

# Whole Blood DNA Methylation Signatures of Diet Are Associated with Cardiovascular Disease Risk Factors and All-cause Mortality

Running title: *Ma et al.; DNA Methylation Signatures of Diet Quality*

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## Abstract:

**Background** - DNA methylation patterns associated with habitual diet have not been well studied.

**Methods** - Diet quality was characterized using a Mediterranean-style diet score (MDS) and the Alternative Healthy Eating Index score (AHEI). We conducted ethnicity-specific and trans-ethnic epigenome-wide association analyses for diet quality and leukocyte-derived DNA methylation at over 400,000 cytosine-guanine dinucleotides (CpGs) in five population-based cohorts including 6,662 European ancestry (EA), 2,702 African ancestry (AA), and 360 Hispanic ancestry (HA) participants. For diet-associated CpGs identified in epigenome-wide analyses, we conducted Mendelian randomization (MR) analysis to examine their relations to cardiovascular disease (CVD) risk factors and examined their longitudinal associations with all-cause mortality.

**Results** - We identified 30 CpGs associated with either MDS or AHEI, or both, in EA participants. Among these CpGs, 12 CpGs were significantly associated with all-cause mortality (Bonferroni corrected p-value  $< 1.6 \times 10^{-3}$ ). Hypermethylation of cg18181703 (*SOCS3*) was associated with higher scores of both MDS and AHEI and lower risk for all-cause mortality (p-value =  $5.7 \times 10^{-15}$ ). Ten additional diet-associated CpGs were nominally associated with all-cause mortality (p-value  $< 0.05$ ). MR analysis revealed eight putatively causal associations for six CpGs with four CVD risk factors (BMI, triglycerides, high-density lipoprotein cholesterol concentrations, and type 2 diabetes; Bonferroni corrected MR p-value  $< 4.5 \times 10^{-4}$ ). For example, hypermethylation of cg11250194 (*FADS2*) was associated with lower triglyceride concentrations (MR p-value =  $1.5 \times 10^{-14}$ ).and hypermethylation of cg02079413 (*SNORA54*; *NAP1LA*) was associated with BMI (corrected MR p-value =  $1 \times 10^{-6}$ ).

**Conclusions** - Habitual diet quality was associated with differential peripheral leukocyte DNA methylation levels of 30 CpGs, most of which were also associated with multiple health outcomes, in EA individuals. These findings demonstrate that integrative genomic analysis of dietary information may reveal molecular targets for disease prevention and treatment.

**Key words:** cardiovascular disease risk factors; all-cause death; Mendelian randomization; diet; DNA methylation; diet quality score; alternative healthy eating index

## Nonstandard Abbreviations and Acronyms

CpG: cytosine–guanine dinucleotide

CVD: cardiovascular disease

n-3 FAs: omega-3 polyunsaturated fatty acids

MDS: Mediterranean-style diet score

AHEI: Alternative Healthy Eating Index

EA: European Ancestry

AA: African ancestry

HA: Hispanic ancestry

FDR: false discovery rate

MR: Mendelian Randomization



# Circulation: Genomic and Precision Medicine

## Introduction

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Epigenetic alterations are involved in the pathogenesis of many human diseases.<sup>1</sup> DNA methylation, which commonly occurs at cytosine–guanine dinucleotide (CpG) sites, is a well-studied epigenetic modification that may affect gene expression and contribute to the development of chronic diseases, including cardiovascular disease.<sup>2-4</sup>

Several lines of evidence suggest that diet may be actively involved in epigenetic regulation, which impacts diet-related disease risk.<sup>5-8</sup> Tremblay et al. measured genome-wide DNA methylation profiles before and after a six-week supplementation of daily dose of 3 grams of omega-3 polyunsaturated fatty acids (n-3 FAs) in 36 participants with BMI between 25 to 40

kg/m<sup>2</sup>.<sup>9</sup> They found that n-3 FAs supplementation caused differential DNA methylation of 308 CpGs, which could be linked to 16 pathways related to cardiovascular disease (CVD) including inflammatory response and lipid metabolism.

While previous studies provide useful evidence that diet plays an important role in regulating the human epigenome, studies of DNA methylation signatures for overall diet quality, however, are few in number and limited by small sample sizes. Diet quality is crucial for chronic diseases prevention.<sup>10-12</sup> In cohort studies, diet quality is often assessed using a variety of diet scores, including the Mediterranean-style diet score (MDS) and the Alternative Healthy Eating Index (AHEI) score.<sup>13-18</sup> These studies showed that a higher diet score was associated with lower disease burden. A thorough insight into the biological mechanisms underlying diet-disease associations is important for disease prevention and treatment. To fill this knowledge gap, we conducted an epigenome-wide association study of diet quality, assessed by MDS and AHEI, with peripheral blood-derived DNA methylation in cohorts with representation of individuals of European as well as non-European ancestries.

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## Methods

The study design is presented in Figure 1. The datasets analyzed in the present study are available at the dbGAP repository phs000280.v5.p1 (ARIC), phs000007.v29.p10 (FHS), phs000741.v2.p1 (GOLDN), phs000209.v13.p3 (MESA), phs000853.v1.p1 (NAS), and phs000821.v1.p1 (LBC; phenotypic data). RS has a protocol for approving data requests (secretariat.epi@erasmusmc.nl). The informed consents given by KORA study participants do not cover data posting in public databases. However, data are available upon request from KORA Project Application Self-Service Tool (<https://epi.helmholtz-muenchen.de/>) Data requests

can be submitted online and are subject to approval by the KORA Board. Methylation data of LBC have been submitted to the European Genome-phenome Archive under accession number EGAS00001000910. For ESTHER and InCHIANTI, the datasets used and/or analyzed during the current study are available from the corresponding author upon request. Data for WHI and CHS can be requested at <https://www.whi.org/researchers/SitePages/Write%20a%20Paper.aspx> and <https://chs-nhlbi.org/node/6222>, respectively. The study protocol was approved by each participating institutions' Institutional Review Board. All participants provided written informed consent. Full descriptions of study populations, phenotypic definitions, DNA methylation profiling, and statistical analyses are available in the Supplemental Material.



