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Epigenomics and metabolomics reveal the mechanism of the APOA2-saturated fat intake interaction affecting obesity

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ABSTRACT

Background: The putative functional variant $-265T>C$ (rs5082) within the *APOA2* promoter has shown consistent interactions with saturated fatty acid (SFA) intake to influence the risk of obesity.

Objective: The aim of this study was to implement an integrative approach to characterize the molecular basis of this interaction.

Design: We conducted an epigenome-wide scan on 80 participants carrying either the rs5082 CC or TT genotypes and consuming either a low-SFA (<22 g/d) or high-SFA diet (≥ 22 g/d), matched for age, sex, BMI, and diabetes status in the Boston Puerto Rican Health Study (BPRHS). We then validated the findings in selected participants in the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study ($n = 379$) and the Framingham Heart Study (FHS) ($n = 243$). Transcription and metabolomics analyses were conducted to determine the relation between epigenetic status, *APOA2* mRNA expression, and blood metabolites.

Results: In the BPRHS, we identified methylation site cg04436964 as exhibiting significant differences between CC and TT participants consuming a high-SFA diet, but not among those consuming low-SFA. Similar results were observed in the GOLDN Study and the FHS. Additionally, in the FHS, cg04436964 methylation was negatively correlated with *APOA2* expression in the blood of participants consuming a high-SFA diet. Furthermore, when consuming a high-SFA diet, CC carriers had lower *APOA2* expression than those with the TT genotype. Lastly, metabolomic analysis identified 4 pathways as overrepresented by metabolite differences between CC and TT genotypes with high-SFA intake, including tryptophan and branched-chain amino acid (BCAA) pathways. Interestingly, these pathways were linked to rs5082-specific cg04436964 methylation differences in high-SFA consumers.

Conclusions: The epigenetic status of the *APOA2* regulatory region is associated with SFA intake and *APOA2* $-265T>C$ genotype, promoting an *APOA2* expression difference between *APOA2* genotypes on a high-SFA diet, and modulating BCAA and tryptophan metabolic pathways. These findings identify potential mechanisms by which

this highly reproducible gene-diet interaction influences obesity risk, and contribute new insights to ongoing investigations of the relation between SFA and human health. This study was registered at clinicaltrials.gov as NCT03452787. *Am J Clin Nutr* 2018;108:188–200.

Keywords: *APOA2*, epigenomics, metabolomics, transcription, gene-diet interaction, obesity, tryptophan metabolism, branched-chain amino acid metabolism, satiety

INTRODUCTION

A common genetic variant within the promoter of apolipoprotein A-II gene (*APOA2*), $-265T>C$ (rs5082), with a frequency of the minor C allele ranging from 0.2 to 0.4, depending on the population, directs *APOA2* expression in vitro (1). Genetic studies in humans have shown associations with obesity and

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Supplemental Tables 1–4 and Supplemental Figures 1–7 are available from the “Supplementary data” link in the online posting of the article and from the same link in the online table of contents at <https://academic.oup.com/ajcn/>.

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Abbreviations: *APOA2*, apolipoprotein A-II; BPRHS, Boston Puerto Rican Health Study; FHS, Framingham Heart Study; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; PCA, principle component analysis; QC, quality control; SNP, single nucleotide polymorphism; TSS, transcription start site.

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postprandial metabolism of triglyceride-rich lipoprotein (1, 2). While this single nucleotide polymorphism (SNP) was not associated with obesity in genome-wide studies, we have demonstrated that this variant interacts with SFA intake on risk of obesity in several populations in the United States, Europe, and Southeast Asia (3–7). Individuals with the CC genotype have an elevated obesity risk when consuming a diet high in SFA (i.e., ≥ 22 g/d) (3, 6), compared with T allele carriers. In addition, CC carriers tend to consume more food, and particularly more high-fat and high-protein foods (7). Despite these consistent observations, the molecular mechanisms underlying this gene-by-diet interaction on obesity remain unknown.

Epigenetic status reflects an individual's profile acquired in response to environmental exposures (8). Early life nutrition, adult lifestyle habits, and other environmental factors can "program" disease risk in later life (9). Gene-diet interactions may reflect, in part, the effects of diet on epigenetic status. Epigenetic changes in promoter and enhancer regions can differentially affect mRNA expression of target genes, modifying their expression and, thus, regulation of specific metabolic pathways, which can be detected through metabolomic analysis (10).

Importantly, *APOA2* CC homozygotes exhibited an increased risk of obesity only when consuming a high-SFA diet, but they have no increased risk of obesity when consuming a low-SFA diet (4–8). This observation suggests that higher SFA intake occurs first, followed by *APOA2* genotype-associated obesity. Thus, we hypothesized that the genotype-dependent response to a high-SFA diet could involve epigenetic changes, leading to altered *APOA2* expression and metabolic changes in energy metabolism and homeostasis. In this study, we examined the correlation between epigenome-wide variation and *APOA2* genotype, conditional on dietary SFA intake, in selected participants of the Boston Puerto Rican Health Study (BPRHS), and then validated our findings in 2 additional populations, the Framingham Heart Study (FHS) and the Genetics of Lipid Lowering Drugs and Diet Network (GOLDN) Study. In the FHS, we further examined how the identified methylation site within the *APOA2* region affects *APOA2* mRNA expression across different *APOA2* genotypes and varying SFA intakes. We then investigated differences in metabolomic profiles between CC and TT genotypes in relation to low- or high-SFA intake in order to understand the relation between epigenetic signatures and metabolic pathways in the context of obesity risk. This study was designed to identify potential mechanisms by which this highly reproducible gene-diet interaction influences obesity risk, and also inform the long-standing debates surrounding saturated fat intake and health outcomes.

METHODS

Boston Puerto Rican Health Study

The BPRHS is a longitudinal cohort study (11). The primary objective is to examine and characterize the relations between nutrition, genetics, and health disparities in Puerto Rican adults. The population comprises 1499 individuals, self-identified as Puerto Ricans, with baseline age ranging from 45 to 75 y (11). To maximize the power to detect the effect of the C allele, we designed the study to focus on the difference between 2 homozygotes (CC compared with TT), which we expected to be maximal. Based on

our published observations of the *APOA2*-SFA interaction, which was consistent in several populations (3–6) and an epigenome-wide association study (12), we determined that a sample size of 20 for each CC and TT genotype with either low- or high-SFA has statistical power of 95% to detect epigenetic signatures at a significance of $\alpha = 1.1 \times 10^{-7}$ (1% methylation difference between CC and TT genotype and an SE of 0.2%) of the *APOA2*-SFA interaction on obesity. Within this study (see **Supplemental Figure 1**), 40 participants with CC genotype at *APOA2* $-265T>C$ were selected, with 20 reporting a low-SFA intake (<22 g/d) and 20 reporting a high-SFA intake (≥ 22 g/d) (3, 6). By matching age, sex, SFA intake, type 2 diabetes status, and BMI for the 40 participants with CC genotype, the second set of 40 participants with TT genotype at *APOA2* $-265T>C$ was selected from the same population, with priority given to participants who were matched most closely for age, sex, SFA intake, and type 2 diabetes (**Table 1**). The Institutional Review Board at Tufts University approved the study protocol. All participants provided written consent for participation in the study.

Genetics of Lipid Lowering Drugs and Diet Network Study

The GOLDN Study is a constituent of the Family Heart Study within the NIH National Heart, Lung and Blood Institute (7). Participants ($n = 1327$), aged ≥ 18 y, from US families of European descent, were recruited at 2 field centers: Minneapolis, MN and Salt Lake City, UT. The main goal of the GOLDN Study was to identify genetic factors that determine individual responses to high-fat meals and 3-wk treatment with fenofibrate, a triglyceride-lowering medication (12). Institutional Review Boards at Tufts University, the University of Minnesota, University of Utah, and the University of Alabama at Birmingham approved the study protocol. In the current study (see **Supplemental Figure 2**), 107 participants with CC genotype and 272 with TT genotype for *APOA2* $-265T>C$, who were not taking medication for hypertension, dyslipidemia, or diabetes, were selected to validate the findings from the BPRHS. Participants of each genotype were further divided into 2 subgroups, based on low-SFA (<22 g/d) and high-SFA intake (≥ 22 g/d) (**Table 1**) (3, 6).

