, to the best of our knowledge, the potential genetic and epigenetic interactions associated with weight loss have not previously been investigated (37-41).

The present study aimed to investigate the genetic and epigenetic alterations associated with weight loss in a population of obese patients participating in a personalized weight loss program, which was designed based on genetic information. Personalized weight loss diets and lifestyle modification plans were provided to study participants according to the combined SNP profiles of five genes associated with energy balance and lipid metabolism. The present study determined the association between a global DNA methylation index (GDMI) and the allelic composition of near insulin-induced gene 2 (*INSIG2*), melanocortin 4 receptor (*MC4R*), adrenoceptor β2 (*ADRB2*), apolipoprotein A5 (*APOA5*) and G-protein subunit β3 (*GNB3*) in 105 obese or overweight patients that participated in a 12 month program.

Materials and methods

Study participants. The patients were referred to the Clinical Genetics laboratory of CGC (Madrid Spain) between January 2009 and June 2010, by either an endocrinologist or a dietitian in order to establish a healthy lifestyle intervention based on the risk of obesity inherent to each polymorphism investigated. Upon arrival at the clinic, the patients answered a questionnaire regarding previous health issues, including surgery, pathologies, diabetes and cardiovascular disease, and their height and present weight was recorded. The Institutional Review Boards of the Johns Hopkins University School of Medicine (Baltimore, MD, USA) approved the protocol for the present study. All participants signed an informed consent form where it was specifically stated that the samples may be used for anonymized research studies and for publications.

Personalized weight reduction program. The patients participated in a personalized weight reduction program guided by their genotypic profile, which included SNP-associated dietary recommendations, for 12 months. The BMI in kg/m^2 was calculated for each participant prior to and after completion of the personalized weight reduction program. Adult obesity was defined as a BMI of ≥ 30 kg/m^2, and patients were classified as overweight if they had a BMI between 25 and 29.9 kg/m^2. The participants underwent a personalized weight reduction program based on their genotypic profile. This method utilized SNP data to develop dietary recommendations. Participants also provided data regarding age at maximum weight recorded, previous dietary interventions and maximum weight loss. Additionally, women provided details regarding age at menarche and pregnancy history, in addition to height and weight prior to and following menarche and pregnancy. The polymorphisms were investigated in our laboratory and the results were disclosed in a post-test genetic counseling session.

The dietary and lifestyle interventions were subsequently tailored to the genotype of each patient by their endocrinologist/dietitian, following the general recommendations provided by the laboratory, as described in Table I. Genetic information and lifestyle modification recommendations were provided during the initial counseling session and adherence was determined at follow-up by the referring endocrinologist/dietitian. Patients were provided with a personalized weight reduction program, with primary and preferential lifestyle interventions, together with supplemental activities for each SNP of the five loci in the panel. The descriptions of sequence variants presented in Table I follow the Human Genome Variation Society 2016 recommended format (42). The five loci selected for this intervention are collectively associated with energy balance and lipid metabolism in ≥1 of the following metabolic pathways or conditions: Development of obesity, high BMI, hyperphagia, hyperinsulinemia, lipolysis control, lipid metabolism homeostasis and exercise-induced weight loss (43-65).

Information on the well-established associations between each SNP and the risk of obesity was also provided to each patient. Each patient received up to three personalized dietary/lifestyle recommendations, based on their individual genotypic mosaic, which included a combination of physical activity and diets low in calories, carbohydrates and/or lipids.

DNA isolation. DNA was extracted from peripheral blood mononuclear cell (PBMC) samples obtained from all participants, and all samples were kept at -80˚C until analysis. Blood was collected in 4 ml vacutainer tubes containing EDTA

Table I. Recommended diet and lifestyle interventions for overweight patients with specific variants of five loci associated with energy balance and lipid metabolism.

