SUPPLEMENTARY DATA

1. Framingham Heart Study (FHS).

Study Sample. The FHS participants were from the Offspring cohort and Third Generation cohort, which have been described in detail elsewhere (1; 2). Briefly, the Offspring cohort recruited 5,124 participants in 1971 and the Third Generation cohort recruited 4,095 participants in 2002. The study sample for the present analysis is a subcohort of 2,740 participants who attended the Offspring examination cycle 8 (2005-2008) or the Third Generation cohort examination cycle 2 (2008-2011) and whose hepatic fat were measured by multidetector computed tomography (CT) between 2008 and 2011. We excluded participants if they had a history of myocardial infarction or stroke, cancer (other than basal cell carcinoma), bariatric surgery, or heavy alcohol consumption (>14 drinks/week in women and >21 drinks/week in men), or missing information on alcohol intake, current smoking status, physical activity, and BMI. After initial exclusion, data collected from 1,496 participants who had whole blood derived DNA methylation profiling were analyzed for the present study. All participants provided written informed consent and the Framingham Heart Study protocols and procedures were approved by the Institutional Review Board for Human Research at Boston University Medical Center.

Genome-wide DNA methylation profiling. Peripheral whole blood samples were collected. We obtained buffy coat fractions and extracted genomic DNA using the Gentra Puregene DNA extraction kit (Qiagen, Venlo, Netherlands). We used EZ DNA Methylation Kit (Zymo Research, Irvine, CA) to perform bisulfite conversion to the extracted genomic DNA. After whole genome amplification, fragmentation, array hybridization, and single-base pair extension, we then quantified DNA methylation in three laboratories using the Illumina Infinium HumanMethylation450 (450K) BeadChip. The number of participants assessed in three laboratories was 578, 135, and 783, respectively. The laboratory-specific DNA methylation results were processed using the wateRmelon R package with the DASEN methodology, which included adjustment of methylated and unmethylated fluorescent intensities and technical variations and quantile normalization of the methylated and unmethylated probes with consideration of two types of assay methods (3). DNA methylation beta values were calculated as the ratio of methylated probe intensity to the overall intensity. Rigorous quality control procedures have been implemented as described elsewhere (4). Briefly, at the study sample level, we excluded participants with a probe missing rate >1%, poor SNP matching to the 65 SNP control probe locations, and outliers by multi-dimensional scaling techniques. We also excluded probes that: have a missing rate of >20% at $p<0.01$, have been previously identified to map to multiple locations (5), or have an underlying SNP (minor allele frequency >5% in European ancestry (EUR) 1,000 genomes project data) at the CpG site or within 10 bp of the single base extension. After quality control, about 430,000 autosomal CpG sites were analyzed subsequently. The FHS methylation data are available at dbGaP under the accession number phs000724.v2.p9.

Hepatic fat measurement. The protocol for assessing hepatic fat in the FHS has been reported previously (6-8). Briefly, participants underwent an abdominal scan with multidetector CT (64-slice scanners, General Electric Health Care). Hepatic fat content was estimated using the ratio of the mean Hounsfield unit from three regions in the liver to the mean Hounsfield unit of a calibration control (phantom) (6). A lower value of the liver-phantom ratio represents higher hepatic fat.

Methylation Quantitative Trait Loci (meQTL). To determine meQTLs, defined as DNA sequences that affect methylation levels at CpG sites, we analyzed the association of SNPs and DNA methylation in 4,170 FHS participants. We obtained SNP data in the FHS using Affymetrix 550K Array and imputed with the 1,000 Genomes Project reference panel (9). We first calculated the residuals for DNA methylation using linear mixed regression models adjusting for age, sex, and technical covariates. We then regressed the residuals on SNPs. We defined *cis*-meQTLs as SNPs associated with DNA methylation at nearby CpGs (±500 kilobases (kb) from CpG, MAF >0.01, imputation r^2 >0.5, p-value

$<1\times10^{-4}$).

Expression Quantitative Trait Loci (eQTL). We conducted eQTL analysis in 5,256 participants in FHS as previously described (10). We excluded eQTLs (SNPs) with MAF ≤0.01, imputation r^2 ≤0.5, and p-value $\geq1\times10^{-4}$. We defined *cis*-eQTLs as SNPs residing within 500kb of a nearby gene.

Covariates assessment. We assessed all covariates when participants visited the research clinic in accordance with standard protocols. Body mass index (BMI) was calculated as weight divided by height squared (kg/m^2). Current smokers were defined as participants who self-reported smoking at least one cigarette per day in the prior year. We used self-reported alcohol consumption for beer, wine, liquor, and spirits. We generated a physical activity score using the intensity and time spent performing each type of activity assessed by the physical activity questionnaire (11). A weighted genetic risk score for nonalcoholic fatty liver disease (NAFLD) was developed using regression coefficients and single nucleotide polymorphisms (SNPs) identified in a genome-wide association study (GOLD consortium) (12).

Epigenome-wide association analysis. We first applied the sva R package to construct surrogate variables in each laboratory. To remove potential batch effects, we calculated the residuals of DNA methylation beta value with adjustment for surrogate variables that associated with hepatic fat at p-value <0.1. In the subsequent analysis, laboratory-specific residuals were pooled together and used as the dependent variable. Hepatic fat was analyzed continuously as the independent variable (liver-phantom ratio). Three models were implemented. Covariates in the first model included age, sex, laboratories, and white blood cell counts (B cells, granulocytes, monocytes, NK cells, CD4+ T-cells, CD8+ T-cells) estimated using the Houseman method (13). The second model additionally adjusted for current smoking status, physical activity level, and alcohol intake. The third regression model additionally adjusted for BMI. The relatedness in our study sample was accounted for using linear mixed models.

2. Rotterdam Study (RS)

Study sample. This study was performed among participants of the prospective population-based RS. In 1989, all 10,275 residents aged 55 years or older in Ommoord, a suburb of Rotterdam, were invited to participate in the study. In 2000, the Rotterdam Study was extended by including 3,011 participants that moved to Ommoord or people who turned 55 (RS-II). The third cohort was formed in 2006 and included 3,932 participants 45 years and older (RS-III). Participants have been re-examined every 3-4 years and have been followed up for a variety of diseases. The RS has been approved by the medical ethics committee according to the Population Screening Act: Rotterdam Study, executed by the Ministry of Health, Welfare and Sports of the Netherlands. All participants in the present analysis provided written informed consent to participate and to obtain information from their treating physicians. A more detailed description of the Rotterdam Study can be found elsewhere (14). For this study, we used data from the first visit of RS-III (a random set of 165 individuals), and another non-overlapping set of the third visit of RS-II and the second visit of RS-III (701 individuals) with both DNA methylation and NAFLD data.

Genome-wide DNA methylation profiling. DNA was extracted from whole peripheral blood (stored in EDTA tubes) by standardized salting-out methods. Genome-wide DNA methylation levels were measured using the Illumina Human Methylation 450K array. In short, samples (500ng of DNA per sample) were first bisulfite-treated using the Zymo EZ-96 DNA-methylation kit (Zymo Research, Irvine, CA, USA). Next, samples were hybridized to the arrays according to the manufacturers' protocol. The methylation proportion of a CpG site was reported as a beta-value ranging between 0 (no methylation) and 1 (full methylation). The data preprocessing was additionally performed in both datasets using an R programming pipeline based on the pipeline developed by Tost & Toulemat (15), which includes

additional parameters and options to preprocess and normalize methylation data directly from idat files. 11,648 probes at X and Y chromosomes were excluded to avoid gender bias. The raw beta values were then background-corrected and normalized using the DASEN option of the WateRmelon R-package.

Hepatic fat measurement. Abdominal ultrasonography was performed by a certified and experienced technician on Hitachi HI VISION 900. Images were stored digitally and re-evaluated by a single hepatologist with more than ten years of experience in ultrasonography. The diagnosis of steatosis was determined by the ultrasound technician according to the protocol by Hamaguchi et al (16). NAFLD was defined by the presence of hepatic steatosis on abdominal ultrasound, in the absence of secondary causes as excessive alcohol consumption (>14 alcoholic beverages weekly), hepatitis B surface antigen, and/or hepatitis C virus positivity and use of steatosis inducing pharmacological agents (such as amiodarone, tamoxifen, corticosteroids, and methotrexate).

Covariates assessment. BMI was calculated by dividing weight in kilograms by height in meters squared. Information on current and past smoking behavior and medication use was acquired from questionnaires. Alcohol intake was assessed in grams of ethanol per day from food frequency questionnaires. Physical activity levels were assessed with an adapted version of the Zutphen Physical Activity Questionnaire (17). To quantify activity intensity, we used the metabolic equivalent of task (MET). We assigned MET values to all activities mentioned in the questionnaire, according to the 2011 updated version of the Compendium of Physical Activities (18). Blood lipids and glucose levels were measured using automatic enzymatic procedures (Roche Diagnostics GmbH, Mannheim, DE). White cell counts were used when available or estimated leukocyte proportions (B-cells, CD4+ T-cells, CD8+ T-cells, granulocytes, monocytes and NK-cells) were calculated as described by Houseman and implemented in the minfi package in R (19; 20).

Epigenome-wide association analysis. We modeled associations between Dasen normalized beta-values of the CpG sites as the dependent variable and NAFLD (Yes/ No) as the independent variable. We used linear mixed effect models adjusting for age, sex, white blood cell proportions and technical covariates (array number and position on array). The second model additionally adjusted for current smoking status, physical activity level, and alcohol intake. In the third model, BMI was additionally added to the model. Technical covariates were modeled as random effects. In addition, we performed a fixed effects meta-analysis using the inverse-variance weighted method implemented in METAL to combine the two non-overlapping samples of our study (21).

3. The Multi Ethnic Study of Atherosclerosis (MESA)

Study sample. The MESA was designed to investigate the prevalence, correlates, and progression of subclinical cardiovascular disease in a population cohort of 6,814 participants. Since its inception in 2000, five clinic visits collected extensive clinical, socio-demographic, lifestyle, behavior, laboratory, nutrition, and medication data (22). DNA methylation and gene expression were measured in purified (CD14+) monocyte samples from the April 2010 – February 2012 examination (exam 5) of 1,264 randomly selected MESA participants from four MESA field centers (Baltimore, MD; Forsyth County, NC; New York, NY; and St. Paul, MN) as previously described (23). The study protocol was approved by the Institutional Review Board at each site. All participants signed informed consent.

Genome-wide DNA methylation profiling. As previously described (23), blood was initially collected in sodium heparin-containing Vacutainer CPT™ cell separation tubes (Becton Dickinson, Rutherford, NJ, USA) to separate peripheral blood mononuclear cells from other elements within 2 h from blood draw. Subsequently, monocytes were isolated with the anti-CD14-coated magnetic beads, using AutoMACs automated magnetic separation unit (Miltenyi Biotec, Bergisch Gladbach, Germany). Based on flow

cytometry analysis of 18 specimens, monocyte samples were consistently >90% pure. DNA was isolated from samples using the AllPrep DNA/RNA Mini Kit (Qiagen, Inc., Hilden, Germany). DNA QC metrics included optical density measurements, using a NanoDrop spectrophotometer.

Illumina HumanMethylation450 BeadChips and HiScan reader were used to perform the epigenome-wide methylation analysis. Bead-level methylation data were summarized in GenomeStudio. Because a two-channel system and both Infinium I and II assays were used, normalization was performed in several steps using the lumi package. "Smooth quantile normalization" was used to adjust for color bias. Next, the data were background adjusted by subtracting the median intensity value of the negative control probes. Lastly, data were normalized across all samples by standard quantile normalization applied to the bead-type intensities and combined across Infinium I and II assays and both colors. QC measures included checks for sex and race/ethnicity mismatches, and outlier identification by multidimensional scaling plots. To estimate residual sample contamination for data analysis, we generated separate enrichment scores for neutrophils, B cells, T cells, monocytes, and natural killer cells. We implemented a Gene Set Enrichment Analysis as previously described (23) to calculate the enrichment scores using the gene signature of each blood cell type from previously defined lists (24). To remove technical error in methylation levels associated with batch effects across the multiple chips, positional effects of the sample on the chip, and residual sample contamination with non-monocyte cell types, we adjusted methylation values for chip, sample position on the chip, and estimated residual sample contamination with neutrophils, B cells, T cells, monocytes, and natural killer cells. The final methylation value for each methylation probe was computed as the beta-value, essentially the proportion of the methylated to the total intensity.

Hepatic fat measurement. Cardiac CT scans were performed either with an ECG-triggered (at 80% of the RR interval) electron-beam scanner (Chicago, Los Angeles, and New York field centers; Imatron C-150; GE Imatron, Milwaukee, WI) or with prospectively ECG-triggered scan acquisition at 50% of the RR interval with a multidetector system that acquired 4 simultaneous 2.5-mm slices for each cardiac cycle in a sequential or axial scan mode (Baltimore, Forsyth Country, and St. Paul field center; Lightspeed (GE Medical Systems, Milwaukee, WI) or Volume Zoom (Siemens, Erlangen, Germany)). Three experienced CT analysts measured liver attenuation on these cardiac CT images using the Advantage Windows Workstation (GE Healthcare, Waukesha, WI) with volume analysis software. Liver attenuation was calculated as the average in Hounsfield units (HU) of three measurements (approximately 1 cm2 each). The three circular regions were consistently placed in the parenchyma of the right lobe of the liver 15 mm from the top.

Covariates assessment. Standard questionnaires were used to collect information on demographics, cigarette smoking, alcohol drinking, and physical activity. Pack-years of smoking was the average number of packs of cigarette smoked per day times the number of years of smoking. Alcohol drinking was categorized into never, former and current. Physical activity was calculated on the basis of duration and intensity of the total intentional exercises, including moderate walking exercise, moderate dance, vigorous team/dual sports, moderate individual activities, and moderate and vigorous conditioning. Weight was measured with a Detecto Platform Balance Scale to the nearest 0.5 kg. Height was measured with a stadiometer (Accu-Hite Measure Device with level bubble) to the nearest 0.1 cm. BMI was defined as weight in kilograms divided by square of height in meters.

Epigenome-wide association analysis. Data were analyzed with multiple linear regression models. We implemented three models. Covariates were age, sex, race/ethnicity, study site, technical covariates (methylation chip and sample position on chips), residual sample contamination with nontargeted cells (e.g., non-monocytes) in model 1. We additionally adjusted for smoking status, physical activity, and

alcohol intake in model 2. In model 3, we adjusted for model 2 covariates and BMI.

4. Coronary Artery Risk Development in Young Adults (CARDIA) Study

Study sample. The CARDIA study is a prospective multicenter study with 5115 adult, Caucasian and African-American participants ages 18–30 years at recruitment. The recruitment was done from four centers as follows: the total community in Birmingham, AL; selected census tracts in Chicago, IL, and Minneapolis, MN; and from the Kaiser Permanente health plan membership in Oakland, CA. Details of the CARDIA study design have been previously published (25; 26). Nighttime examinations were completed beginning with the study initiation in 1985–1986 and in examination years (Y) 0, 2, 5, 7, 10, 15, 20, 25, and 30. We excluded participants if they had a history (at and before Y25, when liver attenuation was measured) of myocardial infarction or stroke, cancer (other than basal cell carcinoma), bariatric surgery, heavy alcohol consumption (>14 drinks/week in women and >21 drinks/week in men), medications (i.e. valproic, methotrexate, tamoxifen, or amiodarone), hepatitis, liver cirrhosis, and HIV..

Genome-wide DNA methylation profiling. Infinium MethylationEPIC BeadChip raw data (IDAT files) were generated from a total of 2,181 blood samples [1,089 at Y15 and 1,092 at Y20] loaded by the R package *minfi*. Quality control and data preprocessing were conducted using the R package *ENmix* (27) with default parameter settings. In the quality control step, low-quality methylation measurements were identified by detection p-value $<10^{-6}$ or number of beads <3 (27). We excluded 6,209 CpGs with a detection rate <95% and 87 samples with a percentage of low-quality methylation measurements >5% or extremely low intensity of bisulfite conversion probes (less than 3 × standard deviation of the intensity across samples below the mean intensity) (27). After excluding low-quality CpGs and samples, we further removed 95 samples that were extreme outliers, as defined by Tukey's method [i.e., $<25^{th}$ percentile – 3 * interquartile range (IQR) or $>75^{th}$ percentile + 3 * IQR] (28) and based on the average total intensity value [intensity of the unmethylated signal (U) + intensity of the methylated signal (M)] or β value [M / (U + M + 100)] across CpG probes. The remaining samples were preprocessed using ENmix, a model-based background correction method which models methylation signal intensities with a flexible exponential-normal mixture distribution, together with a truncated normal distribution to model background noise (27). Dye bias was corrected using *RELIC* (regression on logarithm of internal control probes), which utilizes the intensity values of paired internal control probes that monitor the two-color channels (29). We then separately quantile-normalized M or U intensities for Infinium I or II probes, respectively. Lastly, low-quality methylation values (detection p-value $<10^{-6}$ or number of beads <3) and extreme β-value outliers across samples (defined by Tukey's method) were set as missing. The final clean methylation working dataset contains 860,627 CpG probes and 1999 samples (1042 from Y15 and 957 from Y20).

Hepatic fat measurement. At Y25, measurement of liver attenuation was performed in the right lobe of the liver using CT slices through the upper abdomen and was reported as the average of nine measurements on three slices using circular regions of interest of 2.6 cm^2. The interclass correlation coefficient between different readers on a random selected sample of 156 participants was 0.975 for liver attenuation, indicating high reproducibility of CT measured liver attenuation in CARDIA.