Framingham Heart Study

Established in 1948, the FHS is a free-living multiple generation study of participants recruited in and near Framingham, MA (13). The Offspring Cohort started in 1971 with the enrollment of 5124 participants recruited from the children and spouses of the original FHS (14). In-person physical and clinical examinations occurred every 4–8 y. In exam 8, 2005–2008, blood samples were collected, and 2741 samples were available for DNA methylome and transcriptome analysis (15). In this study (see **Supplemental Figure 3**), to validate the findings from the BPRHS, we include 73 unrelated participants with CC genotype and 170 with TT genotype at *APOA2* $-265T>C$ who did not take medication for hypertension, dyslipidemia, or diabetes. Participants of each genotype were further divided into 2 subgroups based on SFA intake: low, <22 g/d; high, ≥ 22 g/d (**Table 1**) (3, 6).

TABLE 1Characteristics of participants in 3 populations according to CC and TT genotypes of *APOA2* -265T >C¹

	TT		CC	
	Low-SFA intake	High-SFA intake	Low-SFA intake	High-SFA intake
BPRHS				
<i>n</i> = 80	20	20	20	20
Women, %	50	50	50	50
Age, y	59.5 ± 6.8	59.6 ± 6.8	59.5 ± 6.7	58.6 ± 7.6
Drinker, %	30	60	35	60
Smoker, %	10	25	35	30
BMI, kg/m ²	31.6 ± 5.8	31.2 ± 5.6	29.4 ± 6.0	34.0 ± 5.7 ²
SFA, g/d	14.0 ± 4.2	33.7 ± 8.6 ²	14.6 ± 5.0	35.1 ± 12.6 ²
Total energy, kcal	1699 ± 561	3000 ± 630 ²	1522 ± 416	2932 ± 691 ²
GOLDN				
<i>n</i> = 379	107	165	39	68
Women, %	77	42	85	40
Age, y	46.3 ± 16.8	41.5 ± 14.0	43.8 ± 15.8	43.4 ± 12.7
Drinker, %	47	45	64	54
Smoker, %	26	26	31	29
BMI, kg/m ²	27.9 ± 5.5	26.8 ± 5.0	27.1 ± 5.4	29.1 ± 5.9
SFA, g/d	15.3 ± 4.5	38.0 ± 20.8 ²	15.9 ± 4.0	41.1 ± 19.5 ²
Total energy, kcal	1351 ± 383	2599 ± 1407 ²	1367 ± 402	2884 ± 1208 ²
FHS				
<i>n</i> = 243	74	96	34	39
Women, %	73	56	71	62
Age, y	65.1 ± 9.3	62.1 ± 8.2	65.4 ± 9.4	64.4 ± 7.7
Drinker, %	76	72	91	82
Smoker, %	12	4	6	10
BMI, kg/m ²	25.3 ± 4.2	27.5 ± 5.4 ²	25.4 ± 3.7	26.7 ± 4.5
SFA, g/d	16.1 ± 4.2	31.6 ± 9.2 ²	15.9 ± 4.3	30.6 ± 7.6 ²
Total energy, kcal	1532 ± 349	2284 ± 512 ²	1528 ± 337	2336 ± 537 ²

¹Age, BMI, SFA, total energy are expressed as means ± SDs. *APOA2*, apolipoprotein A-II; BPRHS, Boston Puerto Rican Health Study; FHS, Framingham Heart Study; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network.

²Significant differences by *t* test between high- and low-SFA in participants with CC or TT genotype.

Assessment of dietary intake

In the BPRHS, diet was assessed using a specially designed and validated food frequency questionnaire (16), whereas the Diet History Questionnaire was administered in the GOLDN Study (17) and the Willett semi-quantitative food frequency questionnaire was applied in exam 8 of the FHS (18). The dietary intake for each population (16–18) was estimated based on the Harvard University food composition database, the US Department of Agriculture database, and the Minnesota Nutrient System.

DNA extraction

In the BPRHS and the FHS, genomic DNA for methylation analysis was extracted from buffy coats using the QIAamp DNA Blood mini kit or Gentra Puregene DNA extraction kit (Qiagen) (15). In the GOLDN Study, genomic DNA was isolated from CD4⁺ T cells from frozen buffy coat samples using DNeasy kits (Qiagen) (12).

Methylome analysis

Genome-wide DNA methylation of isolated DNA samples in the BPRHS and the GOLDN Study was quantified using Illumina Infinium human methylation 450K arrays (Illumina), as described previously (15, 19). The methylation signal at each

methylation site was estimated as a β score, the proportion of the total methylation-specific signal, and the detection *P* value was the probability that the total intensity for a given probe fell within the background signal intensity. Methylation sites were excluded from further analysis if one of the following criteria was met: 1) detection *P* > 0.01 and 1.5% of samples have missing data, or 2) >10% of samples lack sufficient intensity (12). The methylation signal β scores were further adjusted for batch effects across samples and normalized using the ComBat function in the ChAMP R package (20). To account for heterogeneity of different cell types across all samples, principal components were calculated, based on the β scores of all autosomal CpG sites that passed quality control, using the *prcomp* function in R (v12.12.1). The first 4 principal components were used in all subsequent statistical analyses.

We acquired the FHS DNA methylome data through dbGaP, accession #phg000492.v2, where the methylation statuses of 2741 participants were measured at exam 8, using Illumina Infinium human methylation 450K arrays (15). Methylation signals were processed and normalized as for the BPRHS and the GOLDN Study.

Transcription analysis

We obtained FHS transcriptome data from dbGaP under accession #phe00002.v6. Transcriptome analysis was conducted in

exam 8 using the Affymetrix Human Exon 1.0 ST array with mRNA from whole blood samples collected from 725 Offspring Cohort participants after overnight fasting (21). The quality control and normalization of the initial gene expression data have been published elsewhere (22, 23). In this study, we extracted gene expression data for 201 of 243 participants with CC or TT genotypes who were not taking medication for hypertension, dyslipidemia, or diabetes. Furthermore, to determine if the identified methylation site (i.e., cg04436964) was correlated with gene expression of *APOA2* or nearby genes, we focused analysis on the expression of those 10 genes that passed quality control (QC) of the 31 genes that map within 50 kb upstream or downstream of the identified methylation site. Genes present on the array but not passing QC can be considered as unresponsive to methylation at cg04436964.

Metabolomic profile

Metabolic profiling of plasma samples from those 80 participants of the BPRHS for whom the methylome analysis was performed was conducted by Metabolon, Inc. (24). Briefly, 80 plasma samples were shipped on dry ice to Metabolon, and stored at -80°C until analysis. After proteins were removed with methanol, metabolomic analysis was performed using ultrahigh-performance liquid chromatography-tandem mass spectroscopy. With reference to a library of over 4500 purified standards for retention time/index, mass-to-charge ratio, and chromatographic

data, individual metabolites were identified and quantified by estimating the AUC of the peaks. A total of 808 metabolites that passed QC were identified and assigned to pathway groups.

Statistical analysis

A schematic of the data analysis with sample size information is depicted in **Figure 1**.

