HHS Public Access

Author manuscript

Diabetes Obes Metab. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Diabetes Obes Metab. 2018 September ; 20(9): 2298–2303. doi:10.1111/dom.13333.

Genetic, epigenetic, and transcriptional variations at *NFATC2IP* locus with weight loss in response to diet interventions: The POUNDS Lost Trial

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Abstract

DNA Methylation of *NFATC2IP* was recently identified to be causally related to body mass index. The present study aimed to examine the roles of the genetic variation, methylation, and gene expression at this locus in adiposity changes in a 2-year weight-loss trial. Participants (n=692) were genotyped and randomly assigned to one of the four reduced-calorie diets, and DNA methylation was derived from stored blood samples at baseline (n=48). We found significant interactions of fat intake with the genetic (rs11150675) and transcriptional (ILMN_1725441) variations at the *NFATC2IP* locus on 2-year weight change ($Pi_{nteraction}$<0.01). Similarly, cis-DNA methylation at cg26663590 of the *NFATC2IP* locus showed opposite impact on weight-loss in response to high-fat vs. low-fat diet (effect size: 4.62 vs. -1.24 kg). Additionally, baseline methylation at cg26663590 causally mediated 52.8% of the effect of rs11150675 on 2-year weight-loss in the high-fat diet group (p=0.01), whereas no such mediation in the low-fat diet

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Author Contribution

DS conceptualized and designed the study, contributed to data cleaning and the statistical analysis, drafted the initial manuscript, and approved the final manuscript as submitted. YH, XL, and XS contributed to data cleaning and the statistical analysis, reviewed and revised the manuscript, and approved the final manuscript as submitted. SRS, GAB, and FMS coordinated and supervised the project, critically reviewed the manuscript, and approved the final manuscript as submitted. LQ conceptualized and designed the study, contributed to the statistical analysis, critically reviewed the paper and approved the final manuscript as submitted. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work. LQ is the guarantor of this work, had full access to all the data, and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Conflict of interest

All the authors have no conflicts of interest to disclose.

Trial registration: ClinicalTrials.gov NCT00072995

group. Our findings suggest potentially causal effects of genetic, epigenetic, and transcriptional variations at the *NFATC2IP* locus on adiposity changes in response to dietary fat intake.

Keywords

NFATC2IP gene; genetics; epigenetics; gene expression; diet intervention; dietary fat; weight loss; adiposity; fat distribution; body composition

1 Introduction

Obesity has reached an epidemic level worldwide and led to the rapid rise of its complicated cardiometabolic disorders over the past decades.[1] Diet intervention is among the mainstream approaches to promote weight loss and mitigate cardiometabolic complications.[2] Given that only a limited proportion of individual variability in weight loss interventions can be explained by genetic variations, it suggests that other mechanisms are also involved.[3,4] In a epigenome-wide association study, Wahl et al.[5] identified one and only potential causal pathway linking *NFATC2IP* genetic polymorphism (rs11150675), cis-DNA methylation (DNAm) at cg26663590 CpG sites and body mass index (BMI). However, their study did not analyze whether the *NFATC2IP* pathway affected adiposity changes in response to dietary intervention.

We performed longitudinal analyses to examine the impact of the *NFATC2IP* rs11150675 genotype on adiposity changes (body weight, fat distribution, and body composition) in the 2-year Preventing Overweight Using Novel Dietary Strategies (POUNDS LOST) trial. We particularly tested the potential interactions between dietary interventions and *NFATC2IP* genotype, cis-DNAm, and gene expression on 2-year weight change. Prospective analyses were also performed to access methylation-mediated effects of *NFATC2IP* genotype on weight change. In addition, we evaluated diet-induced changes in *NFATC2IP* gene expression at 6 months and further tested its relation with 2-year weight change.