Covariates assessment. Smoker is defined as participant who is smoking cigarettes regularly (i.e. least 5 cigarettes per week almost every week) for at least three months; former smoker is defined as participant is not smoking cigarettes regularly (regardless of the duration of smoking abstinence); non-smoker is defined as participant who has never smoked cigarettes (other tobacco products, such as cigars, tobacco pipe, chewing tobacco, etc. are permitted). Physical activity used an interviewer-administered questionnaire concerning the frequency of participation in 13 different activities during the past 12

months (30; 31) . Because participants were not asked specifically about duration of physical activity, the activity score is expressed in 'Exercise Units' (EU). A score of 100 EU is roughly equivalent to participation in activities such as a vigorous exercise class or bicycling faster than 10 miles per hour, 2 or 3 h a week for 6 months of the year. Alcohol intake was measured as the total drinks of beer, wine, and liquor.

Epigenome-wide association analysis. Data were analyzed with multiple linear regression models. We implemented three models. Covariates were age, sex, race, and study center in model 1. To account for experimental batch effects and other technical biases, we derived surrogate variables from intensity data for non-negative internal control probes using principal components (PCs) analysis (27). We adjusted for the proportions of different leukocytes estimated using Houseman's method. The top eight PCs explained 95.06% of the variation across the non-negative internal control probes and thus we also included them as covariates in model 1. We additionally adjusted for smoking status, physical activity, and alcohol intake in model 2. In model 3, we adjusted for model 2 covariates and BMI.

5. Genetic Epidemiology Network of Arteriopathy (GENOA)

Study sample. The GENOA study is a community-based study of hypertensive sibships that was designed to investigate the genetics of hypertension and target organ damage in African Americans from Jackson, Mississippi and non-Hispanic whites from Rochester, Minnesota (32). In the initial phase of the GENOA study (Phase I: 1996-2001), all members of sibships containing ≥ 2 individuals with essential hypertension clinically diagnosed before age 60 were invited to participate, including both hypertensive and normotensive siblings. Eighty percent of African Americans (1,482 subjects) and 75% of non-Hispanic whites (1,213 subjects) from the initial study population returned for the second examination (Phase II: 2001-2005). Study visits were made in the morning after an overnight fast of at least eight hours. Demographic information, medical history, clinical characteristics, lifestyle factors, and blood samples were collected in each phase. In an ancillary GENOA study (2009-2011), 657 GENOA African Americans received computed tomography (CT) scans. Written informed consent was obtained from all subjects and approval was granted by participating institutional review boards. The current analysis is limited to African American participants, since CT scans and DNA methylation measures are only available in GENOA African Americans. After exclusions, a total of 150 African American participants were included in this analysis.

Genome-wide DNA methylation profiling. Genomic DNA of 422 participants were extracted from stored peripheral blood leukocytes, bisulfite converted, and then measured for DNA methylation using the Illumina Infinium HumanMethylation450 BeadChip using stored blood samples collected during the Phase I examination. The Minfi R package was used to preprocess, normalize (SWAN), and calculate beta values. The proportion of each cell type were estimated using Houseman's method. Detection p-values were calculated for each site, and beta values were set to missing if a site had detection p-value>0.01. In all samples, > 95% of probes had a detection P-value<0.01; thus, no samples were excluded from analysis. A total of 1,707 probes were removed due to detection P-value>0.01 in >5% of samples.

Hepatic fat measurement. Hepatic fat was measured by CT scanning (GE Lightspeed 16 Pro, Healthcare, Milwaukee, WI) using a standardized protocol (33). A Calcium QCT phantom (Image Analysis, Inc., Columbia, KY) was used to adjust attenuation values as part of quality control. Hepatic fat was measured at the T12 to L1 intervertebral space by calculating the mean Hounsfield units of three regions of interest (ROI's) in the parenchyma of the right lobe of the liver. Participants weighing more than 160 kg were excluded from the CT scan. For participants who weighed more than 100 kg, the tube current (ie, mA) was adjusted upwards (25%). This adjustment was designed to maintain a more

consistent image quality over the spectrum of body sizes. The estimated effective dose was estimated to be 1.5 mSv (150 mrem) for men and 1.9 mSv (190 mrem) for women. CT images were transmitted to the reading center at Wake Forest University. The mean attenuation of the three ROIs and the phantom provided a liver/phantom ratio (LPR) adjusted for scan penetrance. Exclusions made for this analysis include high alcohol consumers (N=1), history of myocardial infarction or stroke (N=43), and medication use (N=2 taking methotrexate, tamoxifen, corticoid steroid, or amiodarone).

Covariates assessment. Smoking was defined categorically (current, former, never). Physical inactivity was defined as having zero hours of heavy activity and 1 or less hours of moderate activity per day. Alcohol consumption was defined as continuous drinks per week, and BMI was continuous (kg/m^2).

Epigenome-wide association analysis. We used linear mixed models to test for association between each phenotype and beta-values of DNA methylation with the technical covariates (array, row and column number) as random effects and the remaining covariates as fixed effects using R (v3.3.2; lme4_1.1-12). Three models were run as follows:
Model 1: Beta value (normalized) ~ Hepatic fat + age + sex + CD8T + CD4T + NK + BCell + Mono + technical covariates (plate, row, and column as random effects)
Model 2: Beta value (normalized) ~ Hepatic fat + (Model 1 covariates) + smoking + physical activity + alcohol consumption
Model 3: Beta value (normalized) ~ Hepatic fat + (Model 2 covariates) + BMI

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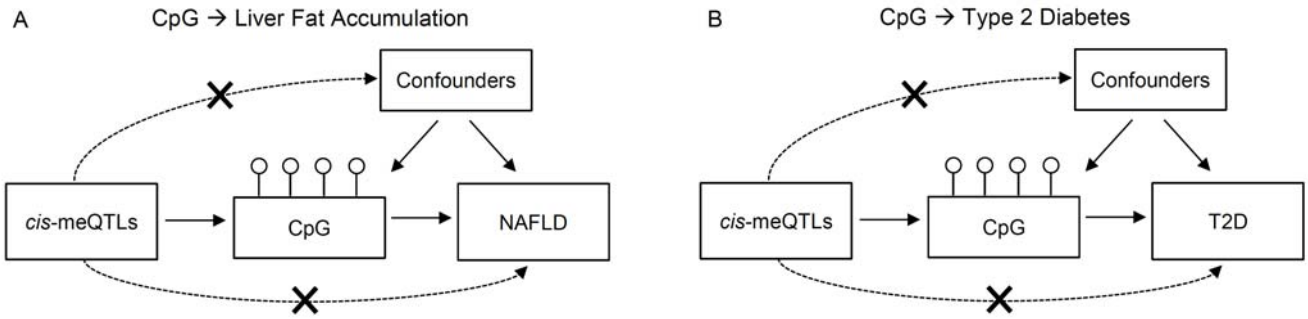
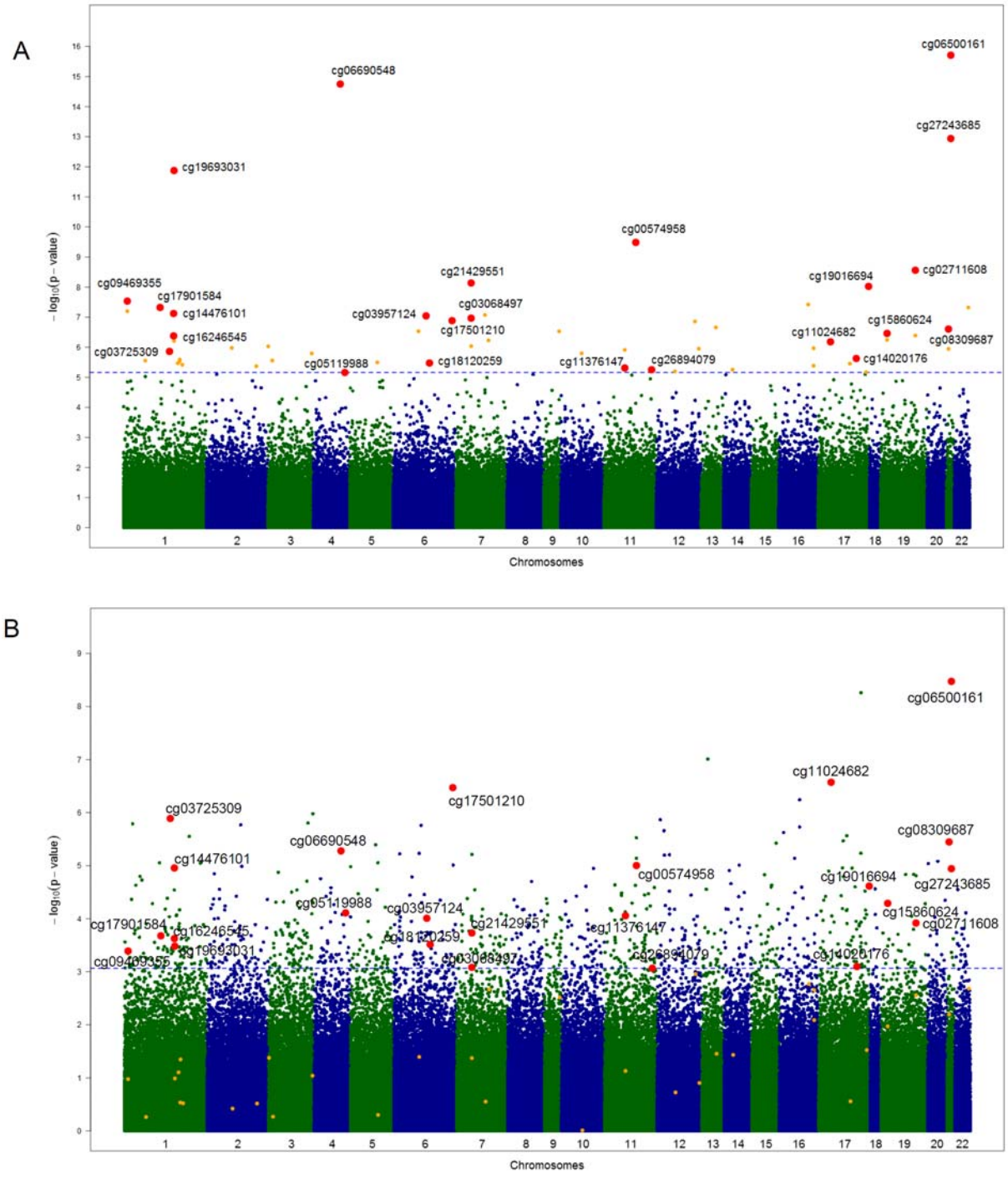
SUPPLEMENTARY DATA

Supplementary Figure S1. Mendelian randomization approach to analyze potential causal association for A) CpG to nonalcoholic fatty liver disease (NAFLD) and B) CpG to type 2 diabetes (T2D) in the Framingham Heart Study. *cis*-meQTLs: single nucleotide polymorphisms (SNPs) associated with DNA methylation at CpG site.



SUPPLEMENTARY DATA

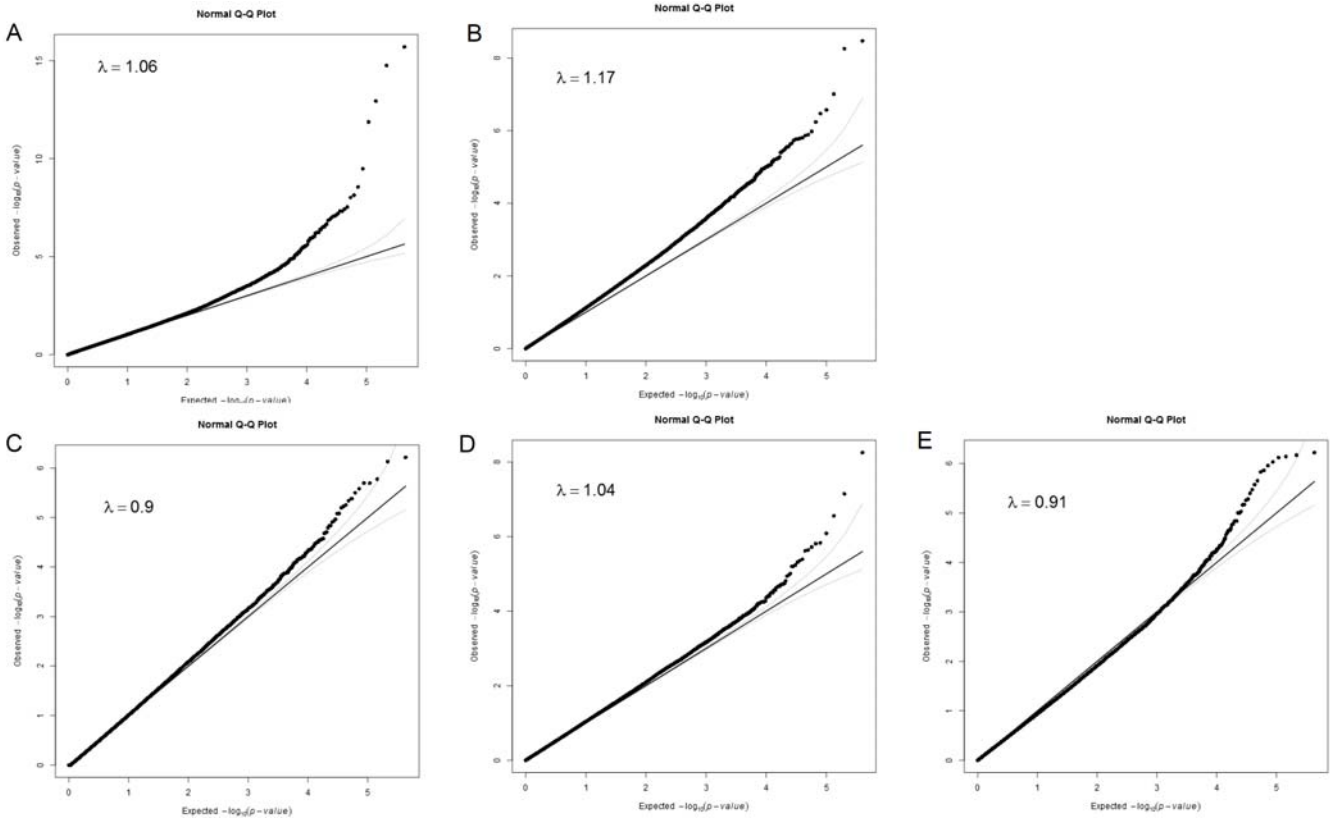
Supplementary Figure S2. Manhattan plots generated using the sex- and age-adjusted model.
Plot 1A represents the discovery analysis in the FHS with a p-value threshold of 6.9x10-6 (dotted line) corresponding to FDR < 0.05. Plot 1B represents the replication meta-analysis of EA participants in the RS, CARDIA, and MESA using the Bonferroni corrected p-value threshold of 8.6x10-4. Orange dots are significant CpGs in the FHS and red dots are replicated CpGs. FDR=false discovery rate. EA=European ancestry. FHS=Framingham Heart Study. RS=Rotterdam Study. MESA=Multi-Ethnic Study of Atherosclerosis study. CARDIA=Coronary Artery Risk Development in Young Adults study. CpG=DNA methylation site.



SUPPLEMENTARY DATA

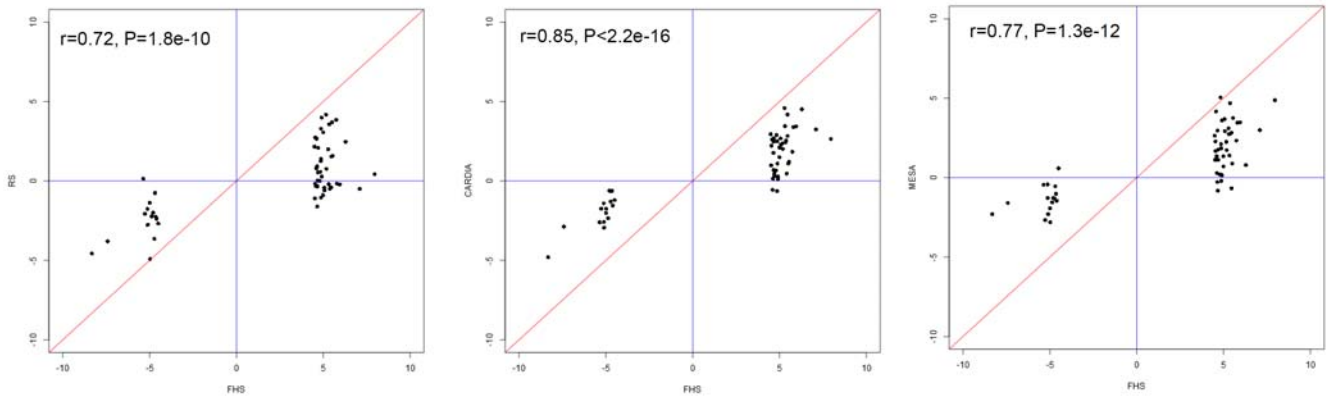
Supplementary Figure S3. QQ plots with lambda values generated using the sex- and age-adjusted model.