## Results

*Epigenome-wide association analysis in European Ancestry (EA) participants.* We analyzed 403,087 autosomal CpGs. For each diet quality score, either MDS or AHEI, we conduct two analyses, a two-step analysis (i.e., discovery and replication) and an one-step analysis (i.e., meta-analysis of all cohorts with internal validation). For MDS, the discovery analysis identified 13 CpGs at false discovery rate (FDR)  $< 0.05$  (corresponding p-value =  $1.5 \times 10^{-6}$ ; Supplemental Table 1; Supplemental Figure 1 [Manhattan plot] and Supplemental Figure 2 [Quantile-Quantile plot]). Of these CpGs, three replicated in the replication samples after Bonferroni correction (corresponding p-value  $< 0.004$ ; Supplemental Table 1). The one-step analysis identified 12 CpGs associated with MDS at FDR  $< 0.05$  (corresponding p-value =  $1.2 \times 10^{-6}$ ; Supplemental Table 2; Supplemental Figure 3 [Manhattan plot] and Supplemental Figure 2 [Quantile-Quantile plot]). Using models with adjustment for sex, age, and energy intake, the two analyses (two-step analysis and one-step analysis) identified 14 CpGs associated with MDS.

For AHEI, in the two-step analysis, the discovery step identified 41 CpGs at  $FDR < 0.05$  (corresponding  $p$ -value =  $6 \times 10^{-6}$ ; Supplemental Table 3; Supplemental Figure 1 [Manhattan plot] and Supplemental Figure 2 [Quantile-Quantile plot]). Two CpGs replicated after Bonferroni correction (corresponding  $p$ -value  $< 0.001$ ; Supplemental Table 3). The one-step analysis identified 24 CpGs at  $FDR < 0.05$  (corresponding  $p$ -value =  $3.1 \times 10^{-6}$ ; Supplemental Table 4; Supplemental Figure 3 [Manhattan plot] and Supplemental Figure 2 [Quantile-Quantile plot]). The combination of the two-step analysis and the one-step identified 24 CpGs associated with AHEI using models adjusted for sex, age, and energy intake.

To reduce potential confounding effects by other lifestyle factors, we additionally adjusted for smoking status, physical activity, and BMI. Among the 14 CpGs associated with MDS, ten CpGs remained significant ( $p$ -value  $< 0.05/14$ ; Figure 2), while all 24 CpGs associated with AHEI remained significant ( $p$ -value  $< 0.05/24$ ; Figure 2). Overall, after adjustment for multiple confounders, we identified 30 CpGs associated with either MDS or AHEI, or both (Table 1). Pairwise correlations of the 30 CpGs were low to moderate, absolute Pearson  $r$  ranging from 0 to 0.66 (Supplemental Table 5). As shown in Supplemental Figure 4, regression coefficients in meta-analyses of all EA participants using MDS and AHEI were highly correlated, e.g., Pearson  $r$  was 0.97 for the regression coefficients of the top 500 CpGs in MDS versus AHEI. We therefore combined the CpGs identified using the two diet scores in the subsequent analyses.

### **Functional and regulatory annotation of diet-associated CpGs.**

Relative to the whole set of CpGs analyzed, the 30 diet-associated CpGs were enriched in gene body regions ( $p$ -value =  $9.3 \times 10^{-4}$ ). The mean whole blood-derived DNA methylation levels of the 30 CpGs were moderately associated with those measured in muscle, omentum, and spleen

(Supplemental Figure 5), with Spearman ranked  $r = 0.56$  ( $n=6$ ),  $0.60$  ( $n=6$ ), and  $0.62$  ( $n=3$ );  $p$ -value =  $1.5 \times 10^{-3}$ ,  $6.1 \times 10^{-4}$ , and  $3.5 \times 10^{-4}$ , respectively.<sup>19</sup>

Among the 30 CpGs, 26 CpGs were annotated to 27 protein-coding genes (Supplemental Table 6). Based on the GTEx expression dataset,<sup>20</sup> the annotated genes were differentially expressed in several tissues (Supplemental Figure 6 and Supplemental Table 7), e.g., differential expression was reported for 17 genes in muscle and 12 genes in small intestine (Bonferroni corrected  $p$ -value = 0.03 and 0.04, respectively). Gene set analyses did not reveal significant enrichment of pathways. Several genes, however, have important biological functions relevant to diet-associated diseases, e.g., *SORBS1* (annotated to cg03190891) and *FADS2* (annotated to cg11250194) play crucial roles in insulin signaling and fatty acids metabolism, respectively.