2 MATERIALS AND METHODS

2.1 Study Population

The POUNDS Lost Trail is a randomized intervention trial in which 811 overweight and obese (BMI 25-40 kg/m^2) individuals were assigned to one of four energy-reduced diets varying in macronutrients, to compare their effects on adiposity changes over 2 years [6]. Two diets were low fat (20%), and the other 2 diets were high fat (40%), and 2 diets were average protein (15%), and the other 2 diets were high protein (25%), which constituted a 2-by-2 factorial design. The two high-fat diets were also low-carbohydrate diets, and two low-fat diets were high-carbohydrate diets. More details of this trial have been described in detail elsewhere [6]. The study was approved by two human subjects committees and by a data safety monitoring board appointed by the National Heart, Lung, and Blood Institute [6]. A written informed consent was assigned by each participant.

2.2 Measurements

Body weight was measured in the morning before having breakfast at baseline, 6, 12, 18 months and 2 years. Body composition was analyzed in a random sample of ~50% of the enrolled participants by a dual-energy X-ray absorptiometry (DEXA) scan using a Hologic QDR 4500A after an overnight fast.[7] Total fat mass (FM), total fat-free mass (FFM), and trunk fat percentage were obtained at baseline, 6 months and 2 years of the intervention. Of these individuals who had DEXA scans, ~50% were randomly assigned to receive computed tomography (CT) scans for total (TAT), visceral (VAT), deep (DSAT) and superficial (SSAT) subcutaneous abdominal fat masses.[7]

2.3 Genotyping, Methylation, and Gene Expression

The genotype success rate was 99%, replicate quality control samples (10%) were included, and genotyped with >99% concordance. Single-nucleotide polymorphism (SNP) rs11150675 near the *NFATC2IP* gene was genotyped in 692 subjects using the OpenArray SNP Genotyping System. Genome-wide DNAm scan was performed in DNA derived from peripheral blood leukocytes with stored baseline blood samples in 48 white participants (high- and low- responders to four weight-loss diets at 2 years), by using the Infinium HumanMethylation450 BeadChip. After quality control, 98% of probes were filtered. Data were normalized using Tost normalization and ComBat normalization function[8], and the relative proportions of six pure cell types were estimated using the R package *Minfi*.[9] Gene expression was measured in subcutaneous adipose tissue using the Illumina HT-12 v3 expression bead chip, biopsied from 96 volunteers (80 whites and 16 blacks). After background correction and normalization, 29,838 out of 48,803 probes were filtered (detection p-value < 0.01) and log2-transformed, and one probe (ILMN_1725441) at *NFATC2IP* gene was extracted. The genome region information at the *NFATC2IP* locus is shown in Figure S3.

2.4 Statistical Analysis

The primary outcome in the present study was weight changes, while changes in body composition, and abdominal fat distribution as our secondary outcomes. Generalized linear mixed models were used to test the potential interactions between the genetic effect (rs11150675), the epigenetic effect (cg26663590), and the transcriptional effect (ILMN_1725441) of *NFATC2IP* gene with diet interventions varying in fat intake, on the trajectory of weight change from baseline to 2 years. Mediation analyses were performed to evaluate the relationship of rs11150675 genotype (predictor), DNAm at cg26663590 at baseline (mediator), and 2-year weight loss (outcome). Effect size and confidence intervals of direct effect (DE) and indirect effect (IDE) were estimated by using the quasi-Bayesian Monte Carlo method with 1000 times simulations. The mediation effect was then calculated from the equation [Mediation Percent (%) = IDE*100% / (DE+IDE)]. All P values were nominal and 2-sided, and a P-value < 0.05 was considered statistically significant. Statistical analyses were performed with R version 3.3.2 (2016-10-31).

3 RESULTS

Study characteristics and a flowchart were presented in Table S1 and Figure S1, respectively. Of 692 participants genotyped, the average age was 51.4 years, 61.1% were females, and 84.2% were Whites. No significant difference was found in age, dietary intake, BMI, weight, body composition, fat distribution, DNAm, and gene expression between the *NFATC2IP* genotypes (P>0.05). Over 2 years' intervention, we found that the genotype determining DNAm at the *NFATC2IP* locus (cis-meQTL rs11150675) was significantly associated with greater reduction in fat mass, rather than lean mass (**Section S2**, Table S2, Figure S4a and S4b). The overall compliance with the dietary intervention was accessed by nutrient intake and biomarkers of adherence (**Section S1**), and we did not observe a significant difference (Table S3).