Plot 2A depicts FHS (discovery). Plot 2B depicts the meta-analysis of EA participants in RS, CARDIA, and MESA (replication). Plot 2C depicts RS EA participants. Plot 2D depicts CARDIA EA participants. Plot 2E depicts MESA EA participants. FHS=Framingham Heart Study. EA=European Ancestry. RS=Rotterdam Study. MESA=Multi-Ethnic Study of Atherosclerosis study. CARDIA=Coronary Artery Risk Development in Young Adults study.



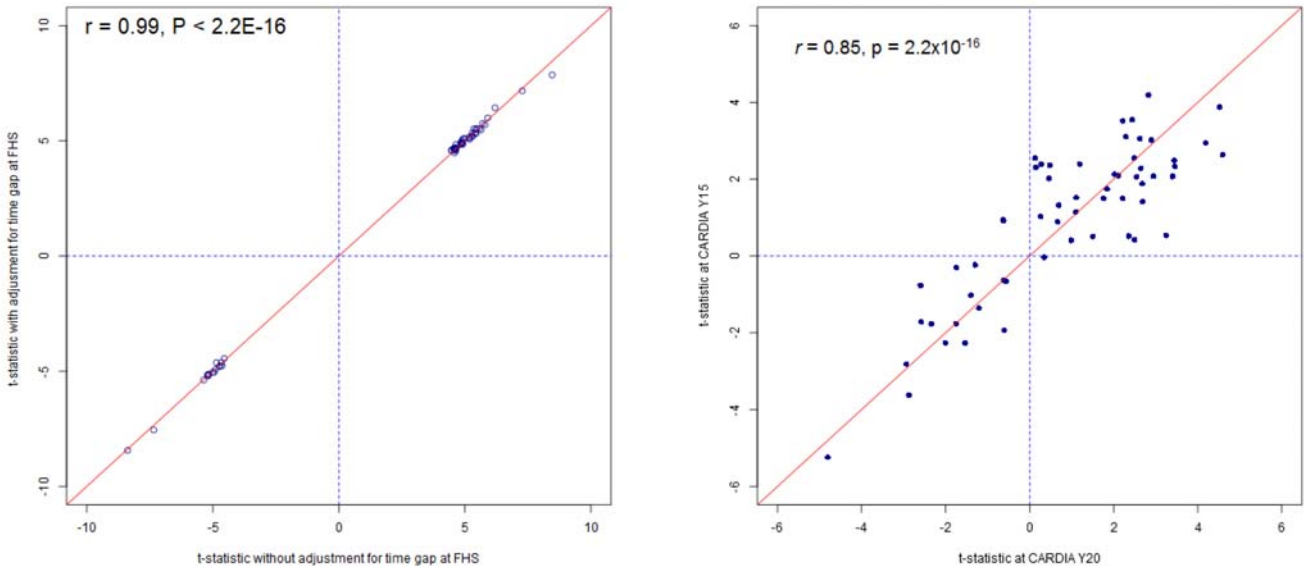
SUPPLEMENTARY DATA

Supplementary Figure S4. Correlation of 58 CpGs discovered in the FHS with replication cohorts. Plots depict the correlation of t-statistics of the 58 CpGs discovered in the FHS at FDR < 0.05 with t-statistics of the same CpGs in EA participants in the RS, CARDIA, and MESA cohorts. FDR=false discovery rate. FHS=Framingham Heart Study. EA=European ancestry. RS=Rotterdam Study. MESA=Multi-Ethnic Study of Atherosclerosis study. CARDIA=Coronary Artery Risk Development in Young Adults study. CpG=DNA methylation site.



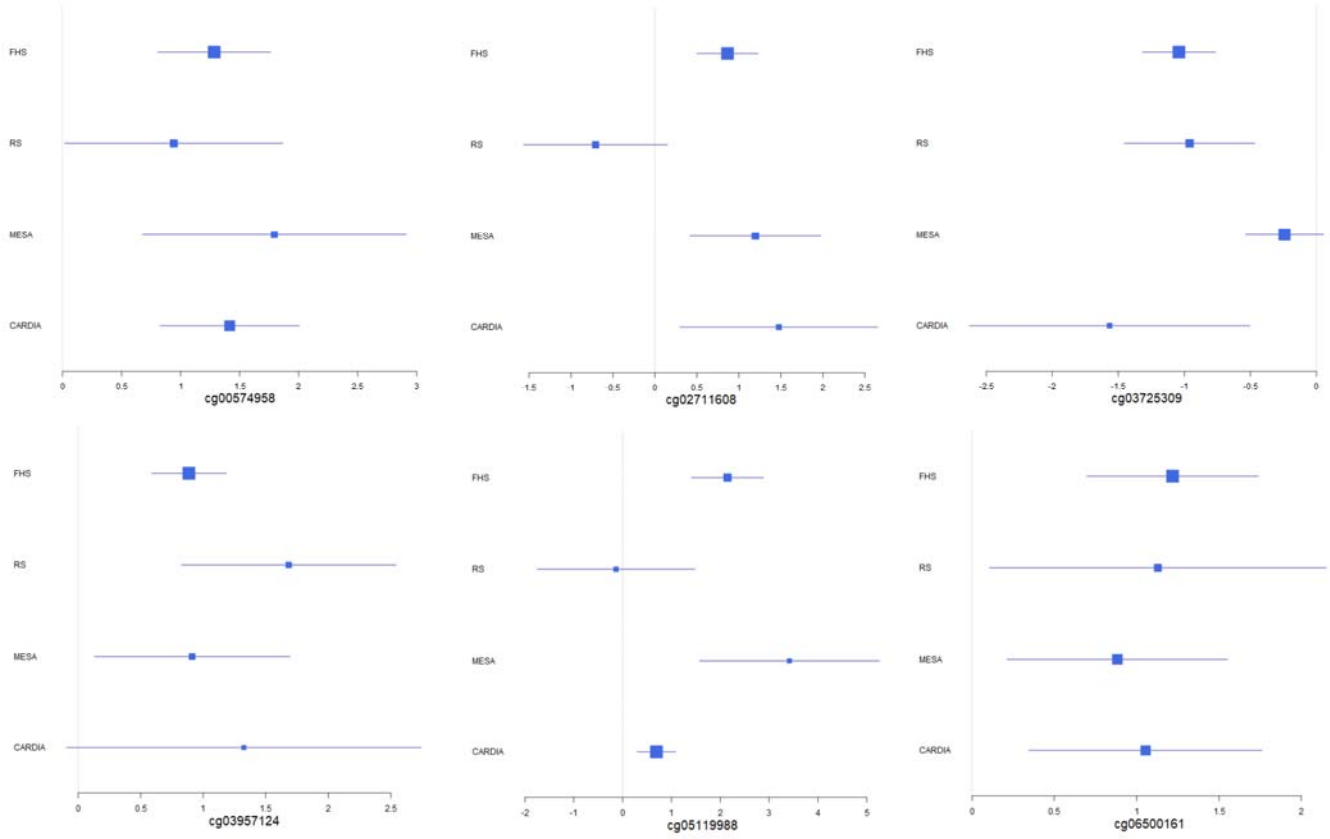
SUPPLEMENTARY DATA

Supplementary Figure S5. Correlation of t-statistics for the associations between DNA methylation and hepatic fat for 58 CpGs significant in the discovery cohort (FHS). Left figure showed t-statistics with and without additional adjustment for the time gap between assessments of DNA methylation and hepatic fat in the FHS. Right figure showed t-statistics generated using DNA methylation data measured at year 15 versus year 20 of the CARDIA study. In the CARDIA study, hepatic fat was measured once using CT at year 20 examination cycle and DNA methylation was measured twice at year 15 and year 20 examinations. CARDIA=Coronary Artery Risk Development in Young Adults study. FHS=Framingham Heart Study. CT=computed tomography. CpG=DNA methylation site.



SUPPLEMENTARY DATA

Supplementary Figure S6. Forest plots for 22 replicated CpGs in European ancestry participants using regression coefficients and standard errors standardized by the cohort-specific standard deviation of the regression coefficients.



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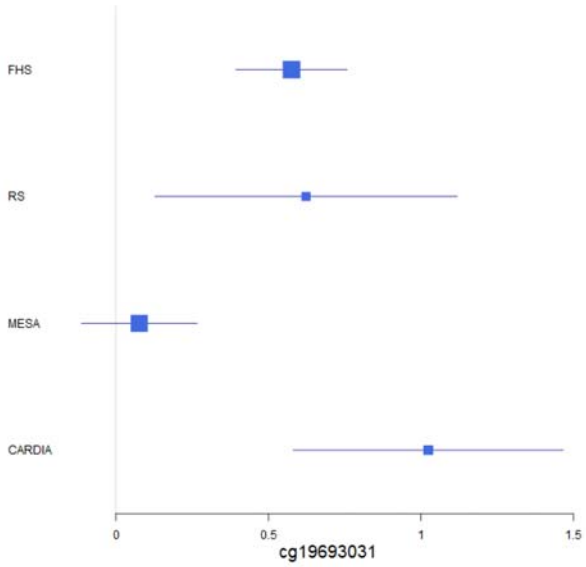
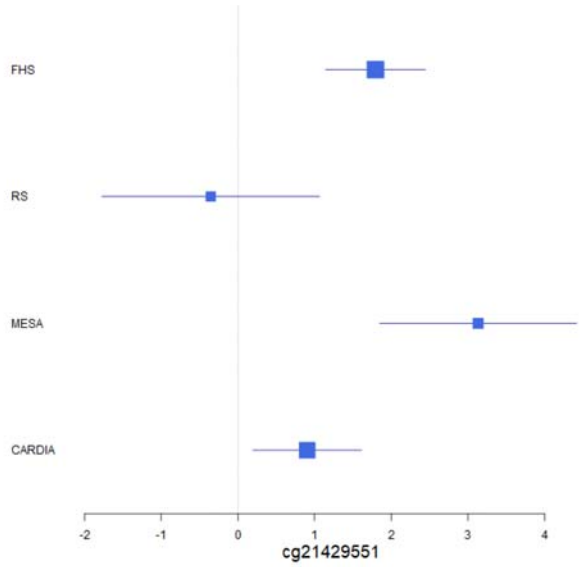
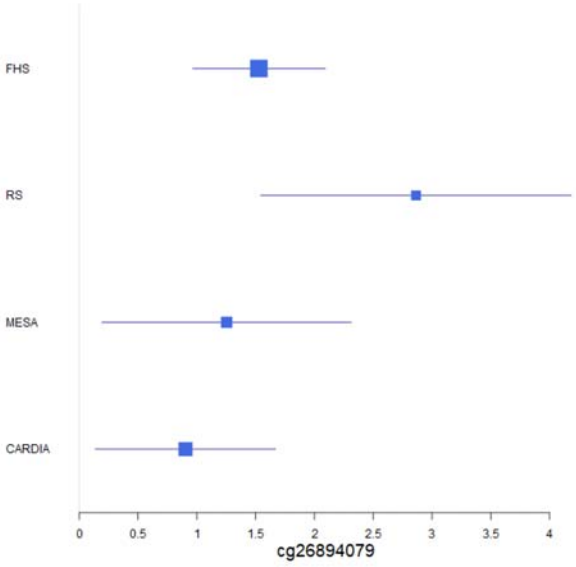
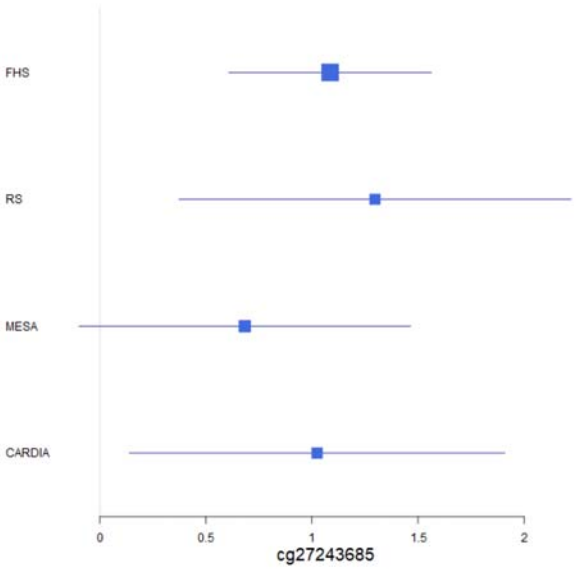