Gene (SNP) and variants	Physical activity	Diet			HGVS name	MAF/minor allele count	Health effects
		Low calorie	Low carbohydrate	Low lipid			
Near *INSIG2* (rs7566605)					NC_000002.12: g.118078449C>G	C=0.2859/1432 (1000 Genomes project) C=0.2798/8146 (TOPMed)	Obesity, dyslipidemia, control of lipid synthesis
GG and GC	NI	NI	NI	NI			
CC	[b]	[a]	NI	[d]			
MC4R (rs17782313)					NC_000018.10: g.60186834T>C	A=0.2400/1202 (1000 Genomes project) A=0.2503/7289 (TOPMed)	Obesity, obesity-related quantitative traits
TT	NI	NI	NI	NI			
CT	[d]	[c]	NI	[d]			
CC	[c]	[b]	NI	[b]			
ADRB2 (rs1042714)					NC_000005.10: g.148826910G>C	G=0.3166/38431 (ExAC) G=0.2043/1023 (1000 Genomes project) G=0.3400/4422 (GO-ESP) G=0.2960/8620 (TOPMed)	Obesity, susceptibility to metabolic syndrome
CC	NI	NI	NI	NI			
CG	[d]	[d]	[b]	NI			
GG	NI	[d]	[b]	[d]			
APOA5 (rs662799)					NC_000011.10: g.116792991G>A	G=0.1629/816 (1000 Genomes project) G=0.1043/3038 (TOPMed)	Obesity, triglyceride metabolism, cardiovascular disease
AA	NI	NI	NI	NI			
AG and GG	[b]	[d]	NI	[a]			
GNB3 (rs5443)					NC_000012.12: g.6845711C>T	T=0.3598/43631 (ExAC) T=0.4498/5850 (GO-ESP)	Plasma triglyceride regulation, essential hypertension
CC	[d]	[d]	NI	[d]			
CT	NI	NI	NI	NI			
TT	[a]	[d]	NI	[d]			

The description of sequence variants follows the HGVS 2016 recommended format. All are genomic sequences. <REFERENCE_SEQUENCE_ID>:<SEQUENCE_TYPE>.<POSITION><CHANGE>. MAF refers to the frequency at which the second most common allele occurs in a given population. The MAF/minimum allele count numbers for a specific SNP represent the frequency of the allele in a sequencing project database (i.e., 1000 Genomes) and the number of times this SNP was observed in that population. SNP information was accessed from dbSNP (https://www.ncbi.nlm.nih.gov/SNP) on July 3, 2017. SNP, single nucleotide polymorphism; HGVS, Human Genome Variation Society; MAF, minor allele frequency; *INSIG2*, insulin-induced gene 2; NI, no intervention; NHLBI, National Heart, Lung and Blood Institute; TOPMed, NHLBI Trans-Omics for Precision Medicine project; MC4R, melanocortin 4 receptor; *ADRB2*, adrenoceptor β2; ExAC, Exome Aggregation Consortium; GO-ESP, NHLBI GO Exome Sequencing Project; *APOA5*, apolipoprotein A5; *GNB3*, G-protein subunit β3. [a]Recommended as primary intervention; [b]recommended as preferential intervention; [c]recommended as primary supplemental activity; [d]recommended as supplemental activity.

(BD Biosciences, Franklin Lakes, NJ, USA). Whole blood was centrifuged at 300 x g for 10 min and the leukocyte layer was separated, adding the same volume of PBS (0.01 M PO4, 0.15 M NaCl, pH 7.2). The resulting mixture was carefully placed on Ficoll-Paque (17-1440-02), followed by centrifugation at 700 x g for 30 min. The corresponding leukocyte portion was separated in another tube and centrifuged at 300 x g for 10 min. The supernatant was discarded and the material precipitated was washed with 1 ml PBS (0.01 M PO4, 0.15 M NaCl, pH 7.2) by centrifugation at 300 x g for 10 min and resuspended in 200 μl NET 100 (5 M NaCl, 1 M Tris-HCl, 0.5 M EDTA, pH 8.0) to be stored at -20˚C. The concentration of cells was determined by manual cell counting using a Neubauer chamber and divided into three equal aliquots.

PBMC DNA samples were sent to the Head and Neck Cancer Research Laboratory of Johns Hopkins School of Medicine where they were digested with 50 μg/ml proteinase K in the presence of 1% sodium dodecyl sulfate at 48˚C for 3 days, which was followed by phenol/chloroform extraction and ethanol precipitation, and finally dissolved in 30 μl LoTE (2.5 mmol/l EDTA and 10 mmol/l Tris-HCl), as previously described (66).