Intriguingly, we found a significant interaction between the rs11150675 genotype and low-/high-fat diet on the 2-year weight change ($P_{interaction}$=0.005) in Figure 1a. In response to the high-fat diet, carrying the A allele was marginally associated with a greater decrease in weight change. Conversely, a significantly less weight loss was observed among the A allele carriers, in response to the low-fat diet. In Figure 1c, we also found dietary fat considerably modified the association between the adipose tissue *NFATC2IP* gene expression (ILMN_1725441) at baseline and the 2-year weight loss ($P_{interaction}$<0.001). Similarly, a lower *NFATC2IP* methylation level at cg26663590 showed opposite associations with weight loss in response to high-fat vs. low-fat diet groups (effect size: -4.62 vs. 1.24) in Figure 1b.

We further performed mediation analyses on the relationships of the *NFATC2IP* genotype (rs11150675 A allele), DNAm (cg26663590), and weight change, as well as the estimated mediation effect (%) through DNAm, stratified by diet intervention groups. In the high-fat group, methylation level at cg26663590 at baseline mediated an estimated 52.8% effect of *NFATC2IP* rs11150675 genotype on weight changes over 2 years (p<0.01 in Figure 2b); whereas no such mediation effects observed in a low-fat group (p=0.80 in Figure 2c). Carrying rs11150675 A allele was associated with a lower methylation level at cg26663590 (p<0.05) (Figure 2a). In addition, we found that a high-fat diet led to a downregulated *NFATC2IP* gene expression (month 0 to 6), which further associated with a less weight-loss (month 6 to 24) (**Section S3** and Figure S2).

4 DISCUSSION

In this 2-year randomized controlled trial, dietary fat intake significantly modified the effect of the genetic (rs11150675), epigenetic (cg26663590), and transcriptional (ILMN_1725441) variations at the NFATC2IP locus on the long-term trajectory of weight change. Further, we found that *NFATC2IP* methylation mediated 52.8% of its genotypic effect on the 2-year weight loss in response to a high-fat diet rather than a low-fat diet.

Our study is the first incorporating the analyses of a genetic variant, DNAm, and transcription in weight loss in diet interventions, and our results showed consistent gene-diet interactions at multiple omics levels. Like a pathway of "*NFATC2IP* rs11150675 A allele, a

lower DNAm at cis (cg26663590), and a lower BMI" Wahl et al.[5] identified, our data not only validated the role of rs11150675 as cis-meQTL for cg26663590, but also supported rs11150675 A allele as well as a lower DNAm at cg26663590 on a greater weight-loss in a high-fat group. We further linked a higher *NFATC2IP* gene expression with a greater 2-year weight loss. As cg26663590 is located in the promoter region of the *NFATC2IP* locus, our finding also fitted the well-accepted mechanism between DNAm and gene expression, that hypomethylation at promoter region leads to upregulation or activation of the cis-downstream gene expression whereas hypermethylation acts as a barrier.[10] Therefore, we inferred a "stitched" pathway of "rs11150675 A allele – lower DNAm at cg26663590 – higher gene expression" at the *NFATC2IP* locus, which was associated with greater weight-loss in respond to high-fat rather than low-fat.

Intriguingly, our mediation analysis further confirmed the interconnected pathway of genetic, epigenetic, and transcriptional variations at the *NFATC2IP* locus in relation to adiposity. We demonstrated a cis-DNAm as a mediator for genetic effect on adiposity changes. Among participants in the high-fat group, we estimated a 52.8% of the "*NFATC2IP* genotype on weight change" impact was mediated by the cis-DNAm at cg26663590, implying that DNAm variation might account for the major genotype effects. The functional role for *NFATC2IP* in adipose biology is unclear. Wahl et al.[5] mentioned that this gene encodes *SH2B1*, a key regulator of leptin affecting obesity. In addition, *NFATC2IP* plays a role in cytokine production[11] and positive regulation of transcription by RNA polymerase II[12], which might be the targeted genes or pathways influenced by calorie-restricted diets in previous weight-loss RCTs[13-15]. Further studies are wanted to localize and validate this mediating process.