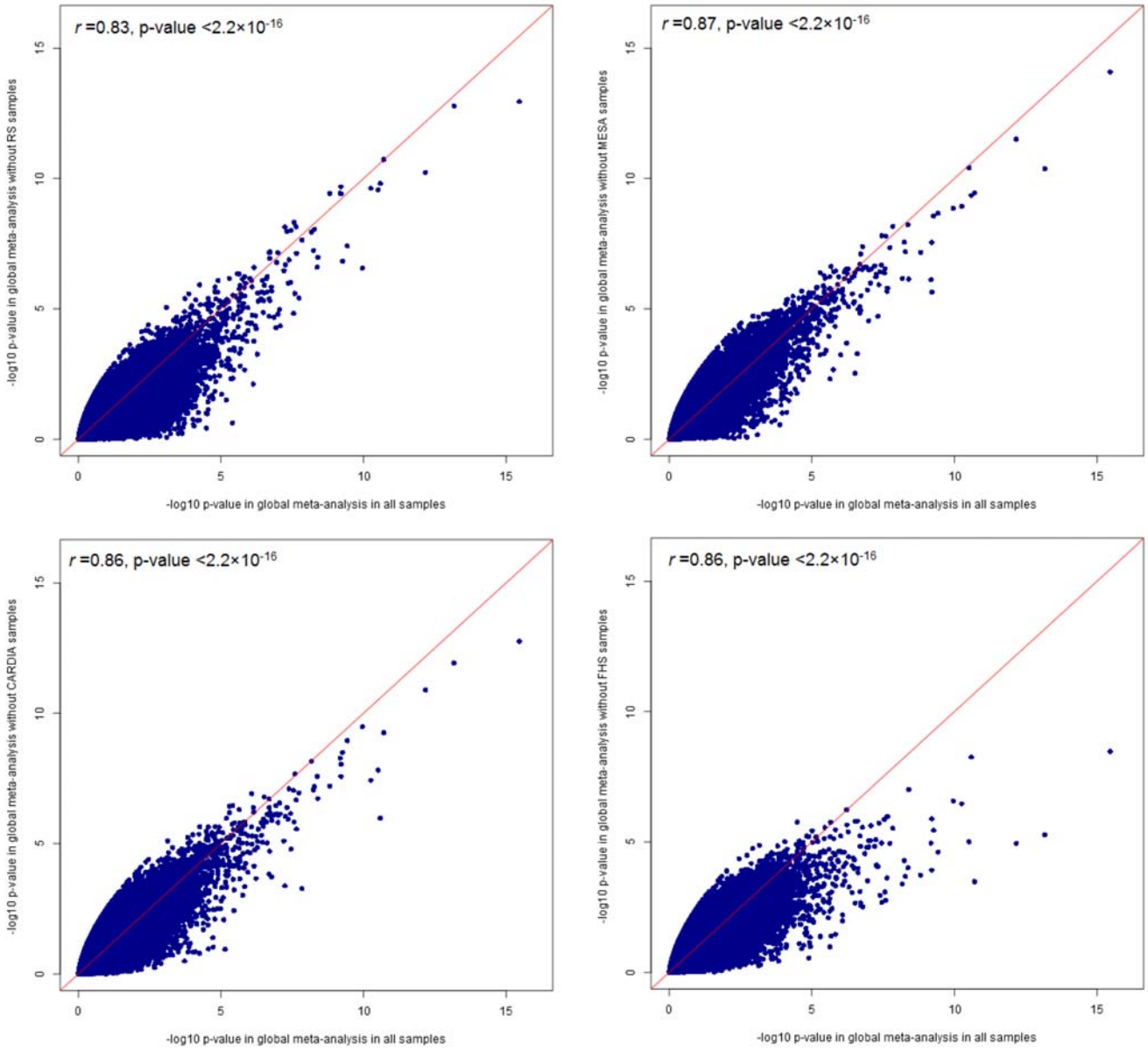






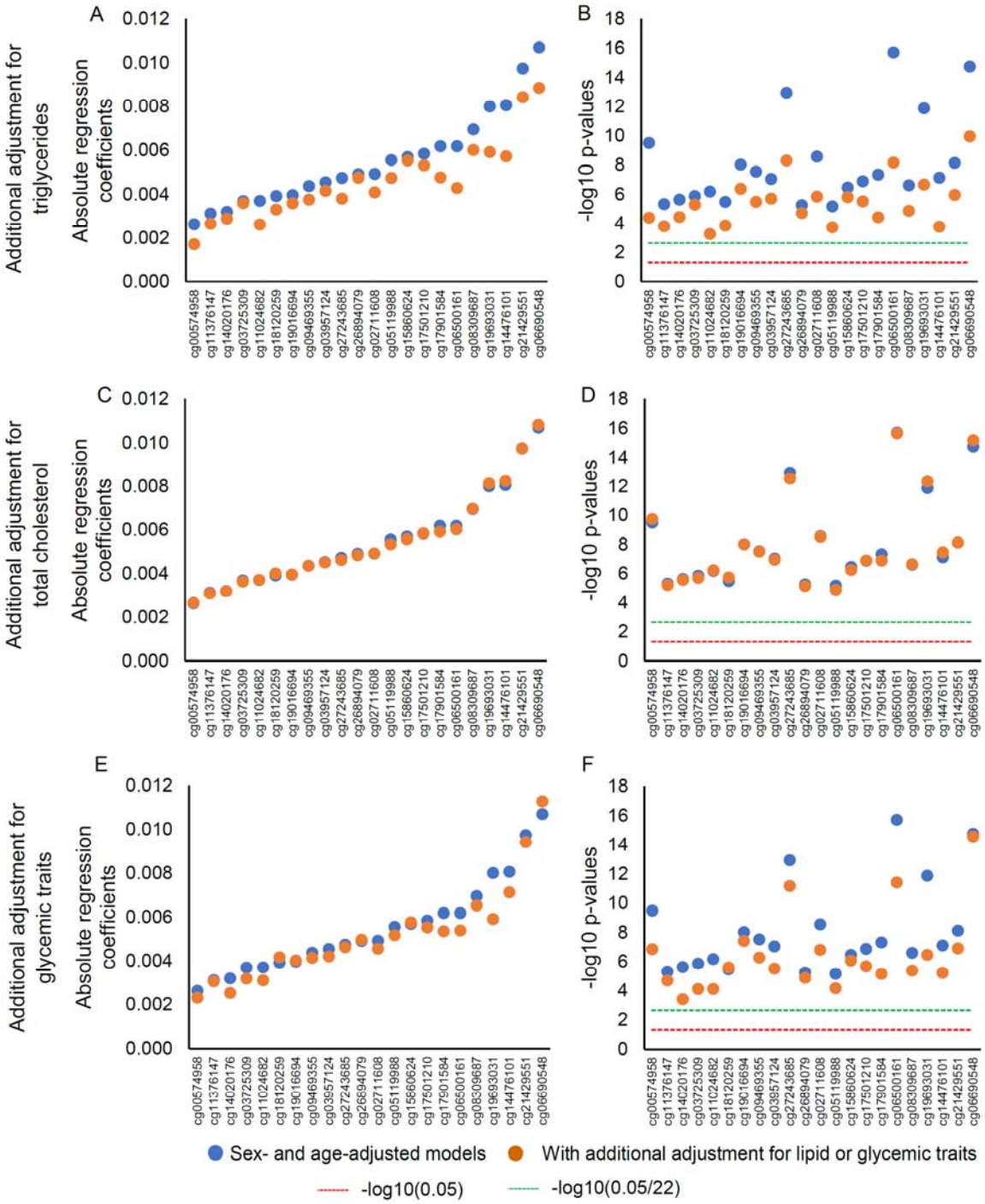
SUPPLEMENTARY DATA

Supplementary Figure S7. Comparisons of leave-one-cohort-out meta-analysis with meta-analysis in all samples for European ancestry participants. Cohort-specific analysis used sex- and age- adjusted model with cohort-specific adjustment for technical variables and family structure



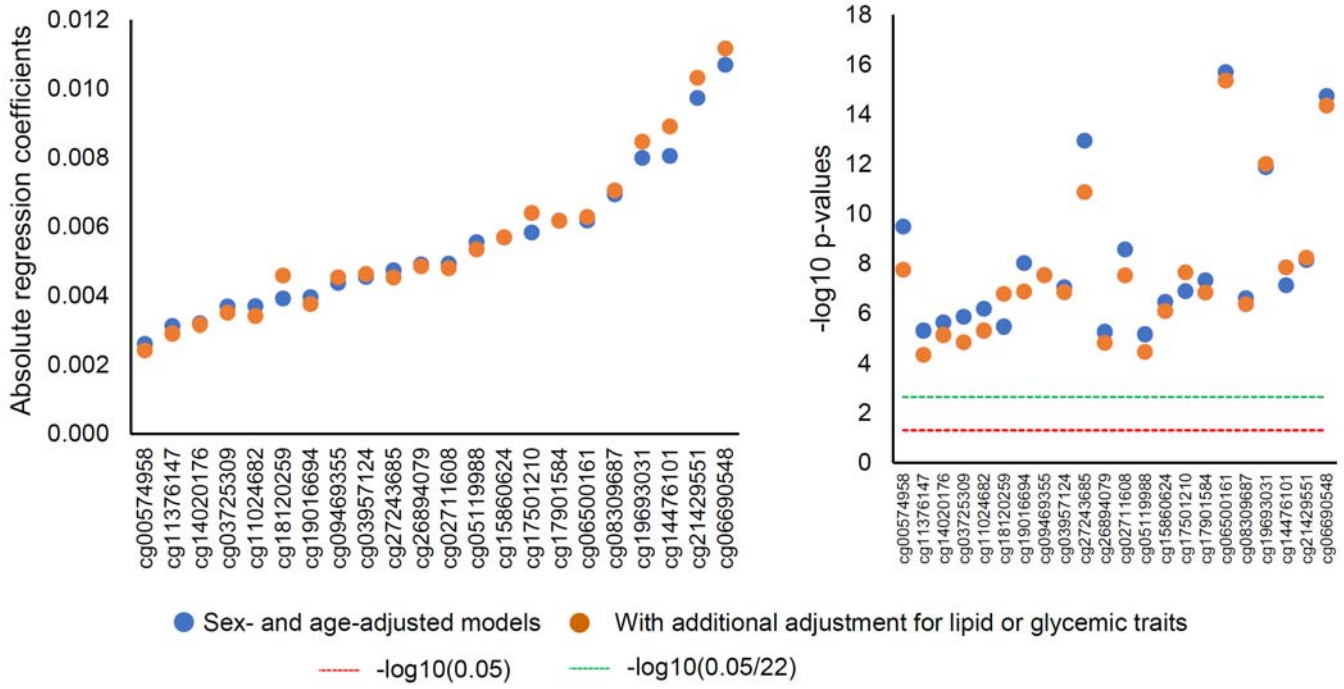
SUPPLEMENTARY DATA

Supplementary Figure S8. Effect of additional adjustment for lipid and glycemic traits on association between the 22 replicated CpG s and hepatic fat in FHS participants. Analysis was based on sex- and age-adjusted models. Upper panel (A and B) were additionally adjusted for fasting triglycerides, middle panel (C and D) were additionally adjusted for total fasting cholesterol, and low er panel were additionally adjusted for prevalent type 2 diabetes, fasting glucose, and HbA1c. Left panel (A, C, and E) were absolute value of regression coefficients, i.e., DNA methylation β value change for per standard deviation change of hepatic fat, and right panel (B, D, and F) were -log10 p-value.FHS=Framingham Heart Study



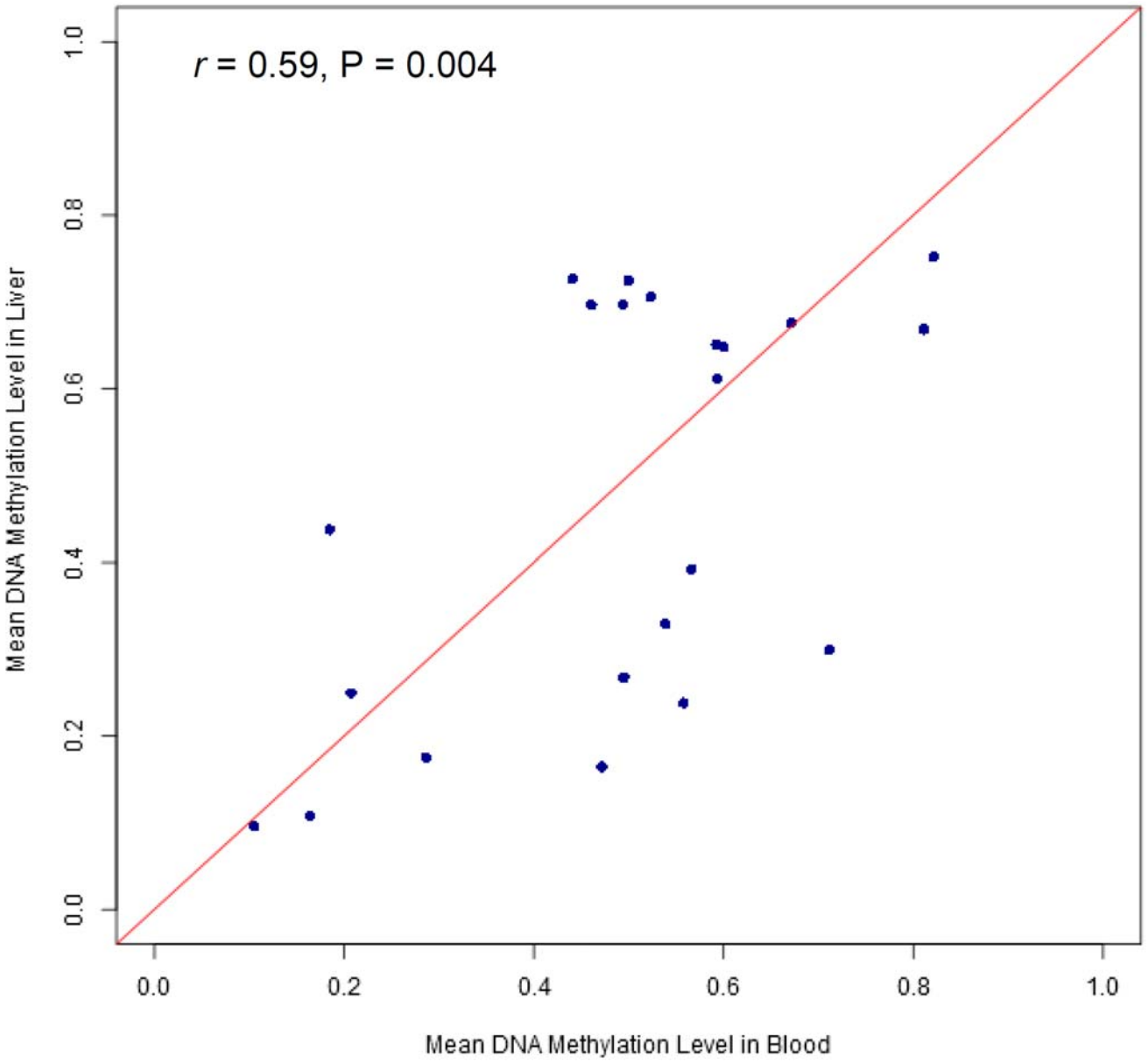
SUPPLEMENTARY DATA

Supplementary Figure S9. Effect of additional adjustment for dietary factors on association between the 22 replicated CpGs and hepatic fat in FHS participants. Analysis was based on sex- and age-adjusted models with and without additional adjustment for caloric intake and total fat intake assessed by food frequency questionnaire in the Framingham Heart Study.



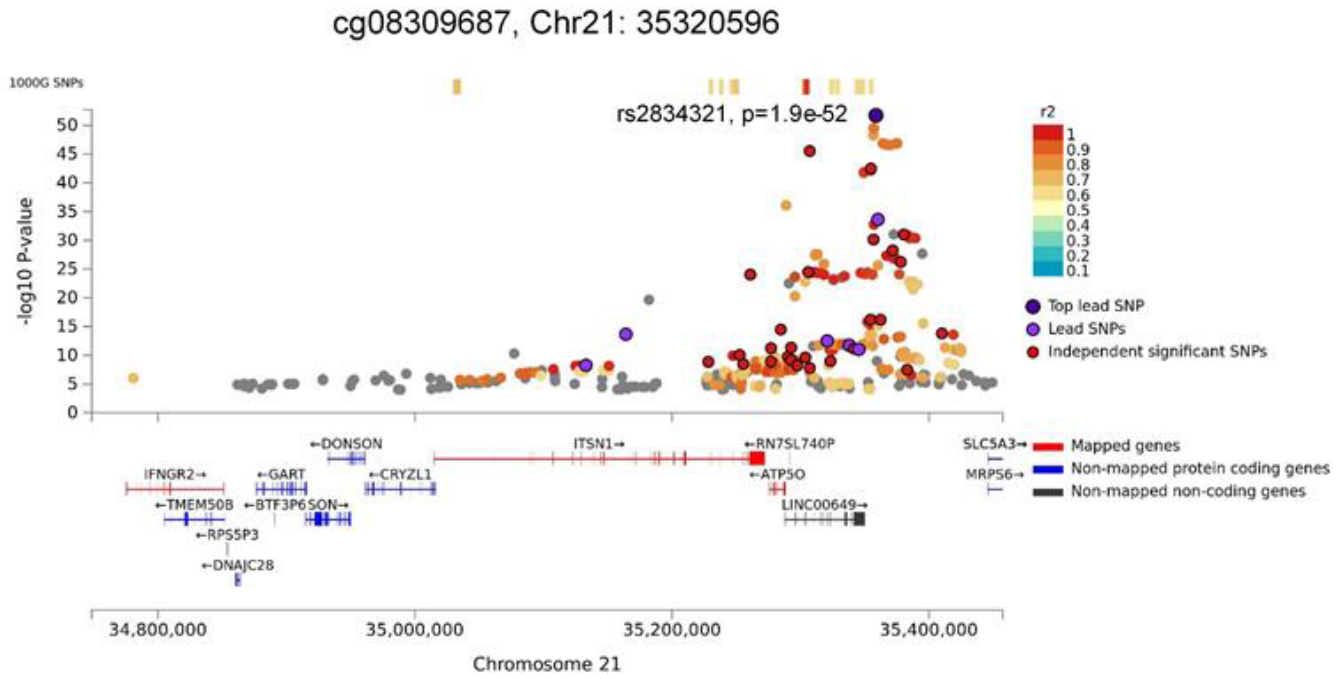
SUPPLEMENTARY DATA

Supplementary Figure S10. Correlation of mean DNA methylation levels for the 22 replicated CpGs in EA participants measured in blood and inliver. CpG=DNA methylation site. EA=European ancestry



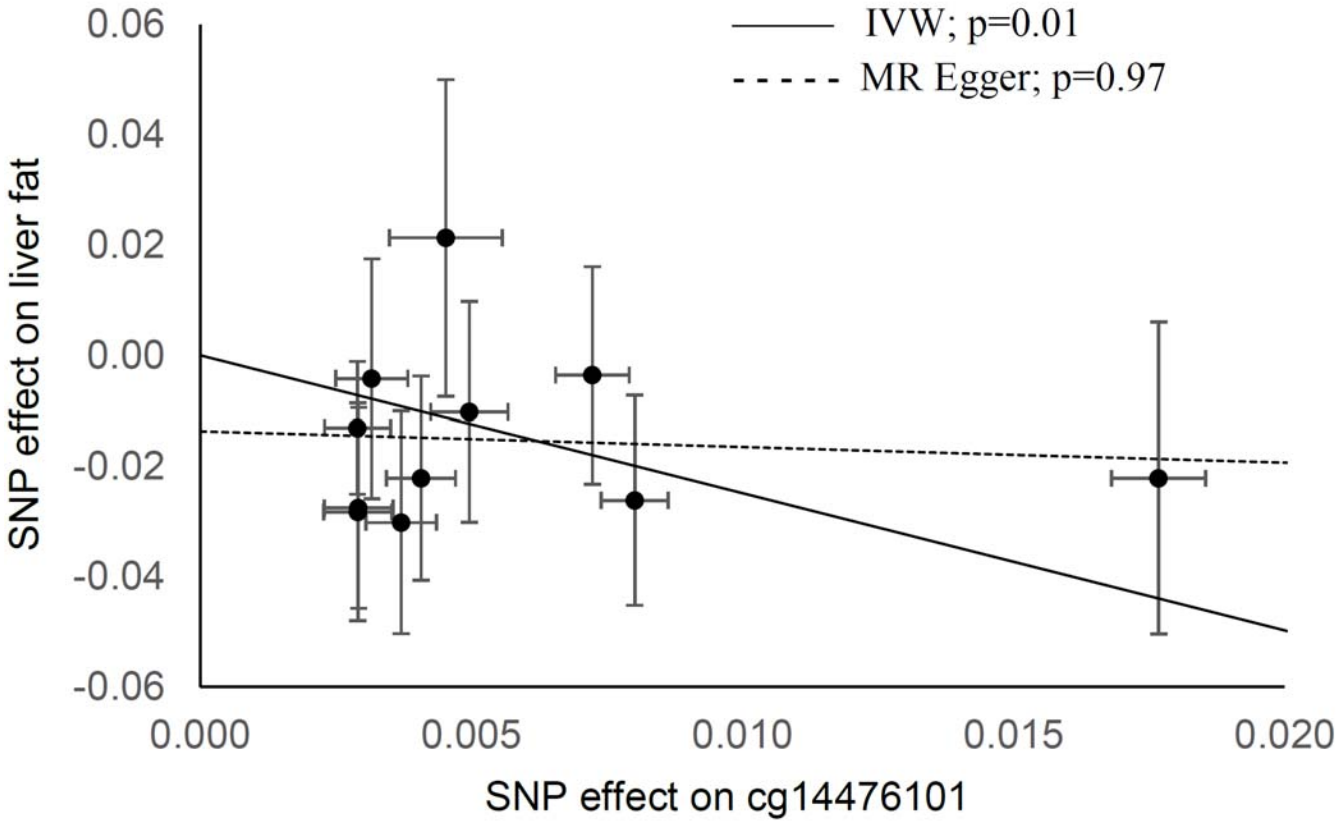
SUPPLEMENTARY DATA

Supplementary Figure S11. Regional plots of *cis*-meQTLs for cg08309687. Plot depicts seven genes (*ATP50*,*ITSN1*, *TMEM50B*, *MRPS6*, *SLC5A3*, and *IFNGR2*) and one long intergenic non-protein coding RNA (LINC00649) for cg08309687. *cis*-meQTLs=*cis*-methylated quantitative trait loci. eQTL=expression quantitative trait loci. FHS=Framingham Heart Study.