Global DNA methylation assays. In total, sufficient DNA levels were obtained from 95 samples for the global DNA methylation analysis. The global DNA methylation levels were determined with an ELISA-based commercial kit (Imprint Methylated DNA Quantification kit; cat no. MDQ1; Sigma-Aldrich, St Louis, MO, USA), according to the manufacturer's protocol. The MDQ1 kit is a high-throughput molecular biology kit, which employs a 96-well plate format to provide accurate differential global DNA methylation absorbance readings with as little as 50 ng genomic DNA. In the present study, 2 μl DNA at a concentration of 100 ng/μl was diluted with 28 μl lysis and binding buffers, and incubated at 37˚C for 60 min. The samples were incubated with capture and detection antibodies and absorbance was read at 450 nm. Quantification of global DNA methylation was performed by calculating the amount of methylated cytosine (5-methylcytosine) in the sample relative to the global cytidine (5-methylcytosine + deoxycytosine) in a positive control that had been previously methylated. All samples were analyzed in duplicate.

SNP analysis. DNA was genotyped using made-to-order TaqMan SNP Genotyping Assays with the following cat no. 4351379 and assay IDs: C_29404113_20 (near *INSIG2*-rs7566605); C_32667060_10 (*MC4R*-rs17782313); C_2084765_20 (*ADRB2*-rs1042714); C_2310403_10 (*APOA5*-rs662799); C_2184734_10 (*GNB3*-rs5443) (Thermo Fisher Scientific, Inc., Waltham, MA, USA).

The following probe sequences were used in each respective genotyping assay: i) rs7566605 (near *INSIG2*) consensus sequence-Chr2: 118078449 on GrCH38: 5-AAGTACTTA ACAATGGATATTTGAT**[C/G]**GTGGTCCTTTAGGTCTG TACCAGGG-3'; ii) rs17782313 (*MC4R*) consensus sequence-Chr18:60183864onGrCH38:5'-GTTTAAAGCAGG AGAGATTGTATCC**[C/T]**GATGGAAATGACAAGAAAA GCTTCA-3'; iii) rs1042714 (*ADRB2*) consensus sequence-Chr5: 148826910 on GrCH38: 5'-TGCGCCGGAC CACGACGTCACGCAG**[C/G]**AAAGGGACGAGGTGTGG GTGGTGGG-3'; iv) rs662799 (*APOA5*) consensus sequence-Chr11: 116792991 on GrCH38: 5'-GAGCCCCAGG AACTGGAGCGAAAGT**[A/G]**AGATTTGCCCCATGAGG AAAAGCTG-3'; and v) rs5443 (*GNB3*) consensus sequence-Chr12:6845711 on GrCH38: 5'-AGAGCATCATCT GCGGCATCACGTC**[C/T]**GTGGCCTTCTCCCTCAGTGG CCGCC-3'.

The two probes for each locus were identical apart from the region highlighted in square brackets, where the nucleotide in this position differed between the two probes, as indicated.

Quantitative polymerase chain reactions (PCR) were performed with two allele-specific TaqMan MGB probes for each of the five SNPs tested on an ABI 7,500 Real-Time PCR instrument. Duplicate reactions were run for each assay using a 25 μl reaction volume on a 96-well plate. PCR was performed with 20 ng input DNA, 1 μmol/l each primer, 0.25 μmol/l each probe and 1x TaqMan master mix (cat no. 4371355; Thermo Fisher Scientific, Inc., Waltham, MA, USA). The cycling program was one cycle of 50˚C for 2 min, one cycle of 95˚C for 10 min, followed by 40 cycles of 95˚C for 15 sec and 60˚C for 1 min. The ROX passive reference provides an internal reference to which the reporter-dye signal can be normalized during data analysis. The Taqman master mix contained AmpliTaq Gold® DNA Polymerase, Ultra Pure (UP) Deoxyribonucleotide triphosphates (dNTPs), ROX passive reference and buffer components optimized for tight endpoint fluorescence clusters, reproducible allelic discrimination and bench top stability. Fluorescence intensities (arbitrary units) of the two probes were plotted and genotype calling was performed using predefined calling parameters.

Statistical analysis. The SNPassoc package (https://CRAN.R-project.org/package=SNPassoc) in R (version 1.9-2) was employed to examine the association between global DNA methylation and five energy balance and lipid metabolism-associated loci. R is a language and environment for statistical computing and graphics, which is similar to the S language and environment (https://www.r-project.org/about.html).