The major strengths of our study include multi-omics analysis, comprehensive and longitudinal measures of body fat and composition, as well as mediation analyses we used to indicate causality under an RCT setting. Even though, several limitations merit discussion. First, although our study is thus far the largest and longest diet intervention weight loss trial, the relatively small sample size in subgroups analyses may lead to wide estimated 95% confidence intervals and limited power. Second, DNAm was measured only once at baseline; thus, longitudinal changes owing to weight loss diets were unable to capture. Third, genetic, epigenetic, and transcriptional variants were all analyses at cis-region, epistasis (DNAm and gene expression at trans-) was not considered. Fourth, methylation was measured in peripheral blood leukocytes and the transcriptome in subcutaneous adipose tissue. Thus, validation in cell and tissue-specific manner is required. Furthermore, the majority of our study participants were white, whether our findings are generalizable to other ethnicities needs to be further investigated.

In summary, our results indicate that the *NFATC2IP* locus may interact with dietary fat at genetic, epigenetic, and transcriptional levels on adiposity changes. Further investigations are warranted to validate our findings.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the participants in The Preventing Overweight Using Novel Dietary Strategies (POUNDS) Lost trial for their outstanding commitment and cooperation. The study was supported by grants from the National Heart, Lung, and Blood Institute (HL071981, HL034594, HL126024), the National Institute of Diabetes and Digestive and Kidney Diseases (DK091718, DK100383, DK078616), the Boston Obesity Nutrition Research Center (DK46200), and United States–Israel Binational Science Foundation Grant2011036. Dr. Qi was a recipient of the American Heart Association Scientist Development Award (0730094N).

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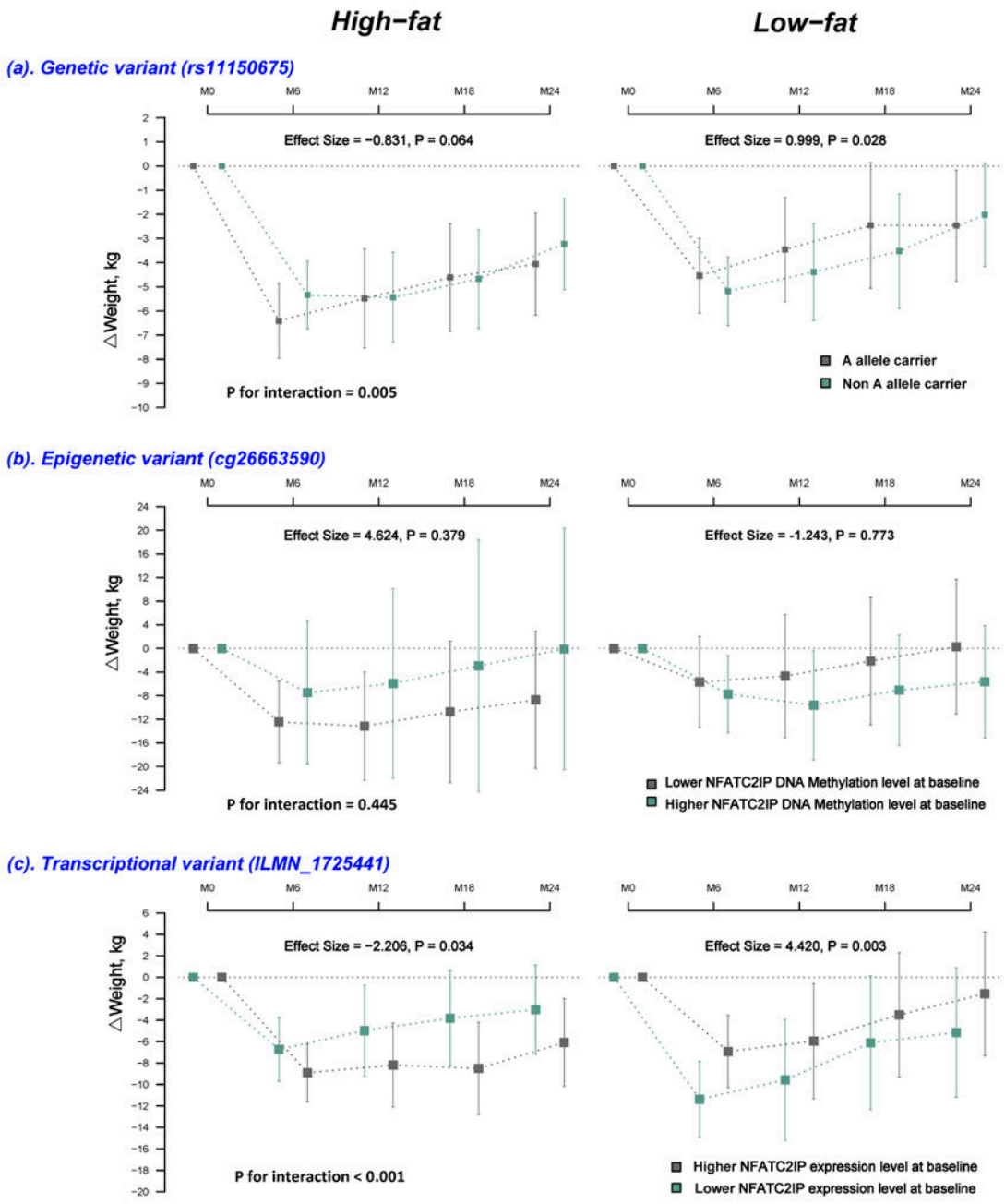