SUPPLEMENTARY DATA

Supplementary Figure S12. Potential causal association of cg14476101at *PHGDH* locus to hepatic fat accumulation using MR analyses. P-value-IVW=0.02, P-value-Egger=0.91, and P-value-Egger-intercept=0.27. MR=Mendelian randomization. IVW=inverse variance weighted. SNP=single nucleotide polymorphism. CpG=DNA methylation site.



SUPPLEMENTARY DATA

Supplementary Table S1. Participant characeristics and methods for measurements of DNA methylation and Hepatic fat.

Cohort	N	Age (years)	Women (%)	Race/Ethnicity	BMI (kg/m^2)	Smoking status (%, current/former/none)	Physical activity score	Alcohol (drinks/day)	DNA Methylation		Hepatic Fat		Time interval between liver fat measurement & blood draw (mdian years) min, P20, median, P75, max)	Family Cohort	White blood cell counts
									Tissue source	Array	Assessment	Liver fat proxy			
FHS	1496	58.8 (10.9)	47.90%	EA	28.2 (5.2)	10.3/58.1/31.6	35.9 (6.0)	4.0 (4.8)	Whole blood	Illumina 450K	CT	Ratio of HU of liver to HU to an external calcium control	+3.0	Yes	Estimated using Houseman method
RS	866	65.4 (7.3)	57.00%	EA	27.6 (4.1)	6/61/33	62.7 (0.25 - 430.35)	41% more than 4 times per week	Whole blood	Illumina 450K	Ultrasound	Yes/No	At the same time	No	blood cell counts
MESA	583	70.2 (9.5)	48.37%	EA	28.3 (5.4)	8.7/54.2/37	2504 (2683)	0.65 (1.31)	CD14+ monocytes	Illumina 450K	CT	Average of three mean liver attenuation measures (Hounsfield unit)	-9.3	No	Estimated from Gene expression signatures measured in same samples
	401	68.4 (9.4)	50.37%	HA	30.0 (5.3)	7/49.9/43.1	2833 (3314)	0.29 (1.15)							
	272	70.0 (9.0)	59.93%	AA	30.6 (5.7)	13/45/42	3630 (6614)	0.29 (0.77)							
CARDIA	455	45.9 (3.3)	48.50%	EA	28.1 (6.0)	10.9/24.7/63.3	363.8 (255)	0.6 (0.9)	Whole blood	Illumina 850K	CT		At the same time	No	
	302	44.3 (3.7)	53.90%	AA	30.7 (6.6)	23.4/12.8/63.5	341.9 (309)	0.4 (0.7)							
GENOA	150	71.6 (6.0)	77.30%	AA	31.4 (6.8)	7.3/36/56.7	inactive/active (119/31)	0.35 (1.35)	Whole blood	Illumina 450K	CT	Ratio of HU of liver to HU to an external calcium control (control roc ~ 100mg Ca)	+11.8	Sibships (but unrelated sample for this analysis)	Estimated using Houseman method

Data are mean (SD), unless otherwise indicated. Physical actiivty score is based on cohort-specific definition. Regarding time interval between liver fat measurement and blood draw, a positive sign means liver fat was measured after DNA methylation measurement. EA: European ancestry. HA: Hispanic ancestry. AA: African ancestry. FHS=Framingham Heart Study. RS=Rotterdam Study. MESA=Multi-Ethnic Study of Atherosclerosis study. CARDIA=Coronary Artery Risk Development in Young Adults study. GENOA=Genetic Epidemiology Network of Arteriopathy. BMI=body mass index. CT=computed tomography. HU=Hounsfield units.

SUPPLEMENTARY DATA

Supplementary Table S2. 58 significant CpGs (FDR <0.05) in the discovery cohort (FHS) for hepatic fat using sex- and age-adjusted Model.

	FHS				RS				CARDIA				MESA					
CpG	Beta	SE	P	N	Beta	SE	P	N	Beta	SE	P	N	Beta	SE	P	N	P for meta-analysis	Direction
cg06500161	6.2E-03	7.4E-04	2.0E-16	1496	9.6E-03	2.1E-03	5.6E-06	865	1.1E+01	2.2E+00	1.5E-06	455	2.0E-04	8.6E-05	2.2E-02	583	3.4E-09	+, +, +, +
cg06690548	-1.1E-02	1.3E-03	1.8E-15	1496	-1.1E-03	2.6E-03	6.7E-01	847	-2.6E+00	9.7E-01	8.2E-03	440	-1.0E-03	2.1E-04	1.4E-06	583	5.3E-06	-, -, -, -
cg27243685	4.7E-03	6.4E-04	1.2E-13	1496	5.7E-03	1.5E-03	1.3E-04	866	5.2E+00	1.8E+00	4.0E-03	454	8.5E-05	5.3E-05	1.1E-01	583	1.1E-05	+, +, +, +
cg19693031	-8.0E-03	1.1E-03	1.3E-12	1496	1.7E-03	3.5E-03	6.3E-01	866	-3.9E+00	1.2E+00	1.2E-03	452	-5.4E-04	1.8E-04	2.9E-03	583	3.3E-04	-, +, -, -
cg00574958	-2.6E-03	4.2E-04	3.3E-10	1496	-3.7E-03	1.5E-03	1.2E-02	865	-3.4E+00	7.5E-01	6.2E-06	452	-2.7E-05	3.4E-05	4.3E-01	583	9.9E-06	-, -, -, -
cg02711608	-4.9E-03	8.3E-04	2.8E-09	1496	5.0E-04	2.4E-03	8.3E-01	866	-5.0E+00	1.5E+00	5.8E-04	454	-4.9E-04	1.4E-04	5.3E-04	583	1.2E-04	-, +, -, -
cg21429551	-9.7E-03	1.7E-03	7.3E-09	1496	8.0E-04	4.9E-03	8.7E-01	865	-2.3E+00	6.7E-01	6.7E-04	455	-1.2E-03	3.3E-04	5.8E-04	583	1.9E-04	-, +, -, -
cg19016694	-4.0E-03	6.9E-04	9.5E-09	1496	-1.0E-02	2.6E-03	1.3E-04	865	-4.4E+00	2.4E+00	6.6E-02	454	-3.2E-04	1.4E-04	2.0E-02	583	2.4E-05	-, -, -, -
cg09469355	-4.4E-03	7.9E-04	2.9E-08	1496	-3.8E-03	2.4E-03	1.2E-01	866	-2.4E+00	2.0E+00	2.3E-01	455	-4.2E-04	1.1E-04	1.9E-04	583	4.1E-04	-, -, -, -
cg08305942	-4.6E-03	8.4E-04	3.8E-08	1496	-8.5E-03	2.3E-03	2.2E-04	866	-2.0E+00	1.8E+00	2.7E-01	455	-1.5E-04	1.6E-04	3.7E-01	583	1.7E-03	-, -, -, -
cg09349128	-4.3E-03	7.9E-04	4.8E-08	1496	9.0E-04	2.2E-03	6.9E-01	866	-7.3E+00	1.7E+00	2.8E-05	455	4.6E-05	6.9E-05	5.1E-01	583	2.1E-03	-, +, -, +
cg17901584	-6.2E-03	1.1E-03	4.8E-08	1496	-5.1E-03	3.3E-03	1.2E-01	866	-3.8E+00	1.3E+00	4.6E-03	455	-5.4E-04	1.9E-04	4.8E-03	583	2.1E-04	-, -, -, -
cg05603985	-3.8E-03	7.1E-04	6.3E-08	1496	8.0E-04	1.9E-03	6.7E-01	866	-8.6E-01	1.9E+00	6.5E-01	455	-2.4E-04	8.5E-05	5.8E-03	583	1.1E-01	-, +, -, -
cg14476101	-8.1E-03	1.5E-03	7.5E-08	1496	2.1E-03	4.3E-03	6.3E-01	866	-3.0E+00	1.2E+00	1.1E-02	455	-1.1E-03	2.3E-04	3.4E-06	583	1.1E-05	-, +, -, -
cg17540192	3.7E-03	6.9E-04	8.4E-08	1496	-3.0E-04	2.1E-03	9.0E-01	863	2.5E+00	9.7E-01	9.4E-03	452	6.0E-05	1.4E-04	6.6E-01	583	2.8E-01	+, -, +, +
cg03957124	-4.5E-03	8.5E-04	9.1E-08	1496	-1.0E-02	2.8E-03	3.9E-04	866	-7.4E+00	3.0E+00	1.5E-02	455	-1.7E-04	1.2E-04	1.7E-01	583	9.8E-05	-, -, -, -
cg03068497	-9.8E-03	1.8E-03	1.1E-07	1496	1.0E-03	5.1E-03	8.5E-01	865	-2.4E+00	6.9E-01	5.5E-04	454	-8.7E-04	3.2E-04	6.2E-03	583	8.2E-04	-, +, -, -
cg17501210	-5.8E-03	1.1E-03	1.3E-07	1496	-5.6E-03	2.8E-03	4.5E-02	866	-4.7E+00	1.0E+00	4.4E-06	454	-6.3E-04	2.0E-04	2.0E-03	583	3.4E-07	-, -, -, -
cg20494738	4.9E-03	9.4E-04	1.4E-07	1496	2.9E-03	1.4E-03	3.8E-02	865	2.0E+00	1.1E+00	8.1E-02	452	2.4E-04	9.1E-05	7.8E-03	583	1.1E-03	+, +, +, +
cg03185794	-4.5E-03	8.7E-04	2.2E-07	1496	-2.3E-03	3.0E-03	4.5E-01	866	-4.0E+00	2.0E+00	4.4E-02	454	-2.6E-04	1.5E-04	8.3E-02	583	3.5E-02	-, -, -, -
cg08309687	-6.9E-03	1.3E-03	2.5E-07	1496	-1.7E-02	4.0E-03	2.8E-05	866	-3.0E+00	1.3E+00	1.8E-02	450	-4.4E-04	1.9E-04	2.5E-02	583	3.6E-06	-, -, -, -
cg05973262	4.1E-03	7.9E-04	2.9E-07	1496	4.4E-03	2.5E-03	7.9E-02	866	2.6E+00	1.8E+00	1.6E-01	454	1.8E-04	1.4E-04	2.0E-01	583	4.0E-02	+, +, +, +
cg13876222	4.9E-03	9.5E-04	3.0E-07	1496	8.6E-03	3.1E-03	5.1E-03	866	1.7E+00	6.6E-01	9.9E-03	452	1.7E-05	4.0E-05	6.7E-01	583	3.0E-03	+, +, +, +
cg15860624	5.7E-03	1.1E-03	3.5E-07	1496	8.8E-03	3.2E-03	6.6E-03	865	4.2E+00	1.4E+00	3.4E-03	455	4.1E-04	1.8E-04	2.2E-02	583	5.1E-05	+, +, +, +
cg07626482	-3.9E-03	7.8E-04	4.1E-07	1496	1.3E-03	2.2E-03	5.4E-01	866	-4.6E+00	2.0E+00	2.2E-02	455	-3.0E-04	1.0E-04	3.5E-03	583	2.8E-03	-, +, -, -
cg16246545	-5.7E-03	1.1E-03	4.2E-07	1496	1.5E-03	3.6E-03	6.7E-01	866	-3.6E+00	1.4E+00	7.4E-03	455	-7.1E-04	1.9E-04	2.4E-04	583	2.4E-04	-, +, -, -
cg24000650	4.7E-03	9.5E-04	5.7E-07	1496	4.1E-03	3.0E-03	1.7E-01	865	2.3E+00	1.2E+00	4.5E-02	455	3.6E-04	1.9E-04	5.5E-02	583	1.1E-02	+, +, +, +
cg22103219	-5.2E-03	1.0E-03	5.9E-07	1496	-9.5E-03	3.1E-03	2.0E-03	866	-3.7E+00	1.8E+00	3.5E-02	454	-1.1E-04	1.5E-04	4.8E-01	583	2.2E-03	-, -, -, -
cg19266329	-4.1E-03	8.3E-04	6.2E-07	1496	2.4E-03	2.7E-03	3.7E-01	866	-2.5E+00	1.7E+00	1.3E-01	454	-1.9E-04	1.4E-04	1.8E-01	583	1.0E-01	-, +, -, -
cg11024682	3.7E-03	7.4E-04	6.6E-07	1496	1.1E-02	2.2E-03	6.4E-07	865	3.8E+00	2.2E+00	8.0E-02	455	2.9E-04	1.0E-04	5.2E-03	583	2.7E-07	+, +, +, +
cg23068772	-4.2E-03	8.6E-04	9.2E-07	1496	-6.0E-04	2.2E-03	7.8E-01	865	-4.3E-01	1.6E+00	7.9E-01	454	-4.8E-04	1.3E-04	3.5E-04	583	4.2E-02	-, -, -, -
cg23032421	-4.5E-03	9.3E-04	9.3E-07	1496	-1.1E-02	2.7E-03	6.8E-05	866	-1.9E-01	1.5E+00	9.0E-01	455	-1.1E-05	7.6E-05	8.8E-01	583	4.2E-02	-, -, -, -
cg03147185	-3.5E-03	7.2E-04	1.1E-06	1496	-3.2E-03	2.3E-03	1.6E-01	865	-3.0E+00	2.8E+00	2.7E-01	454	-1.7E-05	9.2E-05	8.5E-01	583	3.8E-01	-, -, -, -
cg07021906	4.9E-03	1.0E-03	1.1E-06	1496	6.9E-03	3.1E-03	2.4E-02	865	2.9E+00	1.3E+00	1.9E-02	454	2.7E-04	1.7E-04	1.2E-01	583	2.2E-03	+, +, +, +
cg26725076	-4.4E-03	9.1E-04	1.1E-06	1496	2.5E-03	2.4E-03	3.0E-01	865	-4.5E+00	1.8E+00	1.3E-02	454	2.8E-05	1.4E-04	8.4E-01	583	1.2E-01	-, +, -, +
cg27037013	-6.4E-03	1.3E-03	1.1E-06	1496	-1.4E-02	4.1E-03	1.1E-03	866	-2.4E-01	9.2E-01	7.9E-01	455	-2.6E-04	1.4E-04	6.5E-02	583	6.4E-03	-, -, -, -
cg25281677	-4.6E-03	9.4E-04	1.2E-06	1496	-3.0E-03	2.4E-03	2.2E-01	866	1.1E+00	1.8E+00	5.2E-01	449	-2.3E-04	1.1E-04	3.5E-02	583	7.4E-02	-, -, +, -
cg03725309	-3.7E-03	7.6E-04	1.4E-06	1496	-1.6E-03	2.8E-03	5.7E-01	865	-2.9E+00	1.0E+00	3.8E-03	455	-5.1E-04	1.0E-04	6.0E-07	583	1.3E-06	-, -, -, -
cg19939077	-3.8E-03	7.8E-04	1.6E-06	1496	0.0E+00	2.1E-03	1.0E+00	865	-1.0E+00	1.5E+00	5.0E-01	454	-2.2E-05	1.1E-04	8.4E-01	583	9.8E-01	-, -, -, -
cg02504211	3.6E-03	7.6E-04	1.6E-06	1496	4.6E-03	2.3E-03	5.1E-02	863	7.6E-01	1.3E+00	5.4E-01	453	1.5E-04	1.2E-04	2.0E-01	583	9.1E-02	+, +, +, +
cg14020176	3.2E-03	6.8E-04	2.4E-06	1496	8.4E-03	2.3E-03	3.2E-04	866	2.0E+00	1.6E+00	1.9E-01	450	1.6E-04	1.2E-04	1.9E-01	583	8.0E-04	+, +, +, +
cg12593793	-3.9E-03	8.4E-04	2.6E-06	1496	-5.0E-03	2.4E-03	3.4E-02	866	-5.0E-01	1.0E+00	6.3E-01	454	-1.7E-04	9.6E-05	7.5E-02	583	4.5E-02	-, -, -, -
cg02081905	1.7E-03	3.5E-04	2.8E-06	1496	6.0E-04	8.0E-04	4.5E-01	863	9.9E-01	1.6E+00	5.3E-01	450	2.1E-05	3.8E-05	5.9E-01	583	5.4E-01	+, +, +, +
cg19677267	-7.0E-03	1.5E-03	2.8E-06	1496	-2.5E-03	4.6E-03	5.9E-01	866	-2.6E-01	7.6E-01	7.3E-01	455	-2.8E-04	2.5E-04	2.7E-01	583	5.5E-01	-, -, -, -
cg13795986	-5.0E-03	1.1E-03	3.0E-06	1496	-1.0E-04	3.1E-03	9.7E-01	865	-3.5E+00	1.3E+00	7.3E-03	449	1.5E-04	1.9E-04	4.1E-01	583	2.9E-01	-, -, -, +
cg23205886	-3.8E-03	8.2E-04	3.2E-06	1496	9.0E-04	2.7E-03	7.5E-01	866	-1.4E+00	2.1E+00	4.9E-01	455	-2.0E-04	1.7E-04	2.6E-01	583	5.0E-01	-, +, -, -
cg18120259	-3.9E-03	8.4E-04	3.4E-06	1496	4.2E-03	2.6E-03	1.1E-01	866	-4.9E+00	2.0E+00	1.3E-02	455	-4.2E-04	1.4E-04	3.2E-03	583	3.1E-04	-, +, -, -
cg24678869	2.5E-03	5.4E-04	3.4E-06	1496	3.6E-03	1.6E-03	2.4E-02	865	1.7E+00	2.8E+00	5.4E-01	455	8.9E-05	8.8E-05	3.1E-01	583	7.9E-02	+, +, +, +
cg05418719	-4.0E-03	8.7E-04	3.5E-06	1496	-2.5E-03	2.7E-03	3.6E-01	865	-3.7E+00	2.1E+00	7.8E-02	453	3.4E-05	1.2E-04	7.8E-01	583	2.8E-01	-, -, -, +
cg24691964	-3.7E-03	8.0E-04	3.8E-06	1496	-5.3E-03	2.0E-03	7.7E-03	866	-2.4E-01	1.7E+00	8.9E-01	451	-2.9E-05	1.0E-04	7.7E-01	583	3.0E-01	-, -, -, -
cg02203067	3.4E-03	7.3E-04	4.1E-06	1496	5.7E-03	2.4E-03	1.6E-02	865	3.1E+00	2.0E+00	1.2E-01	454	2.0E-04	1.4E-04	1.5E-01	583	8.1E-03	+, +, +, +
cg02640489	-2.9E-03	6.3E-04	4.3E-06	1496	-2.3E-03	2.9E-03	4.2E-01	866	1.1E+00	2.0E+00	5.8E-01	452	-1.6E-04	1.2E-04	1.8E-01	583	3.0E-01	-, -, +, -
cg11376147	-3.1E-03	6.8E-04	4.9E-06	1496	6.0E-04	1.9E-03	7.6E-01	866	-3.7E+00	1.4E+00	8.7E-03	449	-5.2E-04	1.2E-04	3.7E-05	583	8.8E-05	-, +, -, -
cg18819791	-5.1E-03	1.1E-03	5.6E-06	1496	5.0E-04	2.7E-03	8.5E-01	866	-2.7E+00	1.2E+00	2.7E-02	455	-2.7E-04	1.2E-04	2.2E-02	583	3.7E-02	-, +, -, -
cg26894079	-4.9E-03	1.1E-03	5.6E-06	1496	-7.7E-03	2.8E-03	6.8E-03	864	-3.4E+00	1.5E+00	2.7E-02	453	-2.4E-04	1.4E-04	9.5E-02	583	8.6E-04	-, -, -, -
cg22876908	-3.4E-03	7.6E-04	6.4E-06	1496	2.4E-03	2.2E-03	2.8E-01	866	-2.0E+00	2.0E+00	3.2E-01	454	-1.3E-04	1.1E-04	2.6E-01	583	1.9E-01	-, +, -, -
cg11969813	5.0E-03	1.1E-03	6.8E-06	1496	9.1E-03	3.4E-03	7.1E-03	866	1.6E+00	1.3E+00	2.3E-01	455	-2.4E-05	4.0E-05	5.5E-01	583	3.0E-02	+, +, +, -
cg05119988	-5.5E-03	1.2E-03	6.9E-06	1496	-6.7E-03	3.1E-03	3.0E-02	865	-3.5E+00	1.2E+00	3.2E-03	454	-3.1E-04	1.2E-04	8.5E-03	583	7.8E-05	-, -, -, -