Univariate and multivariate analyses were performed to investigate the association between each SNP and global DNA methylation adjusting for sex, age, weight, BMI and weight loss using the association function in the SNPassoc package in R. This function carries out an association analysis between a single SNP and a dependent variable (phenotype) under five different genetic models (inheritance patterns): co-dominant, dominant, recessive, over-dominant and log-additive. The only significant association between global DNA methylation and an SNP, adjusted by BMI and weight loss, was obtained when using the log additive model. $P<0.05$ was considered to indicate a statistically significant difference. All samples were analyzed in duplicate.

Results

Participant characteristics. The characteristics of the study participants are listed in Table II. The majority of the participants were females (57%). The average age of males and females was 45 and 42 years old, respectively. The obese to overweight ratio among males (73% obese and 27% overweight) was different to that observed for female participants (61% obese and 39% overweight). The mean age was 43.5 years old with a standard error of the mean (SEM) of 1.29, the median age was 45 years old, the age range was 11-67 years old and the interquartile range was 15. The mean weight was 85.6 kg with a SEM of 1.89, the median weight was 84.8 kg, the weight range was 55-139 kg and the interquartile range was 25.3. The mean height was 167.5 cm with a SEM of 0.92, the median height was 166 cm, the height range was 149-139 cm and the interquartile range was 25.3. The mean weight loss was 11.4 kg with a SEM of 1.02, the median

Table II. Patient characteristics in DNA methylation study that examined the association between global DNA methylation levels and SNPs.

Sex	
F (%)	60 (57%)
Age	
<34	23
35-39	8
40-44	21
45-49	23
50+	30
Mean (SEM)	43.5 (1.29)
Median (range)	45 (11-67)
Interquartile range	15
Weight (kilograms)	
Mean (SEM)	85.6 (1.89)
Median (range)	84.8 (55-139)
Interquartile range	25,3
Height (centimeters)	
Mean (SE)	167.5 (0.92)
Median (range)	166 (149-190)
Interquartile range	15
Weight loss (kilograms)	
Mean (SE)	11.4 (1.02)
Median (range)	10 (0-54)
Interquartile range	9
BMI	
Mean (SE)	30.1 (0.68)
Median (range)	30 (17-47)
Interquartile range	7
Diet	
Yes (%)	82 (78)
No (%)	22 (21)
Unknown (%)	1 (1)

weight loss was 12 kg, the range of weight loss observed was 0-64 kg and the interquartile range was 9. The mean BMI was 30.1 with a SEM of 0.68, the median BMI was 30, the BMI range was 17-47 and the interquartile range was 7. Furthermore, substantial weight loss was observed across the study (median average, 12 kg) however, marginally higher weight loss was observed in female (median average, 13 kg) compared with male participants (median average, 12 kg). BMI within the obese range was 30 on average, with a difference of 2 points between an average BMI of 29 for females and 31 for males.

Associations between GDMI values and loci associated with energy balance and lipid metabolism. The present study did not identify any associations between global DNA methylation and weight at baseline, BMI, sex or age (data not shown). However, an inverse association between global DNA methylation and weight loss (P<0.05; Fig. 1) was demonstrated. Furthermore, significant associations between the GDMI and *INSIG2*, after adjusting for BMI and weight loss (P<0.05), and significant trends when stratifying by gender (P<0.05) were also observed (data not shown). The frequency of genotypes for near *INSIG2* (rs7566605; 50.53, 41.05 and 8.42% for GG, GC and CC, respectively) and the boxplots for *INSIG2* genotype and their corresponding GDMI values are presented in Fig. 2. No significant associations between global DNA methylation and the other genes were observed (data not shown). The frequency of genotypes for the other four genes were as follows: *ADRB2* (rs1042714), 50.53, 34.74 and 14.74% for CC, CG and GG, respectively; *APOA5* (rs662799), 88.42, 10.53 and 1.05% for AA, AG and GG, respectively; *GNB3* (rs5443), 41.05, 50.53 and 8.42% for CC, CT and TT, respectively; and *MC4R* (rs17782313), 97.89, 1.05 and 1.05% for GG, AG and AA, respectively (Table III). Boxplots of GDMI values stratified by genotype for *ADRB2*, *GNB3*, *APOA5* and *MC4R* are presented in Fig. 3.