Epigenome-wide analysis of *APOA2* genotype, by high- and low-SFA intake, in the BPRHS

To identify epigenetic sites that differed between *APOA2* $-265\text{T}>\text{C}$ CC and TT genotypes, we conducted an epigenome-wide association study in 80 participants selected according to their genotype using the GEM package R tool suite, with methylation signals as dependent variables with the G-model (detecting methylation markers associated with genotype, i.e., methylation quantitative trait loci) (25), adjusting for medication for hypertension, lipid-lowering, cell-type heterogeneity, and population structure. Population structure was adjusted by principle component analysis (PCA), with 50,704 selected SNPs genotyped using Affymetrix Axiom World LA Arrays, designed especially for Hispanic populations, and probe sets to genotype 817,810 SNPs. The 50,704 SNPs were selected based on call rate $>97\%$, MAF $\geq 5\%$, pairwise linkage disequilibrium $R^2 \leq 0.1$, and P value of the Hardy-Weinberg Equilibrium $\geq 10^{-6}$. Using PCA

		Low SFA		High SFA	
Methylome : Discovery Epigenome-wide association with <i>APOA2</i> -265T>C identified cg04436964 in low and high SFA intake	BPRHS	CC vs TT n=20	CC vs TT n=20	CC vs TT n=20	CC vs TT n=20
Methylome : Validation Epigenome-wide association with <i>APOA2</i> -265T>C in two additional populations • Confirmed cg04436964	GOLDN FHS	CC vs TT n=39	CC vs TT n=107	CC vs TT n=68	CC vs TT n=165
Methylome : Meta-analysis Combine results of three populations • Confirmed cg04436964		BPRHS, GOLDN and FHS n=40, 146, and 108		n=40, 233, and 135	
Transcription Association of cg04436964 with mRNA levels of <i>APOA2</i> and neighboring genes	FHS	CC vs TT n=31	CC vs TT n=58	CC vs TT n=34	CC vs TT n=78
Metabolome 635 of 808 metabolites in 63 pathways • pathway enrichment analysis	BPRHS	CC vs TT n=20	CC vs TT n=20	CC vs TT n=20	CC vs TT n=20
Linking Epigenome and Metabolome Correlation between cg04436964 and 635 metabolites in 63 pathways • cg04436964-associated pathway enrichment analysis	BPRHS	CC vs TT n=20	CC vs TT n=20	CC vs TT n=20	CC vs TT n=20

FIGURE 1 Analysis scheme. Experiments, described in the leftmost column, proceeded from top to bottom. Cohort source and sample sizes for the various genotypes at *APOA2* $-265\text{T}>\text{C}$ under conditions of low or high SFA intake are given. For all analyses involving the BPRHS cohort, data from the same participants were used, and from the same data collection period. *APOA2*, apolipoprotein A-II; BPRHS, Boston Puerto Rican Health Study; FHS, Framingham Heart Study; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network.

implemented in SVS (GoldenHelix Inc.), one eigenvalue was selected to represent population structure, based on the Scree plot. This PCA1 principle component variable was used in all regression models to adjust for population structure. Total energy intake was not included as a covariate in the linear regression model as *APOA2* -265T >C is known to be associated with total energy intake (3). The Bonferroni correction with an epigenome-wide significance of $P = 1.1 \times 10^{-7}$ was used to adjust for multiple comparisons (12).

Validation in the GOLDN Study and the FHS

To validate the initial findings from the BPRHS, we conducted a similar epigenome-wide association study in selected datasets from the GOLDN Study (see Supplemental Figure 2) and from the FHS (see Supplemental Figure 3). In the GOLDN Study, 271 participants with TT genotype and 107 participants of CC genotype who did not use medication for hypertension, dyslipidemia, or diabetes were included in the validation analysis (Table 1 and Supplemental Figure 2). To identify methylation quantitative trait loci, a linear Gmodel was implemented with methylation measures as dependent variables and *APOA2* genotype as a predictor using the GEM package R tool suite (25), adjusting for age, sex, center, cell-type heterogeneity, and family relationship. In the FHS, 171 participants with TT genotype and 73 with CC genotype, who did not take medication for hypertension, dyslipidemia, or diabetes, were included for validation (Table 1 and Supplemental Figure 3). A similar linear G-model was implemented using the GEM package R tool suite (25), controlling for cell-type heterogeneity, age, and sex. All analyses were conducted using R3.3.2, and the results were reviewed and formatted using the genome tool of SVS 8.7.

Meta-analysis

A meta-analysis of the results from the 3 populations was conducted using the meta R package (<https://cran.r-project.org/web/packages/meta/index.html>). A comparison of allelic effect (β) of *APOA2* -265T >C from the meta-analysis between low- and high-SFA intake was conducted with SAS 9.4 (SAS Inc.) using a *t* test.

Associations between epigenetic variants and *APOA2* mRNA expression in the FHS

To determine whether identified methylation sites in the *APOA2* region were associated with *APOA2* mRNA expression in whole blood, we examined the correlation between the identified methylation site (cg04436964, which showed differential association with *APOA2* genotype and differed between low- and high-SFA intake) and FHS exam 8 expression data for genes located within a 50-kb region of the methylation site. Gene expression measures were treated as dependent variables, and methylation sites and *APOA2* genotype were tested as predictors, controlling for age, sex, and cell-type heterogeneity. These analyses were conducted using a linear regression model with SAS 9.4 or SVS 8.7.

Metabolic pathway enrichment analysis

All detected metabolites ($n = 808$) were organized into metabolic pathways based on the annotation database of Metabolon Inc. Only pathways that contained ≥ 3 detected metabolites were included in the pathway analysis. For each metabolite, comparisons between CC and TT genotypes were conducted for high- or low-SFA intake. These comparisons were conducted using ANCOVA contrast in a 2-way ANOVA model, which included the fixed effects and interaction terms of genotype and SFA, adjusting for BMI. The mean square error for the overall model was used in the calculation of the individual contrasts. The number of metabolites that differ between CC and TT genotypes at $P \leq 0.05$ was counted within each pathway. Metabolic pathway enrichment between CC and TT genotypes was determined by *z* score calculation for each pathway, separately for low- and high-SFA intake, as: $Z \text{ score} = [r - n(R/N)] / \sqrt{\{n(R/N)[1 - (R/N)][1 - (n - 1)/(N - 1)]\}}$ (26), where $N = 635$ is the total number of metabolites detected in the metabolomic profiling that were included in the 63 pathways, R is the total number of metabolites significantly different between CC and TT genotypes within low- or high-SFA intake, n is the total number of metabolites measured in a specific pathway, and r is the number of metabolites significantly different between CC and TT genotypes within a specific pathway. *P* values of *Z* scores were derived assuming a normal distribution and 2-sided, and were then corrected for multiple testing by the Bonferroni test ($P = 0.05/63, 0.0008$).

Linking the epigenomic and metabolomic signatures

To uncover the connection between epigenomic status and metabolic network, we conducted metabolic network enrichment analysis in relation to the identified methylation site cg04436964, which differed between CC and TT genotypes with a high-SFA intake. We performed an association analysis between cg04436964 and all metabolites using a linear regression model, adjusting for age, sex, population structure, and cell-type heterogeneity. The cg04436964-associated (at $P \leq 0.05$) metabolites were evaluated for overlap with those that differed (at $P \leq 0.05$) between CC and TT genotypes under high-SFA intake. The number of metabolites that met both criteria (associated with cg04436964 at $P \leq 0.05$ and differed between CC and TT genotypes under high-SFA intake) were counted within each pathway. *z* scores and *P* values were calculated as above for each of 63 pathways. The *P* values were corrected for multiple testing using a Bonferroni test. Missing data were excluded from all the analyses.

RESULTS

Epigenome-wide association analysis of *APOA2* stratified by SFA intake in the BPRHS

The general characteristics of 2 groups of selected participants with CC genotypes ($n = 40$) and low- (<22 g/d) or high-SFA intake (≥ 22 g/d) (3, 6) and 2 groups of participants with TT genotypes ($n = 40$) matched to the CC group by SFA intake, age, and sex are shown in Table 1. While the men:women ratio and age are almost identical among the 4 groups, the mean BMI of the CC participants with high-SFA was significantly higher than that

TABLE 2CpG sites that show significant difference in methylation status between *APOA2* genotypes according to saturated fat intake in 3 populations¹

	CpG site	Chr:Position ²	<i>n</i>	CC vs. TT				
				Low-SFA		<i>n</i>	High-SFA	
				β (SE)	<i>P</i> value ³		β (SE)	<i>P</i> value ³
BPRHS	cg04436964	1:161,167,745	20	0.031 (0.006)	1.22×10^{-6}	20	0.035 (0.005)	4.35×10^{-8} *
	cg24429974	1:161,195,180	20	0.010 (0.006)	3.77×10^{-3}	20	0.009 (0.008)	0.255
	cg24847046	1:161,135,079	20	-0.009 (0.006)	0.185	20	-0.017 (0.006)	0.011
GOLDN	cg04436964	1:161,167,745	146	0.009 (0.002)	1.59×10^{-3}	233	0.013 (0.002)	1.02×10^{-10} *
	cg24429974	1:161,195,180	146	0.009 (0.002)	1.51×10^{-4}	233	0.011 (0.002)	1.84×10^{-10} *
	cg24847046	1:161,135,079	146	-0.004 (0.003)	0.106	233	-0.007 (0.002)	2.51×10^{-4}
FHS	cg04436964	1:161,167,745	108	0.031 (0.004)	5.23×10^{-12} *	135	0.040 (0.003)	1.21×10^{-25} *
	cg24429974	1:161,195,180	108	0.022 (0.003)	1.63×10^{-10} *	135	0.021 (0.003)	3.33×10^{-12} *
	cg24847046	1:161,135,079	108	-0.013 (0.003)	3.38×10^{-6}	135	-0.018 (0.003)	2.70×10^{-8} *

¹* *P* value $\leq 1.1 \times 10^{-7}$. *APOA2*, apolipoprotein A-II; BPRHS, Boston Puerto Rican Health Study; FHS, Framingham Heart Study; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network.