Meta-analysis p-values were calculated in RS, CARDIA, and MESA using logit method based on the general fixed effect model. The direction column showed the sign of regerssion coefficients in order of FHS, RS, CARDIA, and MESA. FHS=Framingham Heart Study. RS=Rotterdam Study. MESA=Multi-Ethnic Study of Atherosclerosis study. CARDIA=Coronary Artery Risk Development in Young Adults study. CpG=DNA methylation site. CHR=chromosome. SE=standard error. FDR=false discovery rate

SUPPLEMENTARY DATA

Supplementary Table S3. Pairwise-Pearson correlation of 24 CpGs replicated in the European-ancestry participants

CpG	CHR	Position	cg09469355	cg17901584	cg03725309	cg16246545	cg14476101	cg19693031	cg06690548	cg05119988	cg03957124	cg18120259	cg17501210	cg21429551	cg03068497	cg11376147	cg00574958	cg26894079	cg11024682	cg14020176	cg19016694	cg15860624	cg02711608	cg08309687	cg27243685	cg06500161
cg09469355	1	2161886	1.00																							
cg17901584	1	55353706	0.09	1.00																						
cg03725309	1	109757585	0.25	0.50	1.00																					
cg16246545	1	120255941	0.26	0.28	0.37	1.00																				
cg14476101	1	120255992	0.21	0.31	0.42	0.89	1.00																			
cg19693031	1	145441552	0.30	0.21	0.21	0.26	0.28	1.00																		
cg06690548	4	139162808	0.24	0.19	0.36	0.48	0.57	0.33	1.00																	
cg05119988	4	166251189	0.31	0.10	0.07	0.13	0.02	0.17	0.12	1.00																
cg03957124	6	37016869	0.36	-0.39	-0.16	0.01	-0.14	0.07	-0.01	0.49	1.00															
cg18120259	6	43894639	0.16	0.61	0.54	0.41	0.54	0.30	0.47	-0.13	-0.46	1.00														
cg17501210	6	166970252	0.27	0.35	0.38	0.30	0.40	0.17	0.30	-0.04	-0.17	0.47	1.00													
cg21429551	7	30635762	0.21	0.17	0.44	0.44	0.43	0.15	0.41	0.23	0.15	0.22	0.16	1.00												
cg03068497	7	30635838	0.22	0.27	0.49	0.45	0.44	0.18	0.40	0.23	0.11	0.28	0.17	0.90	1.00											
cg11376147	11	57261198	0.16	0.37	0.43	0.21	0.13	0.21	0.24	0.27	0.11	0.20	-0.09	0.27	0.34	1.00										
cg00574958	11	68607622	0.17	0.36	0.41	0.27	0.27	0.32	0.25	0.06	-0.03	0.32	0.23	0.22	0.29	0.31	1.00									
cg26894079	11	122954435	0.34	0.33	0.43	0.19	0.10	0.19	0.12	0.29	0.17	0.26	0.19	0.23	0.29	0.45	0.28	1.00								
cg11024682	17	17730094	0.01	0.31	0.02	0.12	0.10	0.09	-0.05	-0.08	-0.25	0.29	0.09	-0.07	-0.01	0.01	-0.01	0.11	1.00							
cg14020176	17	72764985	0.06	0.49	0.38	0.26	0.36	0.12	0.19	-0.21	-0.55	0.62	0.36	0.07	0.15	0.07	0.17	0.10	0.40	1.00						
cg19016694	17	80821826	0.35	-0.36	-0.15	0.06	-0.04	0.13	0.04	0.41	0.75	-0.36	-0.04	0.14	0.10	-0.06	0.02	0.13	-0.21	-0.42	1.00					
cg15860624	19	3811194	0.01	0.32	0.21	0.06	0.15	0.08	-0.01	-0.14	-0.28	0.38	0.21	0.00	0.04	-0.01	0.09	0.08	0.30	0.35	-0.24	1.00				
cg02711608	19	47287964	0.30	0.46	0.60	0.45	0.41	0.23	0.41	0.22	0.07	0.43	0.22	0.47	0.53	0.59	0.36	0.43	0.06	0.26	-0.02	0.11	1.00			
cg08309687	21	35320596	0.30	-0.19	-0.04	0.02	-0.09	0.19	0.07	0.39	0.58	-0.27	-0.03	0.08	0.07	0.18	0.10	0.28	-0.24	-0.40	0.56	-0.23	0.08	1.00		
cg27243685	21	43642366	-0.09	0.11	0.05	0.05	0.15	0.00	-0.07	-0.31	-0.36	0.29	0.15	-0.07	-0.04	-0.23	-0.06	-0.11	0.40	0.34	-0.25	0.36	-0.12	-0.29	1.00	
cg06500161	21	43656587	0.03	0.03	0.05	0.02	0.02	0.01	-0.15	-0.08	0.00	0.13	-0.01	0.00	0.06	0.02	-0.06	0.04	0.40	0.18	-0.01	0.33	0.05	-0.09	0.54	1.00

Pearson correlation coefficients were calculated using data in the Framingham Heart Study.

SUPPLEMENTARY DATA

Supplementary Table S4. Sensitivity analysis in EA participants with additional adjustment for lifestyle factors and BMI.

	Discovery				Replication												
	FHS				RS				CARDIA-EA				MESA-EA				P for meta-analysis in replication cohorts
IlmnID	Beta	SE	P	N	Beta	SE	P	N	Beta	SE	P	N	Beta	SE	P	N	
cg06690548	-8.3E-03	1.4E-03	2.6E-09	1496	1.0E-04	2.8E-03	9.7E-01	847	-3.0E+00	1.0E+00	3.6E-03	386	-9.2E-04	2.2E-04	4.0E-05	580	0.004
cg19693031	-6.5E-03	1.2E-03	1.2E-07	1496	1.2E-03	3.9E-03	7.7E-01	866	-1.5E+00	9.2E-01	1.1E-01	376	-4.4E-04	1.9E-04	2.1E-02	580	0.006

Model adjusted for sex, age, smoking, physical activity, alcohol, and BMI. Discovery significance p-value 1.2E-07 (FDR < 0.05); replication significance p-value < 0.025. Meta-analysis p-values were calculated in RS, CARDIA, and MESA using meta-analysis of p-values with logit method based on the general fixed effect model. FHS=Framingham Heart Study. RS=Rotterdam Study. MESA=Multi-Ethnic Study of Atherosclerosis study. CARDIA=Coronary Artery Risk Development in Young Adults study. EA=European ancestry. CpG=DNA methylation site. SE=standard error.

SUPPLEMENTARY DATA

Supplementary Table S5. Mediation analysis by fasting serum triglycerides levels for the association of DNA methylation (CpGs) and hepatic fat

	Triglycerides			
CpG	Mediation	95% CI		P
cg06500161	39%	27%	50%	2.00E-16
cg21429551	15%	6%	27%	2.00E-16
cg19016694	14%	6%	25%	2.00E-16
cg09469355	19%	9%	32%	2.00E-16
cg17901584	29%	17%	50%	2.00E-16
cg14476101	30%	20%	50%	2.00E-16
cg08309687	17%	9%	31%	2.00E-16
cg18120259	23%	12%	42%	2.00E-16
cg05119988	19%	9%	35%	2.00E-16
cg27243685	38%	19%	46%	0.002
cg03957124	13%	5%	24%	0.002
cg11024682	29%	15%	68%	0.002
cg14020176	17%	5%	31%	0.01
cg17501210	13%	2%	29%	0.02
cg19693031	22%	3%	38%	0.03
cg02711608	30%	5%	42%	0.03
cg00574958	47%	11%	93%	0.04
cg26894079	6%	-2%	23%	0.09
cg06690548	20%	-14%	36%	0.14
cg15860624	7%	-3%	20%	0.15
cg11376147	26%	-52%	47%	0.17
cg03725309	3%	-18%	12%	0.75

SUPPLEMENTARY DATA

Supplementary Table S6. Replication of the 22 signficant CpGs identified in the EA participants in the MESA HA cohort (N=401).

CpG	Beta	SE	P
cg19693031	-8.7E-04	2.3E-04	1.7E-04
cg03957124	-4.1E-04	1.4E-04	2.8E-03
cg06500161	2.9E-04	9.8E-05	3.0E-03
cg17901584	-6.1E-04	2.2E-04	6.3E-03
cg19016694	-4.1E-04	1.7E-04	1.6E-02
cg21429551	-6.1E-04	3.4E-04	7.4E-02
cg14020176	2.0E-04	1.4E-04	1.5E-01
cg26894079	-2.6E-04	1.8E-04	1.5E-01
cg00574958	-6.0E-05	4.4E-05	1.7E-01
cg27243685	8.7E-05	6.3E-05	1.7E-01
cg11024682	1.9E-04	1.4E-04	1.8E-01
cg08309687	-3.3E-04	2.5E-04	1.8E-01
cg03725309	-1.5E-04	1.3E-04	2.6E-01
cg06690548	-1.3E-04	1.2E-04	2.9E-01
cg18120259	-1.8E-04	1.7E-04	2.9E-01
cg05119988	-9.6E-05	1.3E-04	4.6E-01
cg15860624	1.3E-04	2.3E-04	5.8E-01
cg09469355	-4.7E-05	1.3E-04	7.1E-01
cg14476101	-8.3E-05	2.5E-04	7.4E-01
cg11376147	-3.8E-05	1.2E-04	7.4E-01
cg17501210	-6.0E-05	2.7E-04	8.3E-01
cg02711608	5.5E-06	1.6E-04	9.7E-01

Model adjusted for sex and age. Bonferroni-corrected p-value threshold is 0.002 (0.05/22 CpGs). MESA=Multi-Ethnic Study of Atherosclerosis study. HA=Hispanic ancestry. EA=European ancestry. CpG=DNA methylation site. SE=standard error.

SUPPLEMENTARY DATA

Supplementary Table S7. Replication of the 22 signficant CpGs identified in the EA participants in AA participants in the MESA, CARDIA, and GENOA cohorts (N=721).

	Model 1												
	GENOA				MESA				CARDIA				
CpG	Beta	SE	P	N	Beta	SE	P	N	Beta	SE	P	N	Meta-analysis P
cg14476101	1.4E-02	5.7E-02	8.1E-01	150	-6.0E-04	2.8E-04	3.6E-02	272	-3.7E+00	1.3E+00	3.5E-03	304	1.1E-02
cg00574958	-1.4E-02	2.0E-02	4.9E-01	150	-1.1E-04	4.9E-05	2.5E-02	272	-1.9E+00	9.0E-01	3.4E-02	296	1.4E-02
cg08309687	5.4E-02	5.1E-02	2.9E-01	150	-6.5E-04	2.6E-04	1.4E-02	272	-1.2E+00	1.1E+00	2.7E-01	303	2.7E-02
cg27243685	2.9E-02	1.4E-02	3.7E-02	150	2.8E-05	6.8E-05	6.9E-01	272	3.2E+00	1.8E+00	6.9E-02	299	5.2E-02
cg21429551	-9.5E-02	5.6E-02	9.5E-02	150	-5.8E-04	4.0E-04	1.4E-01	272	-2.6E-01	8.0E-01	7.5E-01	303	1.7E-01
cg03725309	-5.6E-03	2.3E-02	8.1E-01	150	-1.9E-04	1.4E-04	1.7E-01	272	-1.9E+00	1.0E+00	6.5E-02	302	1.8E-01
cg17901584	6.6E-02	4.5E-02	1.5E-01	150	-2.6E-04	2.9E-04	3.6E-01	272	-7.9E-01	9.6E-01	4.1E-01	302	1.9E-01
cg26894079	7.7E-03	4.1E-02	8.5E-01	150	-9.0E-05	1.8E-04	6.2E-01	272	-4.1E+00	1.5E+00	7.7E-03	302	1.9E-01
cg17501210	-1.2E-01	7.0E-02	8.6E-02	150	-3.9E-04	3.8E-04	3.1E-01	272	-3.3E-01	8.1E-01	6.9E-01	303	2.2E-01
cg06690548	-1.9E-02	2.0E-02	3.4E-01	150	-1.5E-04	1.2E-04	2.3E-01	272	-8.6E-01	1.1E+00	4.2E-01	301	2.4E-01
cg06500161	-2.5E-02	3.2E-02	4.4E-01	150	2.3E-04	1.3E-04	8.1E-02	272	8.3E-01	2.2E+00	7.1E-01	304	2.8E-01
cg19693031	-2.5E-02	4.5E-02	5.8E-01	150	-7.5E-04	2.9E-04	1.0E-02	272	1.1E-01	1.1E+00	9.2E-01	304	2.8E-01
cg03957124	-3.2E-02	3.0E-02	2.9E-01	150	4.6E-05	1.7E-04	7.9E-01	272	-3.5E+00	2.3E+00	1.3E-01	304	3.1E-01
cg14020176	1.5E-02	2.3E-02	5.3E-01	150	-3.4E-05	1.7E-04	8.4E-01	272	-2.4E+00	1.4E+00	8.2E-02	301	4.1E-01
cg15860624	6.6E-02	5.1E-02	2.0E-01	150	7.5E-05	2.9E-04	8.0E-01	272	-8.9E-01	1.1E+00	4.2E-01	304	4.5E-01
cg09469355	6.5E-04	3.8E-02	9.9E-01	150	-1.9E-04	1.5E-04	2.1E-01	272	-3.6E+00	1.9E+00	6.0E-02	303	5.3E-01
cg11376147	5.2E-04	1.6E-02	9.7E-01	150	-2.0E-04	1.3E-04	1.3E-01	272	-1.7E+00	1.3E+00	2.1E-01	302	5.6E-01
cg18120259	-2.8E-02	3.3E-02	4.0E-01	150	-2.3E-05	2.0E-04	9.1E-01	272	-2.6E+00	2.9E+00	3.8E-01	301	6.8E-01
cg02711608	5.3E-03	4.0E-02	9.0E-01	150	-2.7E-04	2.0E-04	1.8E-01	272	-2.7E-01	7.9E-01	7.4E-01	302	7.0E-01
cg05119988	8.2E-03	3.9E-02	8.4E-01	150	-8.3E-05	1.6E-04	6.1E-01	272	-1.2E+00	1.4E+00	4.1E-01	304	7.1E-01
cg19016694	-1.0E-02	3.5E-02	7.6E-01	150	-5.8E-05	2.1E-04	7.8E-01	272	6.5E-01	1.8E+00	7.1E-01	303	8.6E-01
cg11024682	-4.2E-04	3.4E-02	9.9E-01	150	-1.3E-06	1.6E-04	9.9E-01	272	3.8E+00	2.1E+00	6.7E-02	304	9.8E-01

Model adjusted for sex and age. Bonferroni-corrected p-value threshold is 0.002 (0.05/22 CpGs). Meta-analysis p-value was calculated using meta-analysis of p-values with logit method based on the general fixed effect model. MESA=Multi-Ethnic Study of Atherosclerosis study. CARDIA=Coronary Artery Risk Development in Young Adults study. GENOA=Genetic Epidemiology Network of Arteriopathy. EA=European ancestry. AA=African ancestry. CpG=DNA methylation site. SE=standard error.