### **GWAS analysis**

We identified 4,925 *cis*-meQTL variants for 23 of the 30 CpGs in the FHS (Supplemental Material). We found that 68 *cis*-meQTL variants for ten CpGs exactly matched a GWAS reported single nucleotide polymorphism (SNP) in the NHGRI-EBI GWAS Catalog<sup>21</sup> ( $p$ -value <  $5 \times 10^{-8}$ ; Supplemental Table 8). For example, rs174550 for cg11250194 (*FADS2*) was associated with plasma omega-6 polyunsaturated fatty acid concentrations.<sup>22</sup> Overall, these ten CpGs were linked to 35 unique traits, of which many are also diet-associated, such as lipid levels and chronic kidney disease.<sup>23,24</sup>

### **Associations of diet-associated CpGs with CVD risk factors.**

In the EWAS catalog (Supplemental Table 9), we found that 26 (of 30) CpGs have been reported to be associated with one or more CVD risk factors, e.g., hypermethylation of cg18181703 (*SOCS3*) was associated with lower BMI and lower risk of type 2 diabetes.<sup>25-27</sup> We conducted bidirectional Mendelian Randomization (MR) analysis to examine the potential causal relations

between diet-associated CpGs and CVD risk factors, i.e., CpG → CVD trait and CVD trait → CpG. The MR analysis in direction of CpG to CVD trait was performed for 22 (of 30) CpGs that had *cis*-meQTL variants and summary results from the selected GWAS. We found significant putatively causal association for eight CpG-trait pairs after Bonferroni correction for 22 CpGs and five traits (corresponding MR p-value <  $4.5 \times 10^{-4}$ ) and nominally significant putatively causal association for 14 CpG-trait pairs (MR p-value < 0.05; Supplemental Table 10). For example, as shown in Figure 3, hypermethylation of cg11250194 (*FADS2*) was associated with lower triglyceride concentrations (MR p-value =  $1.5 \times 10^{-14}$ ) and hypermethylation of cg02079413 (*SNORA54*; *NAP1L4*) was associated with higher BMI (MR p-value =  $1 \times 10^{-6}$ ). We also observed unexpected associations in the MR analysis. For example, hypermethylation of cg26470501 (*BCL3*) was positively associated with BMI (MR p-value =  $6.5 \times 10^{-5}$ ; Supplemental Table 10; Figure 3), which was not consistent with the positive association that we observed between diet and this CpG and the inverse association between this CpG and BMI.<sup>25,28</sup> In the opposite direction, MR analyses linking CVD traits to CpG, revealed no significant putative causal association after correction for multiple testing (p-value < 0.002; 0.05/30 diet-associated CpGs; Supplemental Table 11). Nevertheless, we observed two nominally significant associations: higher BMI was associated with hypomethylation of cg18181703 (p-value = 0.04) and higher waist-to-hip ratio adjusted for BMI (WHRadjBMI) was associated with hypomethylation of cg25953130 (p-value = 0.02).

### **Relations of diet-associated CpGs with mortality.**

Of the 30 diet score-associated CpGs, the relations of 27 CpGs with all-cause mortality were examined in ten EA cohorts (N up to 10,083). Three CpGs were excluded because of missing data. After adjusting for multiple covariates (Figure 4), we found that 12 CpGs were significantly



associated with all-cause mortality following Bonferroni correction (corresponding p-value <  $1.6 \times 10^{-3}$ ); ten additional CpGs were nominally associated with all-cause mortality (p-value < 0.05). The direction of the associations between CpGs and mortality was concordant with that for the diet-CpG associations, e.g., hypermethylation of cg18181703 (*SOCS3*), which was associated with higher scores of both AHEI and MDS, was associated with lower all-cause mortality (p-value =  $5.7 \times 10^{-15}$ ).

### **Multiethnic analysis**

Although we observed largely consistent directions of effect in AA and HA participants for the 30 CpGs identified in EA participants, none of these CpGs was significant after Bonferroni correction (Supplemental Table 12). The transethnic meta-analysis identified 21 CpGs at FDR < 0.05 including 13 CpGs for AHEI with a corresponding p-value of  $1.1 \times 10^{-6}$  and 10 CpGs for MDS with a corresponding p-value of  $7 \times 10^{-7}$  (Supplemental Table 13). Of the 21 CpGs, ten CpGs were not among the 30 CpGs identified in EA participants and the correlations of the ten CpGs with the 30 CpGs were low to moderate,  $|r|$  ranging from 0 to 0.49 (Supplemental Table 14). The annotated genes for these ten CpGs (Supplemental Table 15) showed enrichment of lipid metabolism-related pathways (Supplemental Table 16). Nine of the ten CpGs were associated with nine unique traits in the EWAS catalog including serum triglyceride and HDL concentrations<sup>29</sup> (Supplemental Table 17).

### **Discussion**

In participants of EA ancestry, we identified 30 CpGs whose methylation in whole blood was associated with diet scores assessed, either MDS or AHEI, or both. Aligning *cis*-meQTL variants for these CpGs with GWAS catalog reported variants revealed that diet-associated differential

DNA methylation can be linked to a series of metabolic and inflammatory disorders. Importantly, we also observed associations between these CpGs and all-cause mortality, which may reflect the importance of diet-induced epigenetic changes on health outcomes. Our study provides novel evidence that integrative genomic analysis of dietary information may be useful to highlight molecular targets for disease prevention and treatment.

Accumulating evidence has shown that epigenetic profiles may be regulated by dietary factors.<sup>6</sup> A recent study found that women who had better adherence to the Mediterranean diet had greater DNA methylation levels at long interspersed nucleotide elements 1 (LINE-1), a surrogate marker of global genomic DNA methylation.<sup>8</sup> In a small subgroup (n=36) of the Prevención con Dieta Mediterránea (PREDIMED) study, genome-wide methylation levels in peripheral blood derived DNA were assessed at baseline and again five years later.<sup>7</sup> This study revealed that adherence to the Mediterranean diet may impact DNA methylation levels of several inflammation-related genes. None of the CpGs identified in this PREDIMED report, however, showed statistically significant differential DNA methylation in the meta-analysis in the present study.