Discussion

The present study demonstrated an inverse association between global DNA methylation and weight loss; as weight loss increased, global DNA methylation decreased. However, no associations between global DNA methylation and weight at baseline, BMI, sex or age were observed. Therefore, global DNA methylation may have potential as a marker for weight loss potential from personalized weight reduction programs based on genotypic profiles. The association between near *INSIG2* (rs7566605) and global DNA methylation indicates that genetic variants may interact with epigenetic events that are ultimately associated with weight loss potential. The present study investigated the association between global DNA methylation and the allelic composition of five genetic loci associated with energy balance and lipid metabolism, and weight loss among participants, in a personalized weight reduction program designed on the basis of genotypic information. These five loci, near *INSIG2* (rs7566605), *MC4R* (rs17782313), *ADRB2* (rs1042714), *APOA5* (rs662799) and *GNB3* (rs5443), are among the most well-characterized SNPs regarding their roles in obesity, energy balance and lipid metabolism.

INSIG2 is located at 2q14.2. The protein product of this gene has a high degree of similarity with the protein encoded by *INSIG1*, both of which are endoplasmic reticulum proteins that inhibit sterol regulatory element binding proteins (SREBP) processing. *INSIG1/2* impair the processing of SREBPs as they bind to SREBP cleavage-activating protein (SCAP), which prevents SCAP from assisting the transport of SREBPs to the Golgi apparatus. Variations in *INSIG2* are reported to be associated with weight gain in certain ethnic subgroups (43,67). The SNP that was investigated in the present study, which is in close proximity to *INSIG2*, is present in 10% of the population and predisposes to the development of obesity in the general population. The CC single SNP predisposes to the development of obesity, is associated with a higher BMI and increases the probability of developing obesity by 40% (44-46). *MC4R* is located at 18q22. The protein product of this gene is a membrane-bound receptor and a member of the melanocortin receptor family.

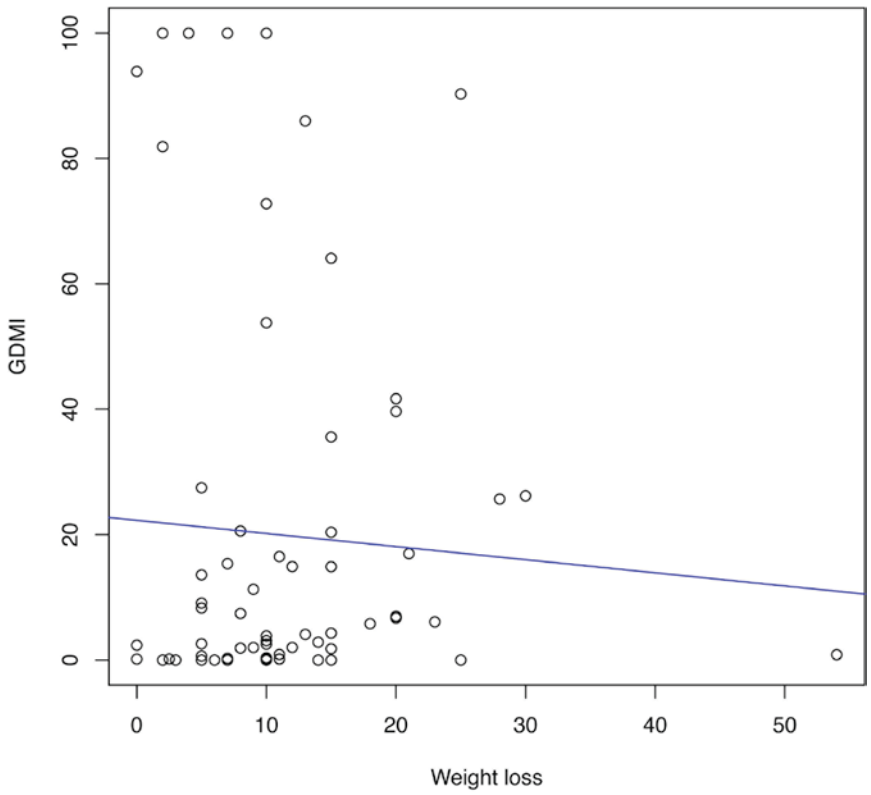

Figure 1. Inverse association between global DNA methylation and weight loss (P<0.05).