²The physical position of CpG sites was based on the genome build 37.

³The threshold of epigenome-wide significance is *P* value $\leq 1.1 \times 10^{-7}$.

of the low-SFA intake group. In addition, SFA and total energy intake were significantly greater in the high-SFA groups than in the low-SFA groups regardless of *APOA2* genotype. These differences are expected, as the participants were selected unbiasedly to represent *APOA2* genotype and SFA intake on obesity (3).

To identify the epigenomic signatures of *APOA2* by SFA interaction, we conducted an epigenome-wide scan to compare the

methylation status of 20 participants with CC genotype and 20 participants with TT genotypes each stratified by low- or high-SFA intake (Figure 1). The Manhattan plots and QQ plots of all methylation sites are depicted in Supplemental Figures 4A and B, and 5A and B. For participants with high-SFA intake (Supplemental Figure 4B), one CpG site, cg04436964, with methylation ranging from 0.77 (77%) to 0.96 (96%), attained genome-wide

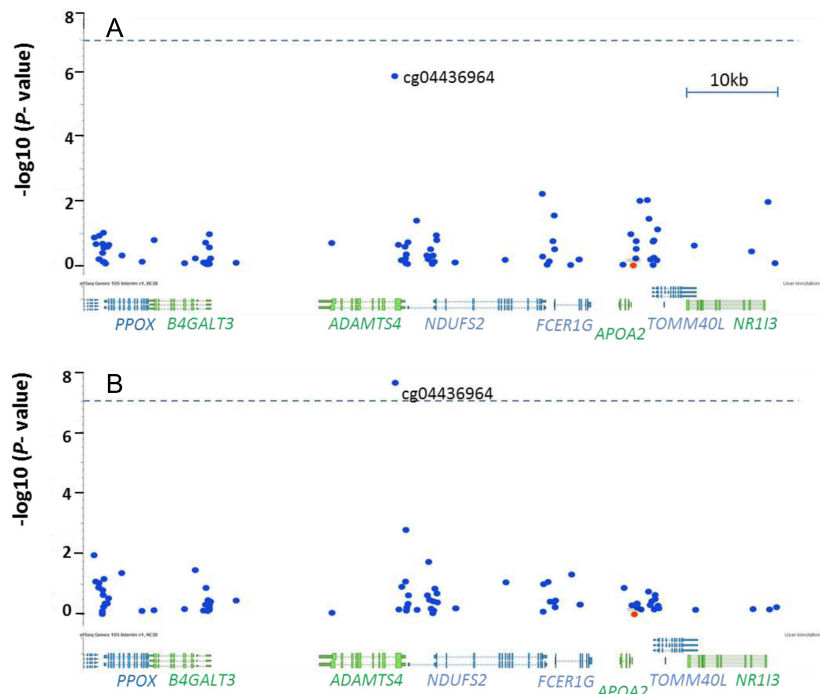


FIGURE 2 Close-up of the 81-kb genomic region surrounding the *APOA2* -265T > C site (rs5082, the orange dot) of an epigenome-wide association study with *APOA2* -265T > C in the BPRHS, GRCh37/hg19 approximate coordinates: chr1:161,134,000–1,612,215,000. (A) Under low saturated fat intake or < 22 g/d (low-SFA), (B) under high saturated fat intake or ≥ 22 g/d (high-SFA). The vertical axis displays the $-\log_{10}(P$ value) of association, whereas the horizontal axis displays the physical position of CpG sites and genes (genome build 37). Each blue dot depicts one CpG site. The dashed line indicates the threshold of epigenome-wide significance at $P = 1.1 \times 10^{-7}$. *ADAMTS4*, a disintegrin and metalloproteinase with thrombospondin motifs 4; *APOA2*, apolipoprotein A-II; BPRHS, Boston Puerto Rican Health Study; *B4GALT3*, Beta-1,4-Galactosyltransferase 3; *FCER1G*, Fc fragment of IgE receptor 1; *NDUFS2*, NADH:ubiquinone oxidoreductase core subunit 2; *NR1I3*, nuclear receptor subfamily 1 group I member 3; *PPOX*, protoporphyrin oxidase; *TOMM40L*, translocase of outer mitochondrial membrane 40 like.

significance at $P = 4.35 \times 10^{-8}$ between CC and TT genotypes (Table 2). This CpG maps ~25.7 kb from the *APOA2* transcription start site (TSS) and ~25.9 kb from $-265T > C$. The physical map of the 50-kb region centered on *APOA2* $-265T > C$ (Figure 2B) indicates the single methylation site that displayed significant differences between CC and TT genotypes. One copy of the C allele is associated with a 3.5% increase in methylation at cg04436964 under a high-SFA diet, assuming an additive model for the epigenetic effect. For participants with low-SFA intake (Figure 2A, Table 2, Supplemental Figures 4A and 5A), however, methylation at no other CpG site differed significantly between CC and TT genotypes after correction for multiple testing ($P > 1.1 \times 10^{-7}$). As obesity may influence epigenetic status, we controlled for BMI and observed that the associations remained consistent. Furthermore, a comparison of methylation under conditions of high- and low-SFA intake within each genotype, CC or TT, indicated that no methylation site showed an epigenome-wide significant difference (between low- and high-SFA) within genotype ($P > 1.1 \times 10^{-7}$, data not shown).

Validation of epigenetic signature of interaction between *APOA2* and SFA intake

To validate the initial finding that cg04436964 methylation status correlates with *APOA2* $-265T > C$ genotype when SFA intake is high in the BPRHS, we conducted in the GOLDN Study an epigenome-wide association study similar to the BPRHS. The general characteristics of 379 participants of either CC ($n = 107$) or TT ($n = 272$) genotype from the GOLDN Study are listed in Table 1, excluding those taking medication for hypertension, diabetes, or dyslipidemia. Similar to the BPRHS, in the GOLDN Study, participants' methylation at cg04436964 showed a significant difference between CC and TT genotypes with high-SFA intake at $P = 1.02 \times 10^{-10}$ (Table 2). In addition, cg24429974, 1.5 kb distal to $-265T > C$, also exhibited a significant genotype-specific methylation difference at $P = 1.84 \times 10^{-10}$ in participants with high-SFA intake, and the significance of the results remained after adjustment for BMI. These significant associations between methylation sites and *APOA2* genotype are depicted for the 80 kb region around *APOA2* $-265T > C$ in Supplemental Figure 6B. One copy of the C allele reflects an increase in methylation of 1.3% at cg04436964 with high-SFA intake (Table 2), assuming an epigenetic effect of an additive model. However, in GOLDN Study participants with low-SFA intake (< 22 g/d) (3, 6), these 2 methylation sites (cg04436964 and cg24429974) did not reach epigenome-wide significance between CC and TT genotypes (Supplemental Figure 6A, $\beta = 0.009$ and 0.009 , $P = 1.59 \times 10^{-3}$ and 1.51×10^{-4} , respectively).