Higher MDS and AHEI scores have been reported to be associated with lower body weight.<sup>17,18</sup> Our observation that diet scores were positively associated with DNA methylation levels of cg18181703 (*SOCS3*) is therefore consistent with the inverse association of cg18181703 and BMI identified in multiple studies.<sup>25,28,30</sup> Overall, by integrating association analysis and MR analysis, our data indicate that diet quality may affect BMI, alter DNA methylation of cg18181703, and impact long-term health. The association between cg18181703 and all-cause mortality also was consistent with observations in a small-scale epigenome-wide study.<sup>31</sup> *SOCS3* is a well characterized gene involved in immune system regulation, which

suggests that the association of diet scores and cg18181703 may be relevant to inflammation and may partly explain the association of cg18181703 with all-cause mortality.

Several diet score-associated CpGs, such as cg19693031 (*TXNIP*) and cg02716826 (*SUGTIP1*; *AQP3*), have been reported to be associated with CVD risk factors.<sup>26,27</sup> *TXNIP*, thioredoxin-interacting protein, is a key regulator of energy metabolism and a therapeutic candidate for type 2 diabetes.<sup>32</sup> *AQP3*, aquaporin 3, is a member of water channel proteins that are associated with a number of diseases such as hypertension and congestive heart failure.<sup>33</sup> Our MR analyses also support a causal link between methylation levels of diet-associated CpGs and CVD risk factors, e.g., hypermethylation of cg11250194 (*FADS2*) was associated with lower triglyceride concentrations. *FADS2* is a key member of the fatty acid desaturase (*FADS*) family.<sup>34</sup> This observation is consistent with the role of diet in the regulation of enzyme activity relevant to fatty acid desaturation.<sup>35</sup> Therefore, the present study provides key evidence that diet may interact with the human genome via epigenetic mechanisms to impact health outcomes.

In a post-hoc analysis, we conducted lookup analysis for diet score-associated CpGs using data reported in a prior epigenome-wide association study of acute coronary syndrome.<sup>36</sup> Although none of the 30 CpGs we report here was significant or correlated with the 47 CpGs identified in this prior study, we found that one of the 47 CpGs, cg14066471 was significantly associated with AHEI ( $p=0.0004$ ) and nominally associated with MDS ( $p=0.004$ ) after Bonferroni correction for 47 CpGs in our trans-ethnic analysis. This observation suggests that our data represent a good resource for other investigators to conduct lookup analyses. It also suggests that a candidate approach may identify additional CpGs associated with diet and diseases.

A major strength of the present study is its large sample size, which includes data from five US and European population-based cohorts, and the use of two common and well-studied diet scores. Several limitations warrant discussion. The diet scores were based on different versions of FFQs, which are prone to measurement errors due to self-reported diet data. In addition, although the associations remained significant for the majority of CpGs after adjustment for lifestyle factors, we cannot rule out the possibility of residual confounding. Although we showed a moderate correlation between peripheral blood-derived DNA methylation profiles and those from other tissues, we lacked data to analyze tissue-specific diet-associated DNA methylation changes which may be more directly related to the development of chronic diseases. Our study may lack power to detect diet-associated DNA methylation markers in AA and HA participants due to the smaller sample sizes (n=2,702 for AA and n=360 for HA) relative to EA participants (n=6,662).

In conclusion, the present study demonstrates that diet quality is associated with differential DNA methylation levels of 30 CpGs in leukocyte-derived DNA among EA participants. Our findings demonstrate that integration of dietary information and genomic data may reveal useful insights into the molecular effects at the intersection of diet, risk factors, and chronic diseases. Future studies with larger sample sizes, deeper coverage of DNA methylation, and more precise dietary measurement are needed to validate our findings and to investigate diet-associated DNA methylation patterns in larger ethnically diverse samples.

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**Table 1.** Diet scores-associated CpGs in European Ancestry (EA) meta-analysis