SUPPLEMENTARY DATA

Supplementary Table S8. 26 CpGs identified (FDR <0.05) in AA participants in the MESA, CARDIA, and GENOA cohorts.

CpG	CHR	Position	Gene	GENOA				MESA				CARDIA				Meta-analysis P	Global meta-analysis in EA population
				Beta	SE	P	N	Beta	SE	P	N	Beta	SE	P	N		
cg00920337	13	52392151	FLJ37307	-4.6E-03	7.9E-03	5.6E-01	150	-7.4E-04	8.6E-05	5.1E-16	272	1.6E-01	1.9E+00	9.3E-01	297	6.6E-10	2.5E-02
cg01077808	19	16607011	C19orf44;CALR3	8.4E-03	9.4E-03	3.7E-01	149	4.4E-04	5.9E-05	1.3E-12	272	3.0E+00	2.4E+00	2.1E-01	295	3.5E-09	2.3E-01
cg15983528	8	1200525		3.3E-03	7.4E-03	6.6E-01	150	-7.1E-04	9.1E-05	1.4E-13	272	-1.2E+00	1.8E+00	4.9E-01	300	4.0E-09	3.9E-01
cg00395894	19	17881640	FCHO1	-1.5E-02	7.1E-03	4.1E-02	150	-6.6E-04	1.0E-04	2.9E-10	272	-3.9E+00	2.0E+00	4.9E-02	295	6.6E-09	7.2E-01
cg15534482	14	78324874	ADCK1	-5.1E-03	1.2E-02	6.7E-01	150	-7.1E-04	9.1E-05	1.1E-13	272	3.8E-01	2.7E+00	8.9E-01	292	1.2E-08	6.0E-01
cg07254987	12	117697516	NOS1	-7.9E-03	6.4E-03	2.2E-01	150	-4.5E-04	6.6E-05	6.3E-11	272	-2.0E+00	1.9E+00	3.1E-01	299	2.8E-08	5.4E-01
cg04350355	22	36962768	CACNG2	-4.2E-02	5.3E-02	4.2E-01	150	-8.4E-04	1.4E-04	5.9E-09	272	-4.9E+00	1.6E+00	1.8E-03	301	2.8E-08	8.6E-01
cg04776860	7	43177046	HECW1	2.3E-02	3.1E-02	4.6E-01	150	-6.8E-04	9.5E-05	1.2E-11	272	-1.3E+00	1.8E+00	4.8E-01	303	3.1E-08	2.6E-01
cg17016145	6	141520754		4.5E-03	2.9E-02	8.8E-01	150	-6.2E-04	8.7E-05	9.3E-12	272	1.3E+00	1.9E+00	5.1E-01	295	1.1E-07	4.5E-01
cg14696064	3	123010055	ADCY5	-7.9E-03	1.8E-02	6.7E-01	150	-5.3E-04	7.9E-05	7.5E-11	272	-1.4E+00	1.5E+00	3.4E-01	299	1.1E-07	8.4E-01
cg06358636	6	133480830		-5.8E-03	1.1E-02	5.9E-01	150	-4.9E-04	7.3E-05	1.9E-10	272	3.1E+00	2.8E+00	2.7E-01	300	1.4E-07	9.7E-01
cg12325049	3	9989666	PRRT3	-3.0E-02	2.2E-02	1.7E-01	150	-4.1E-04	7.2E-05	2.6E-08	272	-3.0E+00	1.4E+00	3.4E-02	283	2.0E-07	6.3E-01
cg22798384	13	114889589	RASA3	-1.4E-02	1.5E-02	3.6E-01	150	-6.2E-04	9.2E-05	1.6E-10	272	6.7E-01	1.7E+00	6.9E-01	300	2.0E-07	3.7E-02
cg27590143	7	51175394	COBL	-4.1E-02	3.3E-02	2.2E-01	150	-9.0E-04	1.4E-04	7.5E-10	272	8.7E-01	1.9E+00	6.5E-01	297	3.2E-07	8.7E-01
cg00334863	22	42779093	NFAM1	6.5E-03	1.9E-02	7.3E-01	150	-8.2E-04	1.4E-04	4.0E-09	272	-3.3E+00	1.8E+00	5.6E-02	295	4.5E-07	2.2E-01
cg13808936	9	136371164		-1.4E-02	1.1E-02	2.0E-01	150	-6.8E-04	1.1E-04	3.2E-09	272	-1.1E+00	1.4E+00	4.6E-01	292	4.7E-07	2.5E-01
cg11626582	10	3761802		-7.9E-03	1.5E-02	6.1E-01	150	-6.6E-04	1.0E-04	9.5E-10	272	1.4E+00	1.6E+00	3.7E-01	301	5.5E-07	3.8E-01
cg13819687	9	35404785	UNC13B	1.7E-02	1.8E-02	3.5E-01	150	-8.9E-04	1.4E-04	6.0E-10	272	-4.8E-01	2.0E+00	8.1E-01	303	7.6E-07	1.6E-01
cg10116904	1	190068092	FAM5C	1.2E-02	1.7E-02	4.9E-01	150	-5.7E-04	1.0E-04	3.6E-08	272	-4.4E+00	2.4E+00	7.1E-02	302	1.2E-06	8.4E-01
cg15775406	7	1333612		2.2E-02	1.9E-02	2.5E-01	150	-5.4E-04	9.2E-05	1.1E-08	272	1.6E+00	2.0E+00	4.3E-01	297	1.2E-06	6.4E-01
cg22201573	4	2278303	ZFYVE28	-5.7E-02	5.1E-02	2.6E-01	150	-1.1E-03	1.8E-04	1.9E-09	272	-1.9E-01	1.4E+00	8.9E-01	299	2.0E-06	8.6E-01
cg02196671	14	104931470		5.6E-02	4.8E-02	2.5E-01	150	-3.7E-04	6.0E-05	2.3E-09	272	2.0E-01	1.7E+00	9.1E-01	303	2.4E-06	5.6E-01
cg12635044	2	235406891	ARL4C	-2.1E-02	2.6E-02	4.2E-01	150	-8.3E-04	1.5E-04	5.3E-08	272	2.3E+00	1.7E+00	1.7E-01	295	2.4E-06	4.5E-01
cg08728732	10	105362705	SH3PXD2A	-1.0E-02	9.9E-03	3.0E-01	150	-5.7E-04	1.0E-04	5.0E-08	272	2.4E+00	2.1E+00	2.7E-01	296	2.5E-06	6.5E-01
cg25709679	15	99206764	IGF1R	-8.2E-03	3.2E-02	8.0E-01	150	-8.5E-04	1.3E-04	3.4E-10	272	4.8E-01	2.7E+00	8.6E-01	285	2.6E-06	9.9E-01
cg19140602	10	28233109	ARMC4	1.3E-02	3.5E-02	7.2E-01	150	-5.7E-04	9.2E-05	2.0E-09	272	-7.1E-01	1.5E+00	6.4E-01	303	2.7E-06	8.7E-01

Model adjusted for sex and age. Meta-analysis in AA participants using p-values with logit method based on the general fixed effect model. The corresponding p-value for false positive rate (FDR) <0.05 was 2.7E-06 in meta-analysis in AA particiants. Bonferroni corrected p-value threshold in lookup in global meta-analysis in EA participants was 0.05/26 (0.002). MESA=Multi-Ethnic Study of Atherosclerosis study. CARDIA=Coronary Artery Risk Development in Young Adults study. GENOA=Genetic Epidemiology Network of Arteriopathy. EA=European ancestry. AA=African ancestry. CpG=DNA methylation site. CHR=chromosome. SE=standard error.

SUPPLEMENTARY DATA

Supplementary Table S9. Functional description for the 18 Illumina annotated genes.

Annotated gene	CHR	Full Name	Gene type	Description
ABCG1	21	ATP binding cassette subfamily G member 1	protein coding	The protein this gene encodes is involved in macrophage cholesterol and phospholipids transport; may regulate cellular lipid homeostasis
ASAM	11	CXADR like membrane protein	protein coding	Expression of this gene in white adipose tissue is implicated in adipocyte maturation and development of obesity.
CPT1A	11	carnitine palmitoyltransferase 1A	protein coding	CPT I is the key enzyme in the carnitine-dependent transport across the mitochondrial inner membrane and the rate of fatty acid beta-oxidation.
DHCR24	1	24-dehydrocholesterol reductase	protein coding	This gene encodes a flavin adenine dinucleotide-dependent oxidoreductase which is involved in cholesterol biosynthesis.
GARS	7	glycyl-tRNA synthetase	protein coding	This gene encodes glycyl-tRNA synthetase, one of the aminoacyl-tRNA synthetases that charge tRNAs with their cognate amino acids.
PHGDH	1	phosphoglycerate dehydrogenase	protein coding	This gene encodes the enzyme which is involved in the early steps of L-serine synthesis in animal cells and other amino acid synthesis.
RPS6KA2	6	ribosomal protein S6 kinase A2	protein coding	This gene encodes a member of the ribosomal S6 kinase family of serine/threonine kinases, which has been implicated in controlling cell growth and differentiation.
SARS	1	seryl-tRNA synthetase	protein coding	This gene belongs to the class II amino-acyl tRNA family. The encoded enzyme catalyzes the transfer of L-serine to tRNA (Ser) and is related to bacterial and yeast counterparts.
SC4MOL	4	methylsterol monooxygenase 1	protein coding	Sterol-C4-mehtyl oxidase-like protein is localized to the endoplasmic reticulum membrane and is believed to function in cholesterol biosynthesis.
SKI	1	SKI proto-oncogene	protein coding	This gene encodes the nuclear protooncogene protein homolog of avian sarcoma viral (v-ski) oncogene. It functions as a repressor of TGF-beta signaling, and may play a role in neural tube development and muscle differentiation
SLC1A5	19	solute carrier family 1 member 5	protein coding	The SLC1A5 gene encodes a sodium-dependent neutral amino acid transporter that can act as a receptor for RD114/type D retrovirus
SLC43A1	11	solute carrier family 43 member 1	protein coding	SLC43A1 belongs to the system L family of plasma membrane carrier proteins that transports large neutral amino acids
SLC7A11	4	solute carrier family 7 member 11	protein coding	This gene encodes a member of a heteromeric, sodium-independent, anionic amino acid transport system that is highly specific for cysteine and glutamate.
SLC9A3R1	17	SLC9A3 regulator 1	protein coding	This gene encodes a sodium/hydrogen exchanger regulatory cofactor.
SREBF1	17	sterol regulatory element binding transcription factor 1	protein coding	This gene encodes a basic helix-loop-helix-leucine zipper transcription factor that binds to the sterol regulatory element-1, which is a motif that is found in the promoter of the low density lipoprotein receptor gene and other genes involved in sterol biosynthesis.
TBCD	17	tubulin folding cofactor D	protein coding	Cofactor D is one of four proteins involved in the pathway leading to correctly folded beta-tubulin from folding intermediates. Cofactor D is believed to play a role in capturing and stabilizing beta-tubulin intermediates in a quasi-native confirmation.
TXNIP	1	thioredoxin interacting protein	protein coding	This gene encodes a thioredoxin-binding protein that is a member of the alpha arrestin protein family. Thioredoxin is a thiol-oxidoreductase that functions as a regulator of cellular redox signaling, cellular metabolism, endoplasmic reticulum stress, and tumor suppression
ZFR2	19	zinc finger RNA binding protein 2	protein coding	unknown

Gene information is taken from NCBI Genes. CHR=chromosome.

SUPPLEMENTARY DATA

Supplementary Table S10. Liver-specific differentially expressed genes in GTEx v6.

Category	Tissue	Number of DEG	Number of input genes overlapping with DEG	Upper tail p-value	Bonferroni corrected p-value	Genes
Up-regulated genes	Liver	1920	6	1.02E-04	5.40E-03	DHCR24, SLC43A1, CPT1A, SREBF1, SLC9A3R1, SC4MOL
Down-regulated genes	Liver	5547	7	1.38E-02	7.29E-01	SARS, TBCD, SLC1A5, ABCG1, RPS6KA2, GARS, SKI

Gene set enrichment analysis depicts genes that are upregulated and downregulated in the liver by overlapping Illumina annotated genes with DEG. GTEx=Genotype-Tissue Expression. DEG=differentially expressed genes.

SUPPLEMENTARY DATA

Supplementary Table S11. The Enriched Biological Processes for Genes Mapped to the 18 Illumina annotated genes.

GO biological process complete	Ref List	Observed	Observed Genes	Expected	Representation	Fold Enrichment	P-value	FDR
positive regulation of cholesterol biosynthetic process (GO:0045542)	5	2	ABCG1, SREBF1	0	+	> 100	1.61E-05	1.94E-02
positive regulation of sterol biosynthetic process (GO:0106120)	5	2	ABCG1, SREBF1	0	+	> 100	1.61E-05	1.80E-02
positive regulation of cholesterol metabolic process (GO:0090205)	6	2	ABCG1, SREBF1	0.01	+	> 100	2.15E-05	2.24E-02
amyloid precursor protein catabolic process (GO:0042987)	9	2	ABCG1, DHCR24	0.01	+	> 100	4.22E-05	3.67E-02
L-alpha-amino acid transmembrane transport (GO:1902475)	41	3	SLC1A5, SLC43A1, SLC7A11	0.04	+	81.03	8.05E-06	1.26E-02
serine family amino acid metabolic process (GO:0009069)	42	3	SARS, GARS, PHGDH	0.04	+	79.11	8.62E-06	1.23E-02
L-amino acid transport (GO:0015807)	61	3	SLC1A5, SLC43A1, SLC7A11	0.06	+	54.47	2.50E-05	2.45E-02
amino acid transmembrane transport (GO:0003333)	72	3	SLC1A5, SLC43A1, SLC7A11	0.07	+	46.14	4.03E-05	3.71E-02
carboxylic acid transmembrane transport (GO:1905039)	112	4	CPT1A, SLC1A5, SLC43A1, SLC7A11	0.1	+	39.55	3.18E-06	2.48E-02
organic acid transmembrane transport (GO:1903825)	112	4	CPT1A, SLC1A5, SLC43A1, SLC7A11	0.1	+	39.55	3.18E-06	1.66E-02
cholesterol metabolic process (GO:0008203)	113	4	ABCG1, SREBF1, DHCR24, SC4MOL	0.1	+	39.2	3.29E-06	1.29E-02
secondary alcohol metabolic process (GO:1902652)	119	4	ABCG1, SREBF1, DHCR24, SC4MOL	0.11	+	37.23	4.01E-06	8.96E-03
sterol metabolic process (GO:0016125)	132	4	ABCG1, SREBF1, DHCR24, SC4MOL	0.12	+	33.56	5.98E-06	1.04E-02
organic acid transport (GO:0015849)	269	5	CPT1A, SLC9A3R1, SLC1A5, SLC43A1, SLC7A11	0.24	+	20.59	3.60E-06	1.13E-02
carboxylic acid transport (GO:0046942)	269	5	CPT1A, SLC9A3R1, SLC1A5, SLC43A1, SLC7A11	0.24	+	20.59	3.60E-06	9.38E-03
organic anion transport (GO:0015711)	414	6	ABCG1, CPT1A, SLC9A3R1, SLC1A5, SLC43A1, SLC7A11	0.37	+	16.05	1.32E-06	2.07E-02
anion transport (GO:0006820)	534	6	ABCG1, CPT1A, SLC9A3R1, SLC1A5, SLC43A1, SLC7A11	0.48	+	12.44	5.64E-06	1.10E-02
small molecule metabolic process (GO:0044281)	1836	8	ABCG1, SARS, SREBF1, SC4MOL, GARS, CPT1A, PHGDH, DHCR24	1.66	+	5.43	1.22E-05	1.59E-02

Ref List quanitifies the number of genes in the database for the GO analysis. The 'Observed' column quantifies the number of genes in the testing set. The 'Expected' column depicts the expected number of genes for the testing set. When comparing the observed versus expected number of genes, '+' denotes over-representation while '-' denotes under-representation. Raw p-values are reported along with the FDR threshold. GO=gene ontology. FDR=false discovery rate.

SUPPLEMENTARY DATA

Supplementary Table S12. Traits associated with cis -meQTLs or proxies of cis -meQTLs using the NHGRI-EBI GWAS Catalog.