To strengthen the validation, we conducted an additional epigenome-wide association study with CC and TT participants in the FHS at exam 8 who were not taking medication for hypertension, diabetes, or dyslipidemia. The general characteristics of the participants are given in Table 1. Similarly, methylation site cg04436964 showed greater significant differences between CC and TT genotypes in participants with high-SFA intake than with low-SFA intake (Supplemental Figure 7, $\beta = 0.040$ vs. 0.031 , $P = 1.21 \times 10^{-25}$ vs. 5.23×10^{-12} , Table 2). In comparison with cg04436964, cg24429974 showed a similar but weaker genotype-related difference between FHS participants with low-

and high-SFA intake (Supplemental Figure 7, $\beta = 0.021$ vs. 0.022 , $P = 3.33 \times 10^{-12}$ vs. 1.63×10^{-10} , Table 2). In addition, cg24847046, 58.6 kb from rs5028, showed a significant association with *APOA2* genotype in participants with low- or high-SFA intake (Supplemental Figure 7, $\beta = -0.013$ vs. -0.018 , $P = 3.38 \times 10^{-6}$ vs. 2.70×10^{-8} , Table 2). Even after adjustment for BMI, the findings remained consistent in all 3 populations. Together, each of the associations described in 3 distinct populations for cg04436964 are directionally identical, reach epigenome-wide significance thresholds, are dependent on SFA intake and rs5028 genotype, and share robustness with the gene-diet interaction described for this variant.

Meta-analysis of methylation differences between CC and TT genotypes

To combine the results on these populations, we conducted a meta-analysis with a fixed-effect model and found that methylation site cg04436964 showed a greater difference between CC and TT genotypes in high-SFA intake (Figure 3B, $\beta = 0.023$, 95% CI: 0.020, 0.026, $P = 9.22 \times 10^{-47}$) than low-SFA intake (Figure 3A, $\beta = 0.015$, 95% CI: 0.012, 0.018, $P = 5.15 \times 10^{-18}$). For cg24429974, the difference in methylation status between CC and TT genotypes was less apparent between high- (Figure 3D, $\beta = 0.014$, 95% CI: 0.011, 0.017, $P = 5.76 \times 10^{-17}$) and low-SFA intake (Figure 3C, $\beta = 0.013$, 95% CI: 0.010, 0.016, $P = 1.59 \times 10^{-15}$). Similarly, the difference in methylation site cg2487046 between CC and TT genotypes was less obvious between high- (Figure 3F, $\beta = -0.011$, 95% CI: -0.014 , -0.008 , $P = 1.29 \times 10^{-11}$) and low-SFA intake (Figure 3E, $\beta = -0.009$, 95% CI: -0.013 , -0.005 , $P = 1.87 \times 10^{-5}$).

To determine differential methylation associated with both SFA intake and $-265T > C$ genotype, we compared the difference in the allelic effect of the C allele on 3 methylation sites between low- and high-SFA intake. Using a *t* test, we found that participants with the CC genotype and high-SFA intake had 0.8% greater methylation at cg04436964, on average, when compared with participants with the TT genotype and low-SFA intake ($P = 0.041$). For cg24429974 and cg24847046, however, the difference between high- and low-SFA intake was not statistically significant (0.1% and -0.2% , $P = 0.565$ and 0.337 , respectively).

Annotation of CpG cg04436964

Data available for human genome build 37 at genome.ucsc.edu indicate that cg04436964 is a solitary CpG, with the nearest CpG island annotated approximately 4.1 kb distant. This information is corroborated by the absence of correlation in methylation status between this CpG and other regional CpGs (data not shown). The cg04436964 CpG appears to be just within or on the edge of an enhancer region, as determined by querying adipose, kidney, and liver cell data at the WashU Epigenome Browser (27). This enhancer is not observed in all assays or cell types, but data indicate overlap with binding sites for RXRA and ZBTB7A, both transcription factors, with roles in the epigenetics of obesity and the regulation of genes highly expressed in adipose, respectively (28–30). However, data are lacking as to whether this region functions as an enhancer under conditions driven by the diet, either habitual or acute (e.g., an intervention). The $-265T > C$ and cg04436964

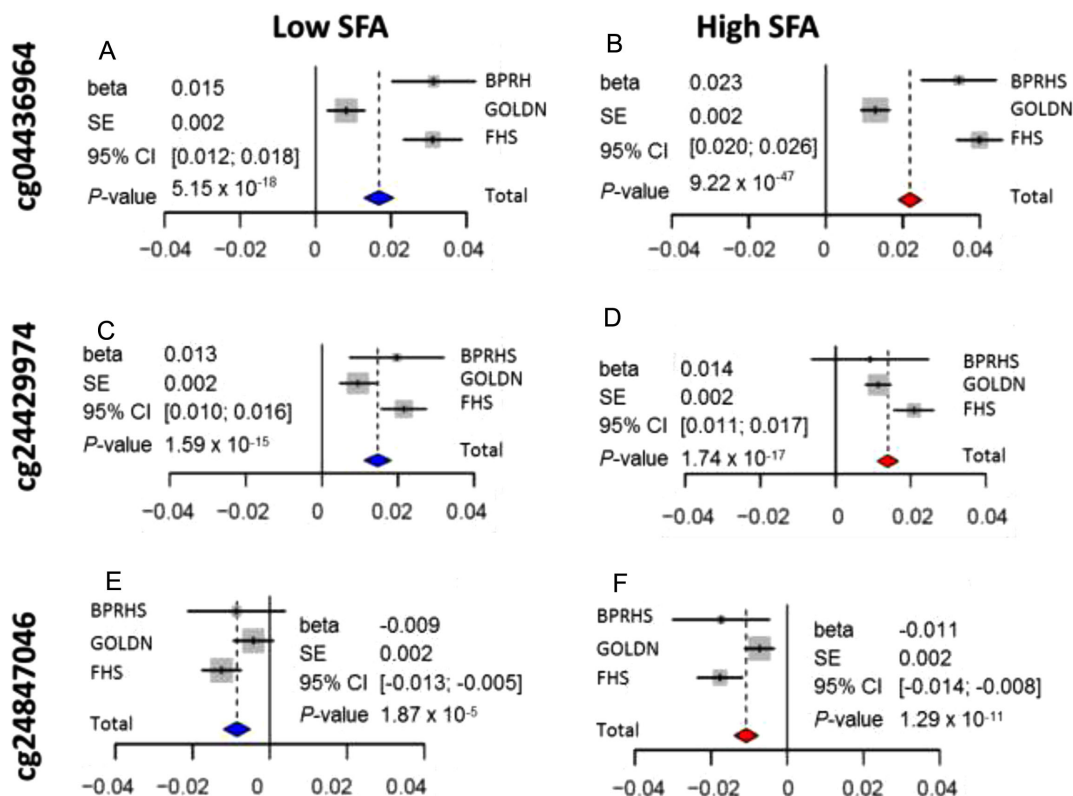


FIGURE 3 Forest plots of meta-analysis of methylation differences at 3 methylation sites over 3 populations (BPRHS, GOLDN, and FHS) between CC and TT genotypes according to low- and high-SFA intake. The summary of statistics (β , SE, 95% CI, P value) of the meta-analysis are listed on each panel: (A) cg04436964 at low-SFA intake; (B) cg04436964 at high-SFA intake; (C) cg24429974 at low-SFA intake; (D) cg24429974 at high-SFA intake; (E) cg24847046 at low-SFA intake; (F) cg24847046 at high-SFA intake. BPRHS, Boston Puerto Rican Health Study; FHS, Framingham Heart Study; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network.

site are ~26 kb apart. The SNP maps with the *APOA2* proximal promoter region, and there is some evidence to indicate that the CpG is within an enhancer (27). Thus, it is plausible to hypothesize that elevated intake of SFA serves to promote a looping of the DNA between these 2 regions, providing a means by which they can interact functionally. This interaction would have a stronger effect on *APOA2* expression only with the CC genotype at rs5082 and under conditions of high intake of SFA.