CpG	CHR	Position	Gene	Diet	Meta-analysis in all EA participants				
					Beta	SE	P	Direction	I-squared
cg04885881	1	11123118		MDS	0.004	0.001	3.2E-07	+, +, +, +, +	0.12
cg24735226	1	65096537	<i>CACHD1</i>	AHEI	-0.004	0.001	1.6E-06	-, -, -, -, -	0
cg07805029	1	92953256	<i>GFII</i>	AHEI	0.003	0.001	3.1E-06	+, +, +, +, +	0
cg19693031	1	145441552	<i>TXNIP</i>	MDS	0.003	0.001	3.1E-07	+, +, +, +, +	0.14
cg24694018	1	145457621	<i>POLR3GL</i>	AHEI	0.002	0.0003	8.3E-07	+, +, +, +, +	0
cg01940273	2	233284934		MDS	0.005	0.001	1.6E-12	+, +, +, +, +	0
cg20842915	7	39665132	<i>RALA</i>	AHEI	0.003	0.001	8.1E-08	+, +, +, +, +	0
cg02508743	8	56903623	<i>LYN</i>	AHEI	-0.002	0.001	2.5E-06	-, -, -, -, -	0
cg27039118	8	116575902	<i>TRPS1</i>	AHEI	0.004	0.001	1.2E-06	+, +, +, +, +	0
cg02716826	9	33447032	<i>SUGT1P1;AQP3</i>	MDS	0.002	0.0005	5.6E-07	+, +, +, +, +	0
cg25953130	10	63753550	<i>ARID5B</i>	AHEI	0.004	0.001	1.2E-08	+, +, +, +, +	0
cg03190891	10	97201172	<i>SORBS1</i>	AHEI	-0.003	0.0005	9.0E-08	-, -, -, -, -	0
cg02079413	11	2986505	<i>SNORA54;NAP1L4</i>	MDS	-0.002	0.0004	3.1E-07	-, -, -, -, -	0.14
cg11250194	11	61601937	<i>FADS2</i>	AHEI	0.003	0.001	1.5E-06	+, +, +, +, +	0
cg11468085	11	67435577	<i>ALDH3B2</i>	AHEI	-0.002	0.0005	1.4E-06	-, -, -, -, -	0.06
cg25909064	11	120082805	<i>OAF</i>	AHEI	0.002	0.0004	8.0E-07	+, +, +, +, +	0
cg03646329	13	48987165	<i>LPAR6;RBI</i>	AHEI	0.003	0.001	1.5E-06	+, +, +, +, +	0
cg16969872	13	79968324	<i>RBM26</i>	MDS	0.004	0.001	1.1E-06	+, +, +, +, +	0
				AHEI	0.003	0.001	3.0E-09	+, +, +, +, +	0
cg09940677	14	103415458	<i>CDC42BPB</i>	MDS	0.003	0.001	1.2E-06	+, +, +, +, +	0.25
				AHEI	-0.001	0.0003	2.9E-06	-, -, -, -, -	0
cg13074055	14	106329206		AHEI	0.005	0.001	1.3E-06	+, +, +, +, +	0
cg27118035	16	31891978	<i>ZNF267</i>	AHEI	-0.003	0.0005	4.9E-09	-, -, -, -, -	0
cg08732950	16	89023389	<i>CBFA2T3</i>	MDS	-0.003	0.0005	2.8E-08	-, -, -, -, -	0
cg02097604	17	17750910	<i>TOM1L2</i>	AHEI	0.002	0.0003	6.6E-09	+, +, +, +, +	0
				MDS	0.002	0.0003	3.6E-08	+, +, +, +, +	0
cg16936953	17	57915665	<i>VMP1</i>	AHEI	0.004	0.001	1.5E-08	+, +, +, +, +	0
cg18181703	17	76354621	<i>SOCS3</i>	AHEI	0.004	0.001	2.0E-12	+, +, +, +, +	0
				MDS	0.004	0.001	3.5E-10	+, +, +, +, +	0
cg19202384	17	79894511	<i>PYCR1</i>	AHEI	0.002	0.0004	9.9E-07	+, +, +, +, +	0.04
cg01294327	19	2291373	<i>LINGO3</i>	AHEI	0.005	0.001	1.4E-06	+, +, +, +, +	0
cg26470501	19	45252955	<i>BCL3</i>	AHEI	0.002	0.0004	2.4E-06	+, +, +, +, +	0.03
cg08884571	19	45901453	<i>PPP1R13L</i>	AHEI	-0.004	0.001	4.6E-07	-, -, -, -, -	0
cg05232694	20	48809539		AHEI	0.004	0.001	3.1E-08	+, +, +, +, +	0

Genome build 37. Regression coefficients are DNA methylation change for per standard deviation change in diet scores from analyses using sex, age, and energy intake adjusted models. Direction order (from left to right): FHS, ARIC, GOLDN, MESA, and RS. AHEI: Alternative Healthy Eating Index. MDS: Mediterranean-style diet score

## Figure Legends:

**Figure 1.** Study design flow chart. AA: African ancestry. EA: European ancestry. HA: Hispanic ancestry. CpGs: cytosine-guanine dinucleotides (DNA methylation sites). AHEI: Alternative Healthy Eating Index. MDS: Mediterranean-style diet score. FDR: false discovery rate. GWAS: genome-wide association study. cis-meQTLs: cis methylation quantitative loci. EWAS: epigenome-wide association study. GEO: Gene Expression Omnibus. GTEx: Genotype-Tissue Expression database. Discovery cohort: Framingham Heart Study (FHS). Replication cohorts: Atherosclerosis Risk in Communities (ARIC) Study, Genetics of Lipid Lowering Drugs and Diet Network (GOLDN), Multi-Ethnic Study of Atherosclerosis (MESA), and Rotterdam Study (RS). Cohorts for all-cause mortality includes: ARIC, FHS, ESTHER study, InChianti Study, Lothian Birth Cohort (LBC) Study 1921 and 1936, Cardiovascular Health Study (CHS), KORA F4 Study, Normative Aging Study (NAS), and Women's Health Initiative (WHI).