Figure 1.

Genetic (a), epigenetic (b), and transcriptional (c) variations at the NFATC2IP locus with weight loss in response to diet interventions varying in fat

Generalized linear mixed models (GLMM) were used to test the potential interactions between the genetic effect (SNP rs11150675), the epigenetic effect (DNA methylation at cg26663590 CpG site), and the transcriptional effect (gene expression at ILMN_1725441) with diet interventions varying in fat intake, on the trajectory of weight change from baseline to 2 years. A genotype-by-diet term (rs11150675 non A/A allele carriers × high-/low-fat diet group) was included in the GLMM, as well as methylation-by-diet term (lower-/higher-

methylation at cg26663590 × high-/low-fat diet group), and gene expression-by-diet term (lower-/higher-expression at ILMN_1725441 × high-/low-fat diet group). In the GLMM, covariates included age, gender, race, smoking status, and BMI at baseline as fixed-effects, and intervention time within individuals as random effects. In addition, GLMM was also used to calculate the regression coefficients (effect size) of genetic (reference group: non-A allele carriers), epigenetic (reference group: participants with a higher *NFATC2IP* DNA methylation level at baseline), and transcriptional variant (reference group: participants with a lower *NFATC2IP* gene expression level at baseline) on weight change in high-fat and low-fat diet groups, separately. We used the medians of methylation and gene expression level as the cutoff points to divide participants into two groups (lower vs. higher).