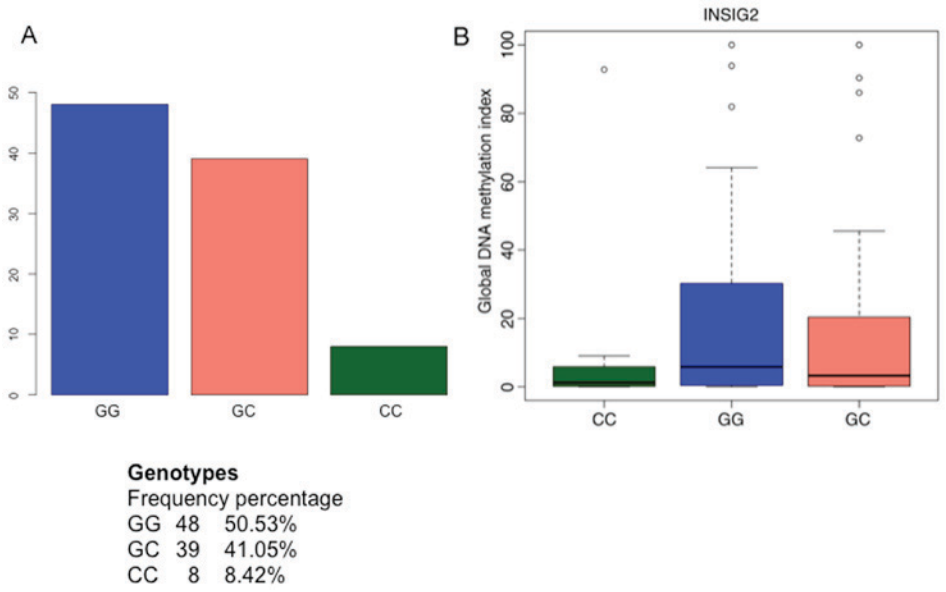

Figure 2. (A) The allelic frequency of near *INSIG2* (rs7566605) was 50.53, 41.05 and 8.42% for G/G, G/C and C/C, respectively. (B) Boxplots for each near *INSIG2* (rs7566605) genotype and their corresponding global DNA methylation index values. *INSIG2*, insulin-induced gene 2.

MC4R is primarily expressed in the central nervous system in areas where energy intake is controlled. The absence of this receptor leads to hyperphagia, hyperinsulinemia and obesity. The protein interacts with adrenocorticotropic and melanocyte-stimulating hormones, and is mediated by G-proteins. Variations in this gene are thought to be associated with waist circumference and insulin resistance, fat mass and obesity (47,48). Heterozygotes (CT) and homozygotes (TT) exhibit a higher energy expenditure, increased glucose oxidation, reduced levels of free fatty acids, a lower BMI and almost half the probability of developing obesity. For these reasons, *MC4R* variants are considered to be protective factors in obesity (49,50,68,69).

ADRB2 is located at 5q31-q32. This gene encodes the β2-adrenergic receptor, which is a member of the G-protein-coupled receptor superfamily. *ADRB2* has an

Table III. Frequency and percentage of alleles and genotypes at five loci associated with energy balance and lipid metabolism in study participants.

A, Frequency and percentage of alleles for each locus in study participants

Gene (SNP) and variants	Frequency	Percentage
ADRB2 (rs1042714)		
C	129	67.89
G	61	32.11
APOA5 (rs662799)		
A	178	93.68
G	12	6.32
GNB3 (rs5443)		
C	126	66.32
T	64	33.68
Near *INSIG2* (rs7566605)		
G	135	71.05
C	55	28.95
MC4R (rs17782313)		
C	187	98.42
T	3	1.58

B, Frequency and percentage of genotypes for each locus in study participants

Gene (SNP) and variants	Frequency	Percentage
ADRB2 (rs1042714)		
CC	48	50.53
CG	33	34.74
GG	14	14.74
APOA5 (rs662799)		
AA	84	88.42
AG	10	10.53
GG	1	1.05
GNB3 (rs5443)		
CC	39	41.05
CT	48	50.53
TT	8	8.42
Near *INSIG2* (rs7566605)		
GG	48	50.53
GC	39	41.05
CC	8	8.42
MC4R (rs17782313)		
CC	93	97.89
TC	1	1.05
TT	1	1.05