CpG methylation and *APOA2* genotype are associated with *APOA2* mRNA expression, depending on SFA intake

As epigenetic status can affect expression of targeted genes, we determined if the identified methylation site in the *APOA2* region was associated with *APOA2* mRNA expression. To do this, we examined the correlation between cg04436964 methylation and mRNA expression in whole blood for the 31 protein- and RNA-coding genes located within 50 kb of this CpG site by mining transcriptome data from FHS exam 8. Among the 31 genes, mRNA expression of 10 genes was detected and passed QC (see **Supplemental Table 1**). For participants with high-SFA intake, cg04436964 methylation exhibited a negative and significant association (**Figure 4B**, $\beta = -0.891$, $P = 0.028$) with *APOA2* mRNA expression. In contrast, those with low-SFA intake, *APOA2* mRNA expression was not associated with methylation at cg04436964 (**Figure 4A**, $\beta = 0.091$, $P = 0.857$). The

results remained the same even after adjusting for the additional potential confounder BMI.

Further, we observed an association between *APOA2* -265T >C genotype and *APOA2* mRNA expression that resembled the association which links methylation and expression, in that the 2 associations were limited to participants with high-SFA intake (**Figure 5**). Among participants with high-SFA intake, we saw that CC homozygotes had significantly lower *APOA2* expression than TT homozygotes [mean expression: 5.79 (CC) compared with 5.90 (TT), $P = 0.010$]. In contrast, among participants with low-SFA intake, there was no significant difference in *APOA2* expression between CC and TT genotypes [mean expression: 5.86 (CC) compared with 5.84 (TT), $P = 0.670$].

In participants with a high-SFA diet, there is no correlation between *APOA2* expression and expression of *NDUFS2* ($P = 0.775$) or *ADAMTS4* ($P = 0.699$). Hence, under a high-SFA diet, there is no influence of expression of the 2 genes on *APOA2* expression. It is possible that the CpG cg04436964 participates in regulating the expression of other genes in tissues other than peripheral blood cells. However, biologically relevant tissues, including liver, subcutaneous and visceral adipose, and kidney, were not readily available for such analysis, especially with regard to *APOA2* genotype and saturated fat intake. Lastly, mining Genotype-Tissue Expression Project data (version 7) shows a weak expression quantitative trait locus for -265T >C in liver and subcutaneous adipose for *TOMM40L*, whose TSS maps about 2.3 kb more distant to cg04436964

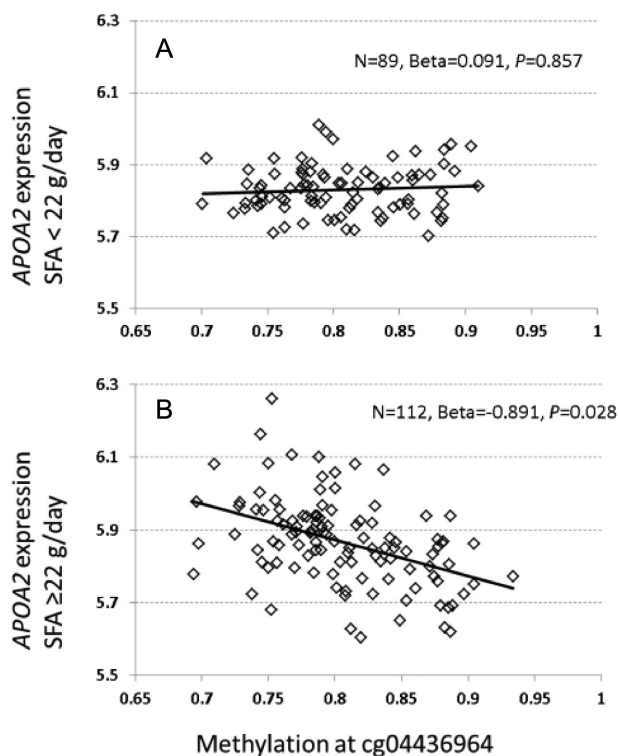


FIGURE 4 Correlation between *APOA2* mRNA expression in whole blood and methylation at cg04436964 in the FHS at exam 8. (A) Under low saturated fat intake or <22 g/d (low-SFA), (B) under high saturated fat intake or ≥ 22 g/d (high-SFA). The y-axis displays a normalized and log₂-transformed signal of *APOA2* mRNA expression adjusted for age, gender, and heterogeneity of cell type, whereas the x-axis displays the methylation level of cg04436964. *APOA2*, apolipoprotein A-II; FHS, Framingham Heart Study.

than the *APOA2* TSS. However, *TOMM40L* expression in blood did not correlate with cg04436964 methylation (Supplemental Table 1).

High-SFA intake highlights metabolic differences between *APOA2* genotypes

To examine the metabolic differences between CC and TT genotypes, metabolic pathway enrichment analysis was performed separately for low- and high-SFA intake datasets, with adjustment for BMI in the analysis. Among 808 metabolites detected in 80 plasma samples from the BPRHS, 635 compounds were grouped into 63 metabolic pathways, each of which contained ≥ 3 metabolites. Comparing CC to TT carriers on the low-SFA diet, 23 and 26 metabolites showed significant increases and decreases at $P \leq 0.05$ (Supplemental Table 2), respectively. Comparing CC to TT carriers under high-SFA conditions, 7 and 64 metabolites displayed significant increases and decreases at $P \leq 0.05$ (Supplemental Table 2), respectively. Metabolites that differed between CC and TT genotypes at $P \leq 0.05$ were counted within each pathway (Supplemental Table 3). For low-SFA intake, there were 4 pathways with a z score of ≥ 1.96 ($P \leq 0.05$), but none passed significance after correction for multiple testing ($P = 0.05/63 = 0.0008$ for 63 pathways). For high-SFA intake, 6 pathways had a z score of ≥ 1.96 ($P \leq 0.05$). After correction for multiple testing, 4 pathways remained significantly

overrepresented (Supplemental Table 3): leucine, isoleucine, and valine metabolism ($P = 2.00 \times 10^{-6}$), monoacylglycerol metabolism ($P = 7.94 \times 10^{-5}$), tryptophan metabolism ($P = 0.0003$), and phenylalanine metabolism ($P = 0.0005$). These 4 pathways highlight important metabolic differences between *APOA2* genotypes (CC vs. TT) with a high-SFA intake.

Linking the epigenomic signature of the *APOA2*-SFA interaction and the metabolic network

To characterize the connections between the epigenomic signature and the metabolic network, we conducted metabolic pathway enrichment analysis to investigate differential methylation between *APOA2* genotypes with high-SFA intake. We examined the correlation between methylation at cg04436964 and 635 metabolites in 63 pathways. The cg04436964-associated (at $P \leq 0.05$) metabolites were merged with those that differed (at $P \leq 0.05$) between CC and TT genotypes under a high-SFA intake. Eighteen metabolites met both criteria, as their levels in blood demonstrated association with cg04436964 methylation at $P \leq 0.05$ and differed between CC and TT genotypes under high-SFA intake (Supplemental Table 4). Enrichment analysis with correction for multiple testing identified 2 pathways as being significantly enriched: tryptophan metabolism (Supplemental Table 4, $Z = 5.029$, $P = 4.92 \times 10^{-7}$) and leucine, isoleucine, and valine metabolism, also called branched-chain amino acid (BCAA) metabolism ($z = 4.048$, $P = 5.16 \times 10^{-5}$). In the tryptophan metabolism pathway (Figure 6), 6 of 18 detected metabolites (33%) were correlated with cg04436964 methylation. In CC carriers, 4 of those metabolites, 3-indoxyl sulfate, indolelactate, indoleacetyl glutamine, and xanthurenate, were at lower concentrations than in TT carriers under high-SFA intake.