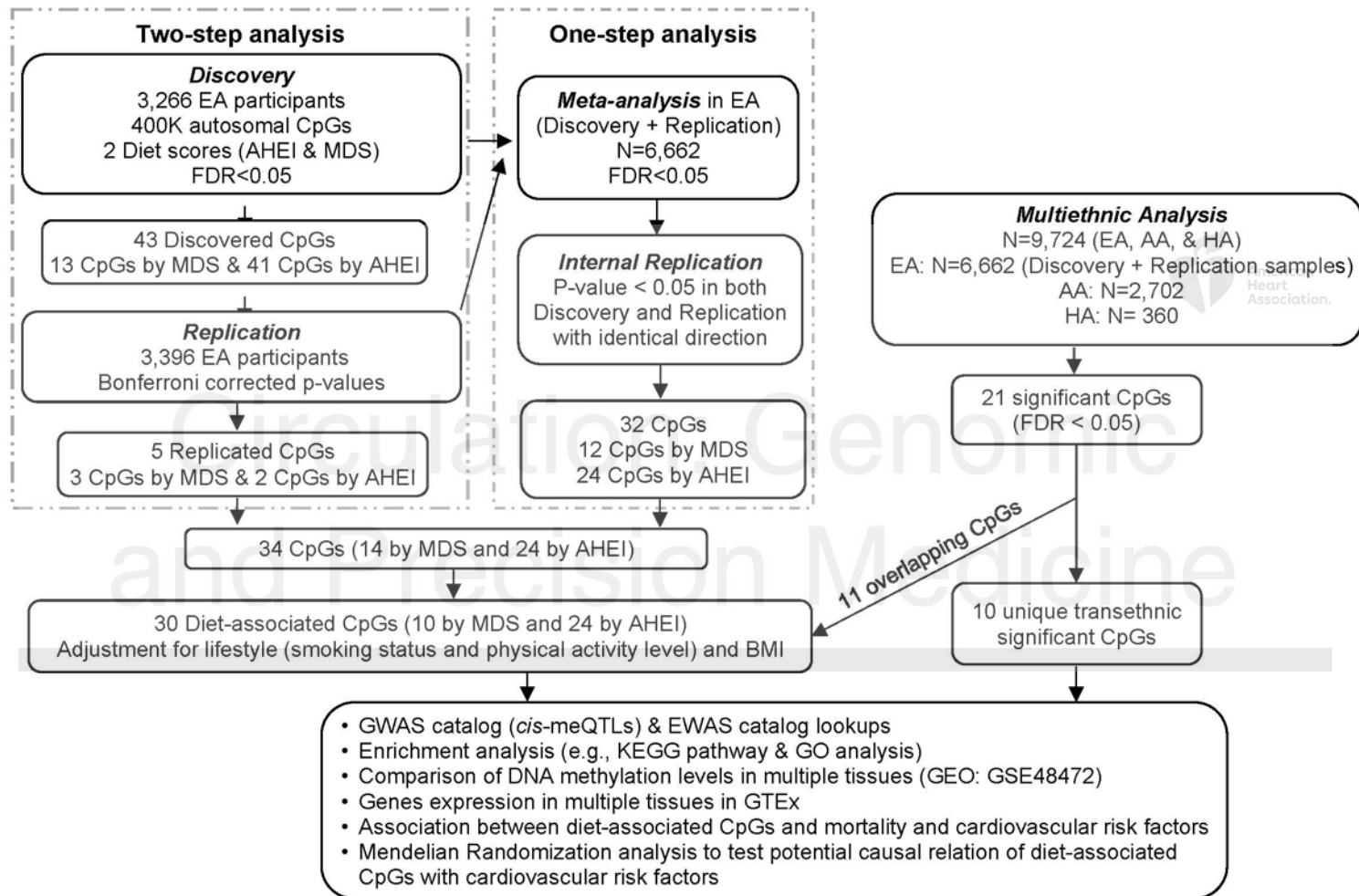
**Figure 2.** Effect of additional adjustment for lifestyle factors (smoking and physical activity) and BMI in European ancestry participants. A and B are 14 CpGs identified using the Mediterranean-style diet score (MDS). C and D are 24 CpGs identified using the Alternative Healthy Eating Index (AHEI). CpGs highlighted in red-colored rectangle are those identified in the two-step analysis alone and CpGs highlighted in green-colored rectangle are those identified in both one-step and two-step analyses. Orange colored dash line represents  $-\log_{10}$  of 0.05 and green colored dash line represents  $-\log_{10}$  of Bonferroni corrected p-value threshold, i.e., 0.05/14 for MDS and 0.05/24 for AHEI. Four CpGs (cg05575921, cg06126421, cg12075928, and cg25189904) in