SNP, single nucleotide polymorphism; *ADRB2*, adrenoceptor β2; *APOA5*, apolipoprotein A5; *GNB3*, G-protein subunit β3; *INSIG2*, insulin-induced gene 2; *MC4R*, melanocortin 4 receptor.

important role in the regulation of energy balance as it leads to increases in lipolysis and thermogenesis (51). Variants in *ADRB2* are associated with obesity, chronic obstructive pulmonary disease and responses to asthma treatment (70-72). The *ADRB2* rs1042714 polymorphism was reported to regulate diet-induced alterations in body weight and composition; women with the CG variant that consumed diets rich in carbohydrates exhibited a 2.5-fold higher risk of developing obesity (52). Furthermore, the *ADRB2* rs1042714 allele is reported to be associated with increased BMI, body fat mass, fat cell volume and waist: hip ratio, in addition to associations with type II diabetes and the inhibition of lipid oxidation (54,55,73-75).

APOA5 is located at chromosome 11q23. This protein is an apolipoprotein that is involved in the regulation of plasma triglyceride levels, is associated with the levels of high-density lipoprotein cholesterol and the susceptibility to coronary artery disease (56,57,76,77). It is a component of high-density lipoprotein and accelerates the catalysis of low-density lipoprotein via the activation of the lipase protein. *APOA5* enhances the catabolism of triglyceride-rich lipoproteins and reduces the production of very low-density lipoprotein, which is the primary triglyceride carrier. The presence of one of its variants in heterozygosity (AG variant) or homozygosity (GG variant) is associated with an increased risk of cardiovascular disease and metabolic syndrome, which may lead to the development of diabetes and obesity (58-60,78). Additionally, the rs662799 SNP of *APOA5*, which was included in the present study, was associated with weight loss following short-term dieting (61).

GNB3 is located at 12p13. This gene is a heterotrimeric guanine nucleotide-binding protein and is also a member of the G protein-coupled receptor superfamily. The *GNB3* SNP rs5443 was reported to be a predictor of successful weight loss under sibutramine therapy (62). This variant has also been associated with various metabolic conditions, including obesity, coronary artery disease, insulin resistance and diabetes, and hypertension. Female carriers of the TT variant were reported to have a 6-fold higher risk of becoming overweight in the postpartum period, though the risk was markedly reduced in women who exercised regularly (63-65,79,80). In addition, associations between rs5443 and patient responses to sildenafil have been reported (81,82).

Epigenetic alterations occur over time and throughout the lifetime of individuals. Examples of these alterations include DNA methylation (83,84) and histone modifications (85,86), which are associated with factors such as diet (87), stress (88) and other modifiable lifestyles, including smoking (89) and alcohol consumption (90). Global DNA methylation levels, measured in PBMCs with Long Interspersed Nucleotide Elements-1 (LINE)-1 as a surrogate endpoint, was reported to be significantly higher in participants with a higher degree of weight loss compared with those who exhibited low responses (<8%) to energy-restricted treatment (91). LINE-1 was reported to be positively associated with healthy energy and micronutrient intake, and inversely associated with body fat mass (92).

It has been demonstrated that weight loss induced by a hypocaloric diet in humans altered the DNA methylation status of certain genes. Baseline DNA methylation patterns