In the BCAA metabolism pathway, 10 of 25 metabolites (40%) displayed negative correlation with methylation of cg04436964, 4 of which differed between CC and TT genotypes with high-

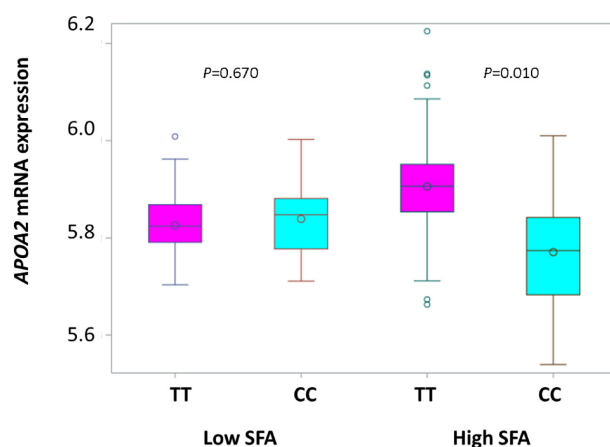


FIGURE 5 Box plot of *APOA2* mRNA expression according to *APOA2* -265T >C (rs5082) genotypes in the FHS at exam 8. The cyan box indicates participants with the CC genotype and the magenta box is for participants with the TT genotype, not taking medication for hypertension, dyslipidemia, or diabetes. Inside the box, the empty circle indicates means and the horizontal lines are medians. The bottom and top edges of the box indicate the range of values between the first and third quartiles (the 25th and 75th percentiles), whereas whiskers indicate the data range outside the box. Beyond the whiskers are the outliers. *APOA2*, apolipoprotein A-II; FHS, Framingham Heart Study.

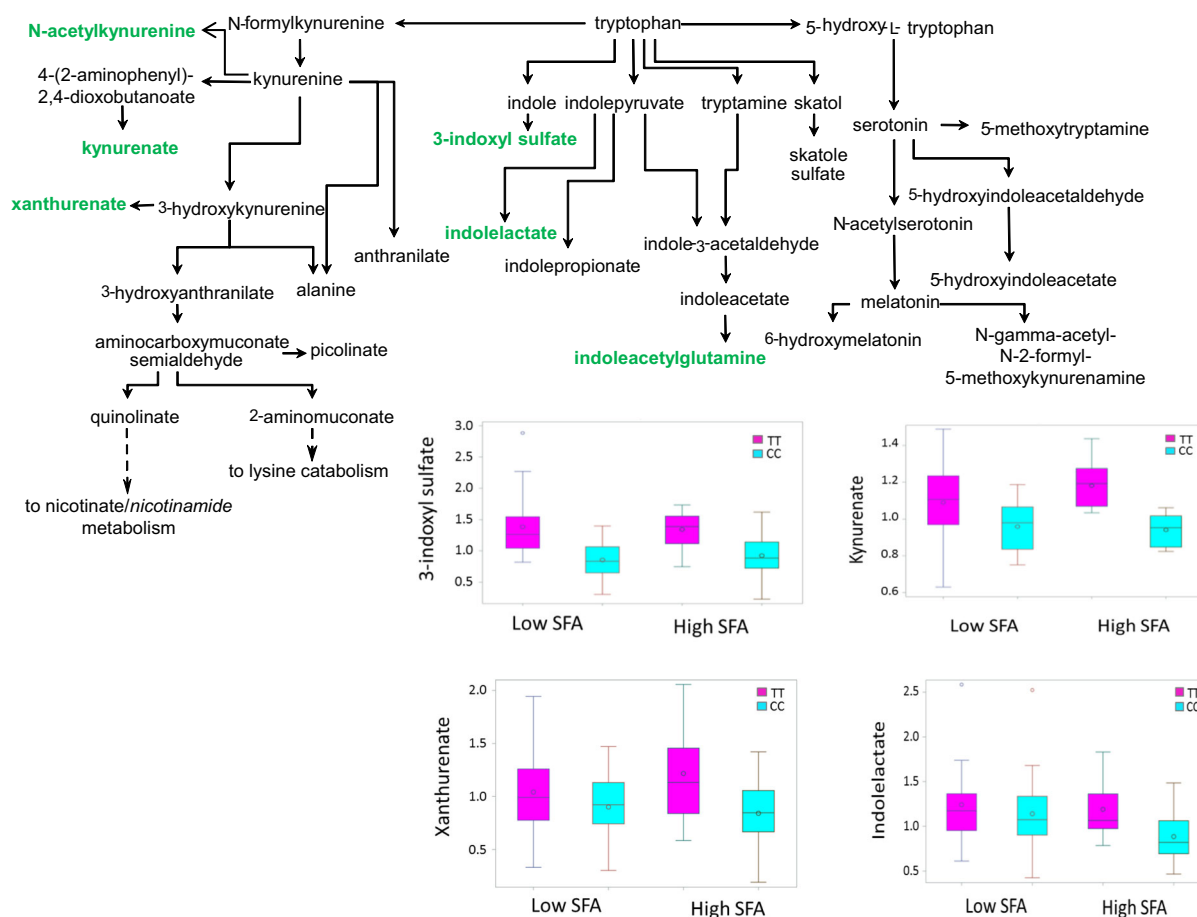


FIGURE 6 CC homozygotes display perturbations in the tryptophan metabolism pathway. The metabolites highlighted in green were significantly ($P < 0.05$) decreased in CC genotype (cyan box) when compared with TT genotype (magenta box) with high-SFA intake. Box plots of 4 metabolites that met 2 criteria—1) differed between CC and TT genotypes, and 2) was associated with cg04436964—were shown according to *APOA2* genotypes and SFA intakes. Inside the box, the empty circle indicates means and the horizontal lines are medians. The bottom and top edges of the box indicate the range of values between the first and third quartiles (the 25th and 75th percentiles), whereas whiskers indicate the data range outside the box. Beyond the whiskers are the outliers.

SFA intake: α -hydroxyisocaproate, β -hydroxyisovalerate, 2-methylbutyrylcarnitine, and 3-hydroxy-2-ethylpropionate. Taken together, these observations of altered metabolite levels imply that with high-SFA intake, tryptophan metabolism and BCAA metabolism are reduced to a greater extent in CC carriers than in TT carriers.

DISCUSSION

A high-SFA diet can synergistically augment the risk of obesity for individuals with a particular genotype or portfolio of risk-increasing alleles (31, 32). The mechanisms by which such gene-diet interactions manifest their effects on obesity are not well characterized. In several populations encompassing various ancestries, we have demonstrated a gene-diet interaction whereby *APOA2* CC homozygotes at $-265T>C$ exhibited an increased risk of obesity when consuming a diet high in SFA compared with TT carriers (3–7), but they (CC homozygotes) had no increased risk of obesity when consuming a low-SFA diet (4–8). Thus, this replicated interaction between *APOA2* genotype and SFA provides a useful platform for dissecting and

characterizing the mechanisms of a gene-by-diet interaction on obesity. To achieve this objective, we report methylome, transcription, and metabolomics analyses based on 3 populations. We showed that participants with the CC genotype and high-SFA intake had increased methylation at cg04436964, near the *APOA2* gene, compared with TT carriers, echoing the differences in BMI that have been replicated in 6 populations (3–7). Just as BMI did not differ by $-265T>C$ genotype with low-SFA intake, genotype-based differences in methylation were also less apparent when SFA intake was low. The current study broadens our understanding, in that increased methylation at cg04436964 is associated with decreased expression of *APOA2*, again under high-SFA intake conditions. Both the methylation differences and the altered gene expression reflect the signals of the gene-diet interaction between *APOA2* genotype and dietary SFA intake.

A comparison of the metabolomic profiles for CC and TT genotypes in the BPRHS for high-SFA intake, but not for low-SFA intake, identified 4 metabolic pathways that were significantly overrepresented: phenylalanine, monoacylglycerol, tryptophan, and BCAA. These results suggest that key differences between CC and TT carriers with high intake of SFA are

explained by the metabolism of lipids and amino acids. We further demonstrated that the correlation between cg04436964 methylation and genotype-specific metabolites was significantly enriched in 2 of these pathways, namely tryptophan metabolism and BCAA metabolism. This indicates that, under conditions of elevated intake of SFA, CC carriers have dysregulated tryptophan metabolism and BCAA pathways to a greater degree than do TT carriers.