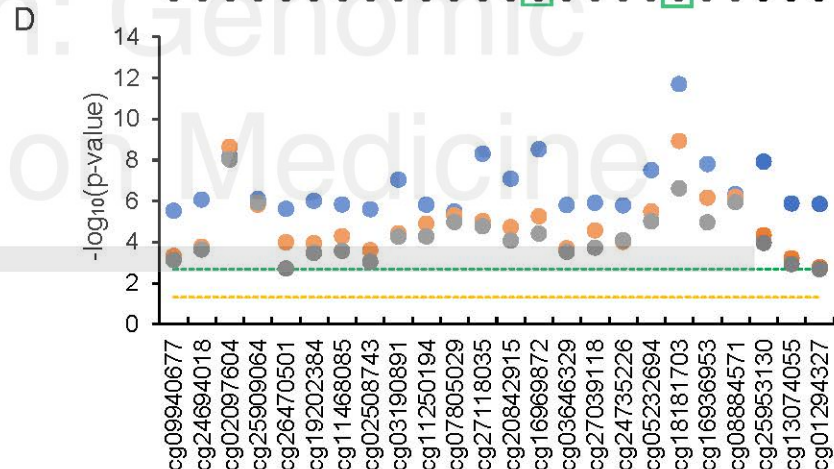
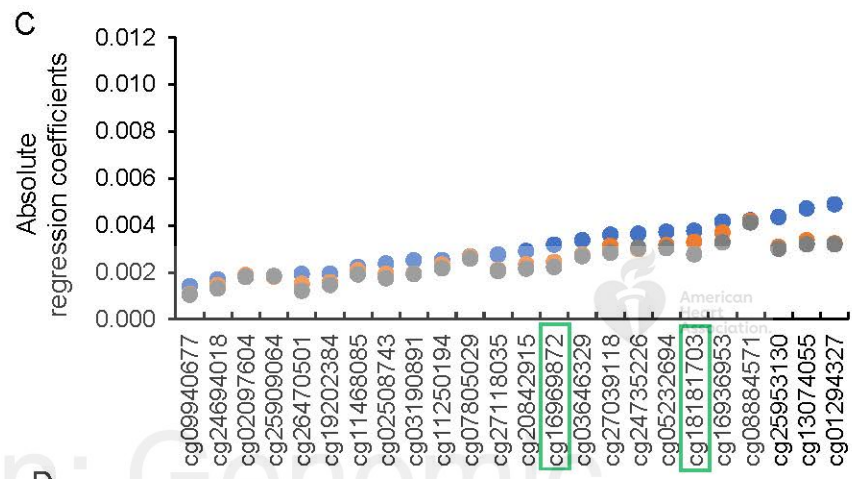
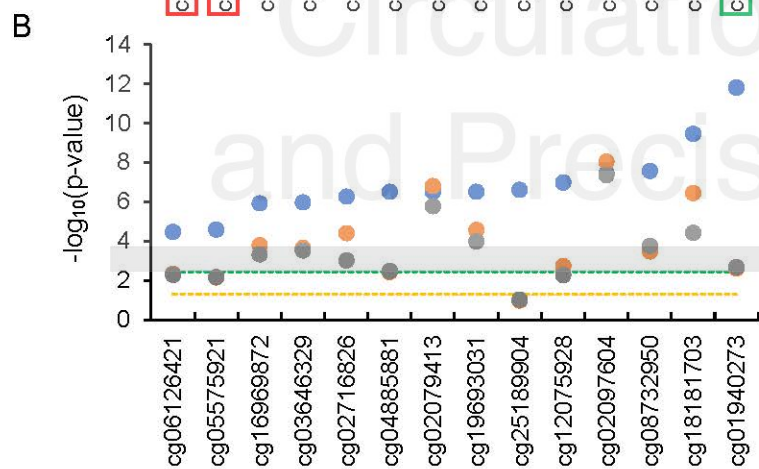
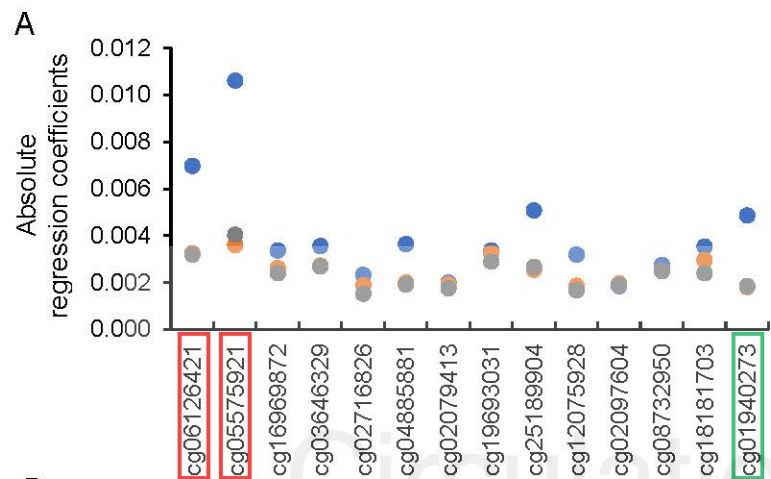
MDS analysis became non-significant after Bonferroni correction in models with adjustment for lifestyle factors and BMI, whereas all 24 CpGs in AHEI analysis remained significant.

**Figure 3.** Mendelian Randomization (MR) analyses for associations between cg11250194 (FADS2) and triglycerides (TG), between cg02079413 (SNORA54; NAP1L4) and BMI, and between cg26470501 (BCL3) and BMI. IVW: inverse variance weighted. Symbols and bars represent effects size and standard errors of instruments variables (cis-meQTL variants) used in MR analysis. Solid line is for MR-IVW analysis and dashed line is for MR-Egger analysis. No horizontal pleiotropy effect was detected for all MR analyses.

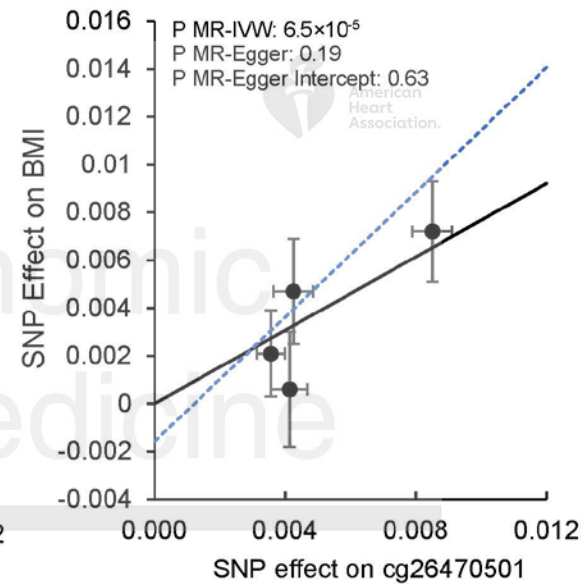
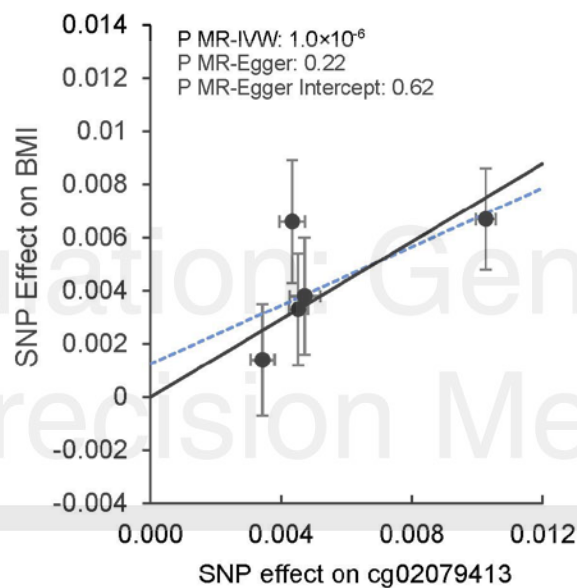
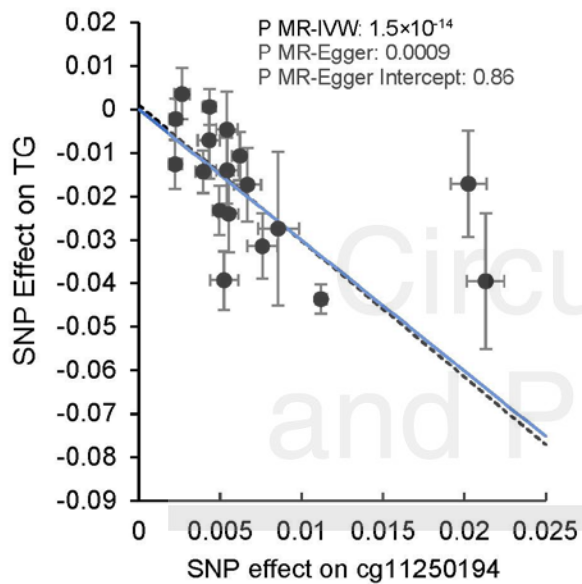