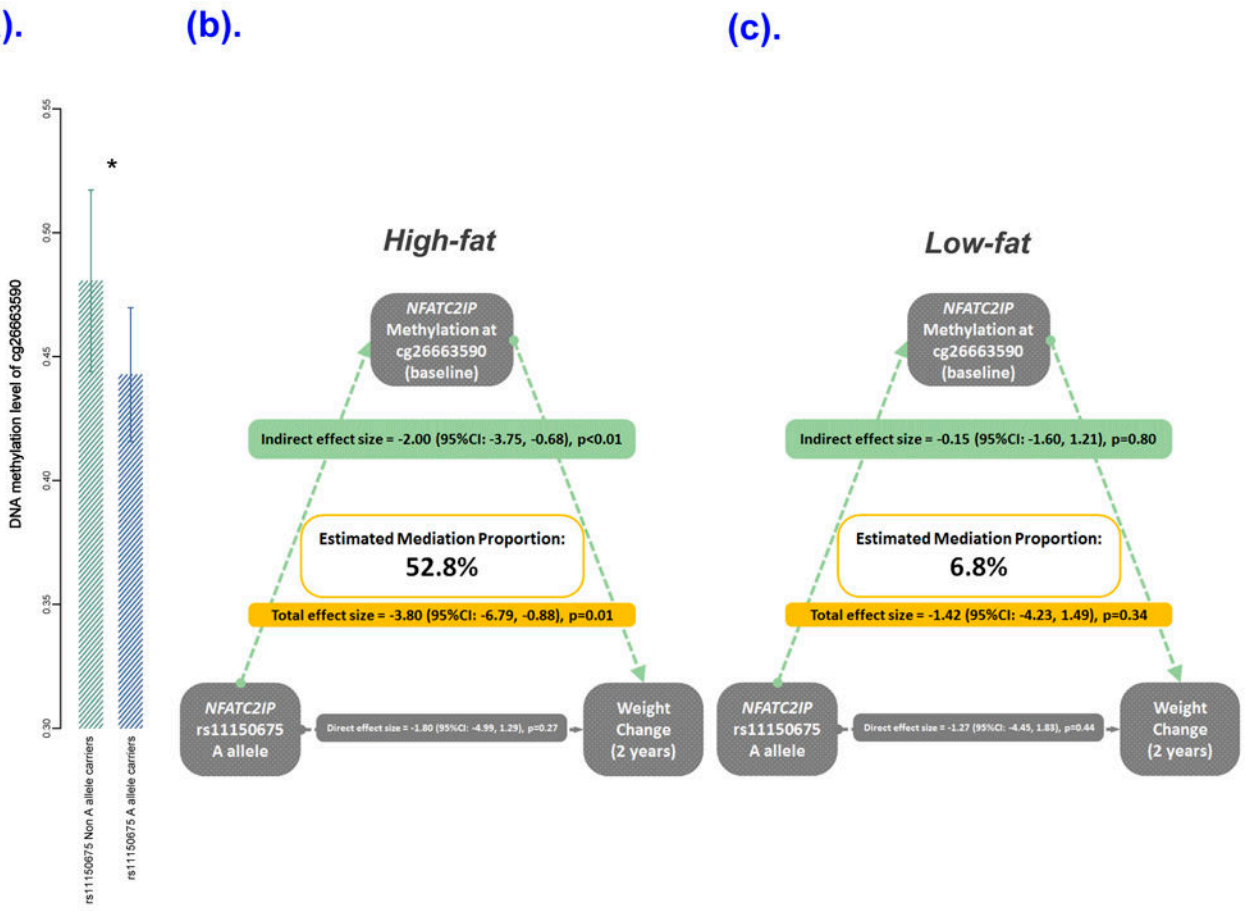

Figure 2.

(a) DNA methylation levels between the NFATC2IP genetic variant rs11150675 non-A allele carriers and A allele carriers. (b) mediation analyses of NFATC2IP genetic variant rs11150675 A allele (predictor), DNA methylation at cg26663590 CpG site at baseline (mediator), and trajectories of weight change over 2 years (outcome) in high-fat diet group (n=24). (c) mediation analyses of NFATC2IP genetic variant rs11150675 A allele (predictor), DNA methylation at cg26663590 CpG site at baseline (mediator), and trajectories of weight change over 2 years (outcome) in low-fat diet group (n=24).

In Figure 3a, a general linear model (GLM) was used to compare the difference in DNA methylation levels at cg26663590 CpG site between the *NFATC2IP* genetic variant rs11150675 non-A allele carriers and A allele carriers, adjusted for age, gender, and smoking status at baseline. In Figure 3b and Figure 3c, GLMs and GLMMs were fitted for the mediator and outcome models respectively, adjusted for age, gender, BMI, and smoking status at baseline.