In mammals, tryptophan metabolism is a powerful regulator of feeding and satiety (33, 34). Over 95% of tryptophan is metabolized through the kynurenine pathway (35, 36). While plasma serotonin cannot enter the brain, kynurenine easily passes the blood-brain barrier. Thus, plasma kynurenine concentration reliably reflects the status in brain of kynurenic acids or kynurenate (35). Recently, kynurenic acid has been characterized as regulating food-dependent behavioral plasticity in *Caenorhabditis elegans* via a system whose elements are conserved with mammals (37). Fasting depletes kynurenic acid, and low kynurenic acid activates NMDA-receptor-expressing interneurons to promote food cravings through a neuropeptide-Y-like signaling axis and serotonin release upon feeding (37). In our study, under a high-SFA diet, CC carriers exhibited reduced concentrations of tryptophan metabolites in the kynurenine pathway (Figure 6), including xanthurenate and kynurenic acid, relative to TT carriers. Appetite in CC carriers would then be expected to be greater than in TT carriers, hence CC individuals would consume more food. Indeed, CC carriers, in general, consume more kilocalories than TT carriers (4, 5), which elevates their obesity risk. Furthermore, CC individuals also showed low levels of 3-indoxyl sulfate, indolelactate, and indoleacetyl glutamine, perhaps indicating reduced doleamine-2,3-dioxygenase-independent tryptophan catabolism (35). Lastly, associations between eating behavior and variants in genes encoding monoamine oxidase A and serotonin receptor 2A underscore the role of the tryptophan-serotonin-kynurenine pathway in appetite (38). Thus, altering tryptophan catabolism can lead to an imbalance between energy intake and expenditure via impact on appetite, thereby augmenting the risk of obesity.

In the enriched BCAA metabolism pathway, where metabolites differed between CC and TT genotypes and were associated with cg04436964 methylation and *APOA2* expression, CC homozygotes with high-SFA intake uniquely showed a decrease of 4 BCAA catabolites: 3-hydroxy-2-ethylpropionate, α -hydroxyisocaproate, β -hydroxyisovalerate, and 2-methylbutyrylcarnitine. Impaired flux through the BCAA metabolic pathway is a sign of overall metabolic inefficiency. Specifically, with high-fat diets, reduced flux through the BCAA metabolic pathway was associated with obesity onset in mini-pigs (39), hindering BCAA-driven modulation of glucose metabolism in the liver, thereby elevating insulin resistance (40). Furthermore, when the metabolic capacity of the liver is compromised by high-fat feeding, BCAA homeostasis is less stably maintained, and this impairs the liver-skeletal muscle axis of cooperative BCAA catabolism (41). In parallel, defects in BCAA oxidation in muscle serve to disrupt normal lipid metabolism (42), possibly by curbing mTOR-mediated mitochondrial biogenesis (43), which could be of greater effect with high-SFA intakes. Together, the observed changes in metabolites within the Trp/KynA and BCAA pathways, examined in conjunction with reduced succinyl carnitine of the TCA cycle (Supplemental Table 2), serve

as dual indicators of altered signaling—appetite/feeding and energy balance—that promote the development of obesity.

Our application of omics in 3 populations is a major strength of this study. Beginning with the multiethnic BPRHS, we initially detected methylation differences between *APOA2* genotype in participants with high-SFA intake, examining 20 participants of CC genotype and 20 participants of TT genotype. One may argue that the small sample size of the BPRHS discovery cohort limits the power to identify other methylation sites that are associated with the *APOA2* genotype differentially under low- and high-SFA intake. However, we have validated our findings by conducting 2 additional epigenome-wide associations in the FHS and GOLDN populations. Three methylation sites—cg04436964, cg24429974, and cg24847046—were identified at the threshold of epigenome-wide significance after correction for multiple testing. Meta-analysis of these results confirmed that only the methylation at cg04436964 differed between *APOA2* genotypes and correlated with *APOA2* mRNA under high-SFA intake. CC carriers with high-SFA intake tend to have high BMI, so obesity may be contributing to the differences in epigenetic status. Throughout our analyses, we have controlled for BMI in all 3 populations and the results remain consistent. In the FHS, we observed a significant difference in cg04436964 methylation between CC and TT carriers with low-SFA intake, which was not observed in the BPRHS or the GOLDN Study. Detection of the difference in the FHS alone cannot be attributed to greater power stemming from greater sample size because the sample size was greatest in the GOLDN Study. Instead, our ability to detect the difference in the FHS could be explained by the possibility that CC carriers modified SFA intake over time, i.e., some participants might have consumed higher SFA intake early and then changed to a low-SFA diet at a later time point. Indeed, when we examined the SFA intake across 4 examinations—exams 5–8 of the FHS—we found that only 15 CC (6.2%) and 24 TT (9.9%) carriers of 243 participants (without taking medications for lowering lipids, diabetes and hypertension) maintained a high-SFA diet (≥ 22 g/d) (3, 6), whereas 23 CC (9.5%) and 38 TT (15.6%) carriers maintained a low-SFA diet (< 22 g/d) across all 4 exams. An epigenome-wide scan with 23 CC and 38 TT participants with consistent low-SFA intake across all 4 exams indicated that the difference in cg04436964 methylation between CC and TT genotypes did not reach epigenome-wide significance ($P = 1.7 \times 10^{-6} > 1.1 \times 10^{-7}$). On the other hand, for 15 CC and 24 TT participants with consistent high-SFA intake across all 4 exams, the difference in cg04436964 methylation between CC and TT genotypes did reach epigenome-wide significance ($P = 4.11 \times 10^{-11}$). This observation further confirms that the SFA-dependent cg04436964 methylation differs between CC and TT genotypes consistently in all 3 distinct populations.

Some limitations of the current study must be noted. First, the epigenome analysis of the GOLDN Study used CD4 + T cells from a frozen buffy coat, whereas the BPRHS and FHS analyses were conducted using whole-blood buffy coats. This difference could introduce inconsistencies in estimating the population-specific effect sizes associated with methylation. Indeed, the β value for methylation associated with the C-allele was smaller in the GOLDN Study than in the BPRHS and FHS, but the directions were consistent in all cohorts. Second, the dietary patterns and food sources of SFA and their corresponding impacts on health could vary across groups because of regional

and cultural differences. In this regard, the BPRHS, which studied a population of Caribbean Hispanic origin, is quite different from the FHS and the GOLDN Study. Population level differences in lifestyle, including diet, could also contribute to epigenetic differences between populations. Lastly, although we employed a targeted metabolomics approach, this is inherently limited to those metabolites that have been identified.

In summary, we applied multiomics approaches to investigate the mechanistic foundations of one of the most consistently replicated gene-diet interactions, that of SFA and the *APOA2* genotype. Our findings from 3 populations of diverse ancestries strongly support that the epigenetic status of the *APOA2* regulatory region is associated with SFA intake and the *APOA2* -265T>C genotype, leading to differential diet-dependent and genotype-dependent *APOA2* expression. As appropriate for a gene-diet interaction linked to the disruptive condition of obesity, we also uncovered plausible dysregulation of select metabolic pathways. The epigenetic signature of the gene-diet interaction is correlated with the down-regulation of the kynurenine pathway of tryptophan metabolism and BCAA metabolism, with possible implications for food intake. Collectively, these findings illustrate the effectiveness of multiomic approaches to well-established gene-diet interactions, and contribute novel evidence to ongoing explorations of the impact of saturated fat on human health.

The authors' responsibilities were as follows—C-QL and JMO: conceived and designed the study; C-QL, CES, LDP, Y-CL, DA, and DC: acquired the data; C-QL, CES, LDP, MAP, and KLT: analyzed and interpreted the data; JMO and C-QL: performed statistical analysis and drafted the manuscript; C-QL, CES, LDP, JMO, and KLT: critically revised the manuscript for intellectual content; JMO, KLT, and DKA: provided funding and supervision; and all authors: read and approved the final manuscript. The Genotype-Tissue Expression Project was supported by the Common Fund of the Office of the Director of the National Institutes of Health, and by the NCI, NHGRI, NHLBI, NIDA, NIMH, and NINDS. The data used for the analyses described here were obtained from the Genotype-Tissue Expression Portal on 10 January 2018. None of the authors had a conflict of interest. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the US Department of Agriculture. The USDA is an equal opportunity provider and employer.

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