**Figure 4.** Meta-analysis of association between 30 diet-associated CpGs and all-cause mortality in 10 cohorts of European ancestry participants (n≈10,000). A positive sign for diet indicates that a higher dietary scores (MDS or AHEI, or both) were associated with DNA hypermethylation, whereas, a hazard ratio of over 1.0 indicates that DNA hypermethylation was associated with increased all-cause mortality. Models were adjusted for baseline covariates including sex, age, smoking status, physical activity level, alcohol intake, BMI, and prevalence disease status of hypertension, type 2 diabetes, cardiovascular disease, and cancer. Estimated leukocyte counts, technical variables, and kinship (for related study samples) were also considered. Hazard ratios and 95% confidence interval were estimated using Cox proportional hazard models and meta-analyzed using random effect models. X-axis is in logarithmic scale.





● Sex, age, & energy intake-adjusted ● Additional adjustment for lifestyle factors ● Additional adjustment for lifestyle factors and BMI

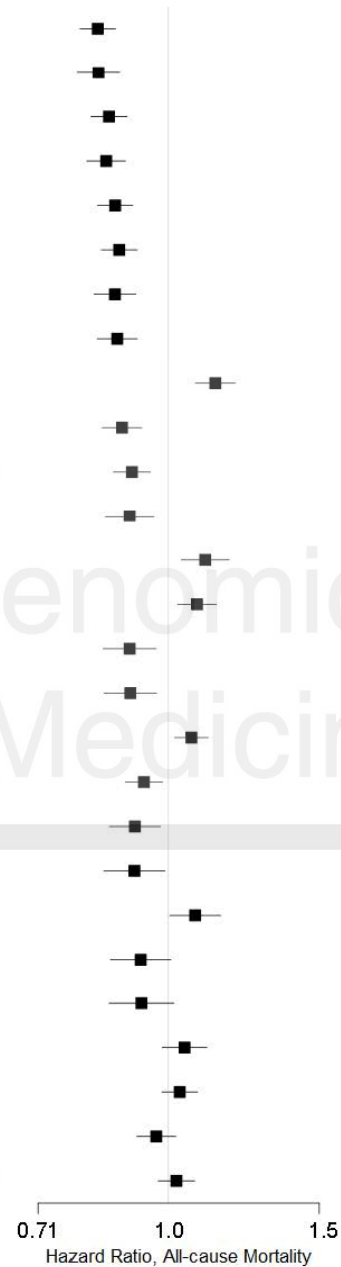


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CpG	CHR	Gene	Diet	P.Mortality
cg18181703	17	SOCS3	+	5.7e-15
cg26470501	19	BCL3	+	1.2e-10
cg02716826	9	SUGT1P1;AQP3	+	1.3e-10
cg01940273	2		+	3.5e-10
cg25953130	10	ARID5B	+	3.5e-09
cg24694018	1	POLR3GL	+	8.9e-08
cg04885881	1		+	5.6e-07
cg27039118	8	TRPS1	+	6.4e-07
cg11468085	11	ALDH3B2	-	3.8e-06
cg02097604	17	TOM1L2	+	4.8e-06
cg16936953	17	VMP1	+	0.0001
cg05232694	20		+	0.002
cg09940677	14	CDC42BPB	-	0.002
cg03190891	10	SORBS1	-	0.004
cg03646329	13	LPAR6;RB1	+	0.004
cg19202384	17	PYCR1	+	0.005
cg08732950	16	CBFA2T3	-	0.008
cg25909064	11	OAF	+	0.01
cg01294327	19	LINGO3	+	0.01
cg16969872	13	RBM26	+	0.03
cg27118035	16	ZNF267	-	0.04
cg19693031	1	TXNIP	+	0.07
cg11250194	11	FADS2	+	0.10
cg24735226	1	CACHD1	-	0.15
cg02508743	8	LYN	-	0.21
cg08884571	19	PPP1R13L	-	0.23
cg02079413	11	SNORA54;NAP1L4	-	0.39



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