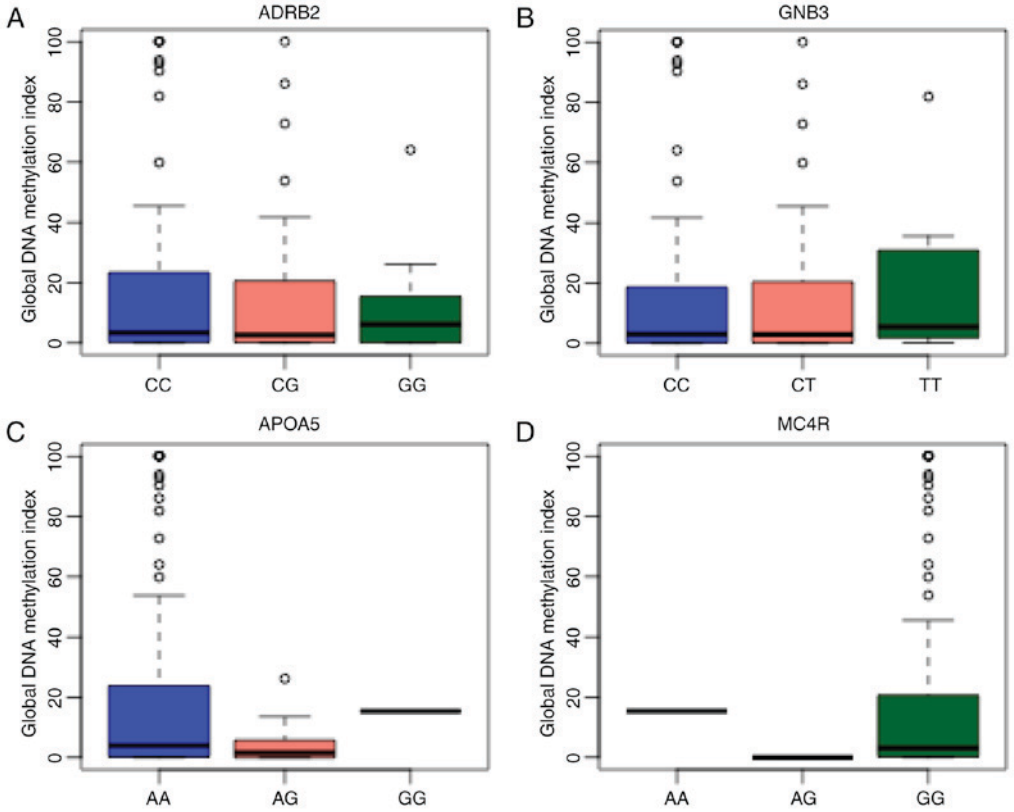

Figure 3. Boxplots of global DNA methylation index values stratified by genotype for (A) *ADRB2*, (B) *GNB3*, (C) *APOA5* and (D) *MC4R*. *ADRB2*, adrenoceptor β2; *GNB3*, G-protein subunit β3; *APOA5*, apolipoprotein A5; *MC4R*, melanocortin 4 receptor.

have previously been employed as epigenetic markers that may allow the degree of weight loss to be predicted in obese patients (52). In another study, epigenetic scores were used to predict alterations in body weight (7), and also identified five genes (aquaporin 9, dual specificity phosphatase 22, homeodomain-interacting protein kinase 3, troponin T1 slow skeletal type and troponin I3 cardiac type) that were differentially methylated between participants with high and low responses to a weight loss intervention program. The study also reported that subjects with the highest methylation in these regions exhibited a significantly enhanced response to the weight loss treatment program. While these studies demonstrate that differential methylation at specific loci may have an effect on weight loss, the results of the present study also demonstrate that global differential DNA methylation may also be associated with weight loss.

Global and gene-specific DNA methylation alterations, which vary with age, sex and socioeconomic status, may also be predictive biomarkers of weight loss response to intervention programs (91). Global DNA methylation and inflammatory gene promoter hypermethylation are reported to be early biomarkers of adiposity and metabolic alterations (93). Global DNA methylation and hydroxymethylation may functions as biomarkers in obesity and associated comorbidities. DNA methylation patterns are reported to behave differently depending on the choice of intervention in obesity (diet or surgery) (94).

The major strength of the present study is the simultaneous analysis of SNP loci and DNA methylation in the context of weight loss in obese patients. Interactions between germline variants of genes with somatic changes in epigenetic modifications may provide insights into pathologic causality in obesity and weight loss. The present study also demonstrates that non-invasive methods of assaying molecular biomarkers, such as those employed in the current study, may translate well in the clinic. Additionally, as global DNA methylation does not appear to be associated with initial BMI, initial weight, sex or age, further studies of this type should consider including additional weight-associated variable measurements that may be associated with global and gene-specific DNA methylation levels.

The results of the current study indicate that precision weight loss programs designed based on genetic and epigenetic information, which involve the creation of personalized interventions for individuals, may be beneficial for obese patients (95,96). These personalized programs may incorporate data from previous studies that have identified associations between DNA methylation, diet and weight loss. At present, is difficult to interpret how the interaction between *INSIG2* and global DNA methylation modulates the weight loss response. Therefore, additional studies should consider the concurrent associations of established and unknown energy balance and lipid metabolism SNPs to improve the understanding of the role of DNA methylation in obesity and the weight loss responses.

Acknowledgements

The present study was supported by the National Cancer Institute (grant nos. K01-CA164092 and U01-CA8498).

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