CpG	Independent *cis* -meQTL (1)	P-value for independent *cis* -meQTL with CpG	*cis* -meQTL overlapped with GWAS (2)	P-value for *cis* -meQTL overlapped with GWAS with CpG	LD R-squared b/w (1) and (2)	CHR for *cis* -meQTL overlapped with GWAS	BP for *cis* -meQTL overlapped with GWAS	Trait	P-value in GWAS	PMID
cg19016694	rs78483419	2.6E-12	rs9896933	NA	0.92	17	80870884	Bone mineral accretion in asthma (oral corticosteroid dose interaction)	3.0E-08	26025128
cg18120259	rs9472155	1.2E-82	rs9472155	1.2E-82	1	6	43897727	Vascular endothelial growth factor levels	2.0E-26	21757650
cg17501210	rs239934	7.1E-24	rs2236313	3.7E-14	0.68	6	167360389	Vitiligo	1.0E-16	20526339
cg17501210	rs239934	7.1E-24	rs2149085	6.4E-23	0.98	6	167371110	Crohn's disease	8.0E-12	23850713
cg17501210	rs239934	7.1E-24	rs1819333	5.9E-23	0.98	6	167373547	Inflammatory bowel disease	7.0E-21	23128233
cg17501210	rs239934	7.1E-24	rs1819333	5.9E-23	0.98	6	167373547	Crohn's disease	2.0E-19	26192919
cg17501210	rs239934	7.1E-24	rs1819333	5.9E-23	0.98	6	167373547	Inflammatory bowel disease	1.0E-06	27569725
cg17501210	rs239934	7.1E-24	rs1819333	5.9E-23	0.98	6	167373547	Crohn's disease	2.0E-20	28067908
cg17501210	rs239934	7.1E-24	rs1819333	5.9E-23	0.98	6	167373547	Inflammatory bowel disease	9.0E-15	28067908
cg17501210	rs2345568	1.8E-16	rs9355610	2.0E-16	0.97	6	167383075	Graves' disease	7.0E-10	21841780
cg17501210	rs239934	7.1E-24	rs444210	2.4E-21	0.92	6	167390242	Inflammatory bowel disease	2.0E-17	26192919
cg17501210	rs239934	7.1E-24	rs9457247	1.8E-23	0.98	6	167392174	Crohn's disease	2.0E-18	26192919
cg17501210	rs2345568	1.8E-16	rs4710154	7.8E-16	0.98	6	167394634	Basal cell carcinoma	1.0E-08	27539887
cg17501210	rs239934	7.1E-24	rs415890	2.3E-23	0.98	6	167406633	Crohn's disease	3.0E-12	21102463
cg17501210	rs2038580	1.8E-16	rs2301436	1.6E-17	0.76	6	167437988	Crohn's disease	1.0E-12	18587394
cg17501210	rs2038580	1.8E-16	rs2301436	1.6E-17	0.76	6	167437988	Crohn's disease	6.0E-08	20570966
cg17501210	rs2038580	1.8E-16	rs6456156	1.4E-06	0.6	6	167522300	Primary biliary cholangitis	6.0E-07	28425483
cg17501210	rs2038580	1.8E-16	rs3093024	2.7E-07	0.71	6	167532793	Rheumatoid arthritis	8.0E-19	20453841
cg17501210	rs2038580	1.8E-16	rs1854853	1.9E-06	0.65	6	167533062	Rheumatoid arthritis	2.0E-10	24782177
cg17501210	rs2038580	1.8E-16	rs3093023	1.2E-07	0.73	6	167534290	Rheumatoid arthritis	2.0E-11	20453842
cg17501210	rs2038580	1.8E-16	rs3093023	1.2E-07	0.73	6	167534290	Rheumatoid arthritis	4.0E-09	24782177
cg17501210	rs2038580	1.8E-16	rs1571878	3.5E-07	0.71	6	167540842	Rheumatoid arthritis	5.0E-35	24390342
cg14476101	rs866321	1.3E-37	rs1163251	4.3E-36	0.98	1	120209755	Blood metabolite levels	7.0E-27	24816252
cg14476101	rs666930	3.3E-09	rs637868	3.6E-09	0.97	1	120257110	Alanine aminotransferase (ALT) levels after remission induction therapy in acute lymphoblastic leukemia (ALL)	4.0E-07	28090653
cg14476101	rs11583993	2.7E-127	rs894079	4.7E-64	0.66	1	120263657	Severe gingival inflammation	1.0E-06	28459102
cg14476101	rs41276626	3.5E-15	rs12144094	4.9E-15	0.98	1	120264823	Height	2.0E-09	25282103
cg11376147	rs2511992	9.7E-06	rs2729354	NA	0.98	11	57358343	Blood protein levels	8.0E-14	28240269
cg11024682	rs7214988	6.5E-16	rs11658311	3.1E-14	0.97	17	17470526	Obsessive-compulsive symptoms	7.0E-06	26859814
cg11024682	rs12936587	1.8E-10	rs12936587	1.8E-10	1	17	17543722	Coronary heart disease	4.0E-10	21378990
cg11024682	rs12936587	1.8E-10	rs12936587	1.8E-10	1	17	17543722	Coronary artery disease or large artery stroke	2.0E-10	24262325
cg11024682	rs12936587	1.8E-10	rs12936587	1.8E-10	1	17	17543722	Hip circumference	3.0E-08	25673412
cg11024682	rs12936587	1.8E-10	rs12449964	2.2E-10	0.98	17	17544704	Coronary artery disease or ischemic stroke	2.0E-08	24262325
cg11024682	rs941444	1.1E-20	rs12941356	9.4E-19	0.71	17	17716531	Resting heart rate	5.0E-08	27798624
cg11024682	rs1889014	2.6E-22	rs8082590	1.1E-05	0.67	17	17958402	Schizophrenia	2.0E-08	25056061
cg11024682	rs1889014	2.6E-22	rs8082590	1.1E-05	0.67	17	17958402	Schizophrenia	2.0E-08	26198764
cg08309687	rs4817600	6.1E-09	rs2834288	2.3E-05	0.62	21	35264397	Gut microbiota (bacterial taxa)	2.0E-08	27694959
cg08309687	rs2032314	6.4E-17	rs2032314	6.4E-17	1	21	35354523	Red blood cell traits	8.0E-10	23222517
cg02711608	rs7247877	2.7E-13	rs314669	4.8E-10	0.72	19	47168129	Glaucoma (primary open-angle)	2.0E-06	27001270
cg02711608	rs11668911	1.2E-21	rs11083846	1.0E-17	0.8	19	47207654	Chronic lymphocytic leukemia	4.0E-09	18758461
cg27243685	rs57137919	2.9E-09	rs4148087	8.0E-09	0.86	21	43622267	Eating disorder in bipolar disorder	4.0E-07	26433762

cis -meQTL=cis -methylation quantitative trait loci. CpG=DNA methylation site. CHR=chromosome. BP=base pair. GWAS=genome-wide association study. PMID=PubMed identification number

SUPPLEMENTARY DATA

Supplementary Table S13. Three-way association of CpGs, gene expression, and hepatic fat in the FHS.

CpG	Gene	CHR	CpG position	TSS	P-value for CpG and gene association	P-value for gene and liver fat association	Direction for CpG and liver fat association	Direction for CpG and gene association	Direction for gene and liver fat association
cg06500161	ABCG1	21	43656587	43619809	2.1E-60	1.2E-30	+	-	-
cg27243685	ABCG1	21	43642366	43619809	1.2E-37	1.2E-30	+	-	-
cg00574958	CPT1A	11	68607622	68522090	1.9E-20	2.0E-17	-	-	+
cg17901584	DHCR24	1	55353706	55302242	6.3E-17	1.2E-01	-	-	+
cg06690548	SLC7A11	4	139162808	139060406	6.1E-14	3.7E-01	-	-	+
cg14476101	PHGDH	1	120255992	120202441	3.7E-12	3.2E-02	-	-	+
cg17501210	RNASET2	6	166970252	167271494	9.4E-11	7.7E-02	-	-	-
cg19693031	TXNIP	1	145441552	145438489	8.2E-08	7.8E-03	-	-	-
cg08309687	ATP5O	21	35320596	35273997	2.2E-04	8.7E-01	-	+	+
cg08309687	TMEM50B	21	35320596	34804794	6.3E-04	4.1E-05	-	+	-
cg08309687	ITSN1	21	35320596	35014716	6.5E-03	2.3E-02	-	+	+
cg08309687	SLC5A3	21	35320596	35467498	1.4E-02	2.0E-01	-	-	-
cg08309687	MRPS6	21	35320596	35322421	4.5E-02	5.5E-01	-	-	+

Gene expression levels were derived from whole blood in the FHS. Regarding direction, '+' sign represents a positive association and '-' sign represents an inverse association. CpG=DNA methylation site. FHS=Framingham Heart Study. CHR=chromosome. TSS=transcription start site.

SUPPLEMENTARY DATA

Supplementary Table S14. MR analysis for CpGs association to nonalcoholic fatty liver disease (NAFLD)

	IVW method			Egger method			Egger method intercept (horizontal pleiotropy)					F-statistics of Ivs			Explained variance by SNPs		Heterogeneity	
CpG	Beta	SE	P	Beta	SE	P	Beta	SE	P	number of IVs	IVs	Mean	Min	Max	CpG	T2D	Q statistics	P-for-Q statistics
cg02711608	-0.73	3.05	0.81							4	rs1060043, rs1862335, rs3027961, rs8102896	31	11	52	0.08	3.5E-04	2.50	0.47
cg06500161	-0.38	3.88	0.92							2	rs225443, rs9978671	29	17	42	0.03	1.1E-04	0.81	0.37
cg08309687	-4.99	1.36	2.5E-04	-3.97	2.57	0.17	-0.01	0.01	0.66	9	rs11702633, rs17655956, rs2070393, rs2244966, rs2834311, rs2834321, rs743316, rs9636874, rs9978794	55	10	162	0.15	2.7E-03	6.04	0.64
cg09469355	1.01	10.76	0.92							3	rs12049067, rs3107125, rs4648832	20	10	33	0.01	6.8E-04	4.60	0.10
cg11024682	2.45	2.76	0.37							4	rs1006656, rs11656629, rs11658477, rs1889014	73	17	139	0.09	2.3E-04	0.89	0.83
cg14020176	-1.39	5.82	0.81							2	rs895690, rs939543	31	16	47	0.03	1.4E-05	0.04	0.83
cg14476101	-2.44	1.00	0.01	-0.08	1.80	0.97	-0.02	0.01	0.15	11	rs10802118, rs1177389, rs1886736, rs372814, rs3862258, rs4659015, rs4844381, rs6667104, rs667226, rs7528420, rs866321	85	10	382	0.22	1.7E-03	6.71	0.75
cg15860624	6.69	5.49	0.22							3	rs10410543, rs12459781, rs2060249	25	21	29	0.02	8.3E-04	2.12	0.35
cg17501210	2.95	4.95	0.55							2	rs239935, rs9356478	50	30	70	0.04	4.3E-04	2.29	0.13
cg17901584	-1.14	4.66	0.81							1	rs3170766	38	38	38	0.02	8.3E-06		
cg18120259	-2.31	2.38	0.33							4	rs866730, rs9472153, rs9472155, rs9472159	50	12	151	0.12	1.8E-03	10.47	0.01
cg19016694	3.93	2.45	0.11							5	rs12943585, rs3791160, rs4789817, rs4986117, rs6416854	49	14	163	0.10	7.4E-04	2.67	0.62
cg21429551	-3.94	3.00	0.19							1	rs6462211	34	34	34	0.01	2.4E-04		
cg27243685	-5.22	6.39	0.41							2	rs221954, rs748319	34	30	38	0.02	2.3E-04	0.96	0.33

A positive beta value means that the instrument SNPs (cis-meQTLs) increased DNA methylation and increased hepatic fat, whereas a negative beta value means that the instrument SNPs increased DNA methylation but reduced hepatic fat. The inverse variance weighted (IVW) approach was implemented for all CpGs and the MR-Egger method was implemented for CpGs with three or more instrumental variables (IVs) and significant using IVW. MR=Mendelian randomization. CpG=DNA methylation site. SNP=single nucleotide polymorphism. SE=standard error. CpG Supplemental

SUPPLEMENTARY DATA

Supplementary Table S15. Cross-sectional association between hepatic fat-associated CpGs with prevalence of type 2 diabetes in 4,068 Framingham Heart Study participants

	Model 1			Model 2		
CpG	OR	95% CI	P	OR	95% CI	P
cg09469355	0.78	0.78 (0.67, 0.9)	1.2E-03	0.85	0.85 (0.73, 1)	4.7E-02
cg17901584	0.44	0.44 (0.37, 0.53)	1.9E-18	0.53	0.53 (0.43, 0.63)	2.9E-11
cg03725309	0.51	0.51 (0.42, 0.62)	8.7E-12	0.62	0.62 (0.5, 0.76)	8.1E-06
cg14476101	0.70	0.7 (0.62, 0.79)	3.3E-08	0.79	0.79 (0.69, 0.91)	8.8E-04
cg19693031	0.47	0.47 (0.41, 0.53)	5.6E-30	0.48	0.48 (0.42, 0.55)	1.5E-26
cg06690548	0.82	0.82 (0.74, 0.91)	2.5E-04	0.91	0.91 (0.81, 1.03)	1.3E-01
cg05119988	0.65	0.65 (0.57, 0.74)	1.0E-11	0.68	0.68 (0.6, 0.77)	5.1E-09
cg03957124	0.66	0.66 (0.53, 0.83)	2.7E-04	0.83	0.83 (0.66, 1.05)	1.2E-01
cg18120259	0.77	0.77 (0.64, 0.93)	7.7E-03	0.91	0.91 (0.74, 1.11)	3.5E-01
cg17501210	0.74	0.74 (0.65, 0.85)	9.2E-06	0.87	0.87 (0.76, 1)	5.1E-02
cg21429551	0.71	0.71 (0.62, 0.8)	9.8E-08	0.79	0.79 (0.69, 0.9)	5.6E-04
cg11376147	0.59	0.59 (0.48, 0.72)	4.1E-07	0.67	0.67 (0.54, 0.82)	1.6E-04
cg00574958	0.44	0.44 (0.38, 0.52)	1.2E-22	0.54	0.54 (0.46, 0.64)	9.2E-13
cg26894079	0.91	0.91 (0.77, 1.07)	2.5E-01	1.01	1.01 (0.86, 1.18)	9.1E-01
cg11024682	1.49	1.49 (1.32, 1.69)	1.9E-10	1.33	1.33 (1.17, 1.5)	6.6E-06
cg14020176	1.50	1.5 (1.28, 1.75)	4.6E-07	1.40	1.4 (1.19, 1.64)	5.8E-05
cg19016694	0.86	0.86 (0.72, 1.03)	1.0E-01	1.02	1.02 (0.85, 1.21)	8.5E-01
cg15860624	1.18	1.18 (1.03, 1.35)	1.9E-02	1.09	1.09 (0.95, 1.25)	2.2E-01
cg02711608	0.57	0.57 (0.47, 0.68)	7.3E-10	0.66	0.66 (0.55, 0.79)	8.5E-06
cg08309687	0.73	0.73 (0.63, 0.84)	1.8E-05	0.81	0.81 (0.7, 0.94)	4.7E-03
cg27243685	1.50	1.5 (1.31, 1.72)	1.0E-08	1.34	1.34 (1.16, 1.54)	5.0E-05
cg06500161	1.90	1.9 (1.67, 2.17)	1.6E-21	1.66	1.66 (1.45, 1.91)	4.7E-13

Model 1 adjusted for sex, age, alcohol intake, smoking status, physical activity level, laboratory for DNA methylation assessment, DNA methylation chip ID, row, and column, top three PCs, and estimated leukocyte composition.
Model 2 adjusted for model 1 covariates and BMI.

SUPPLEMENTARY DATA

Supplementary Table S16. MR analysis for causal associations from CpGs to type 2 diabetes

CpGs	IVW method			Egger method			Egger method intercept (horizontal pleiotropy)			number of IVs	Ivs	F-statistics of Ivs			Explained variance by SNPs		Heterogeneity	
	Beta	SE	P	Beta	SE	P	Beta	SE	P			Mean	Min	Max	CpG	T2D	Q statistics	P-for-Q statistics
cg02711608	-0.27	1.06	0.80							6	rs11668911, rs151217961, rs1862335, rs3027960, rs7260181, rs8102896	26	11	52	0.09	1.7E-05	3.91	0.56
cg06500161	1.84	1.41	0.19							2	rs225443, rs9982016	29	17	41	0.03	7.3E-06	0.01	0.91
cg08309687	-0.47	0.53	0.37							16	rs10222139, rs117631025, rs190361590, rs2243871, rs2834259, rs2834311, rs2834321, rs35070015, rs7277233, rs73199882, rs78070167, rs78449000, rs78713112, rs879489, rs9636874, rs9978794	38	13	162	0.20	9.5E-05	21.03	0.10
cg09469355	-2.41	2.14	0.26							3	rs3107125, rs56117848, rs870207	22	11	37	0.02	8.6E-06	0.60	0.74
cg11024682	1.10	2.54	0.67							5	rs11655294, rs11656699, rs1889014, rs35367147, rs9916193	80	18	156	0.13	1.5E-04	33.17	1.1E-06
cg14020176	-9.75	1.95	5.6E-07							2	rs62084914, rs939543	33	16	50	0.03	1.0E-04	0.003	0.96
cg14476101	0.02	0.29	0.95							17	rs10802118, rs11583993, rs1163550, rs12065721, rs12121447, rs148882526, rs190242852, rs347911, rs4659015, rs4844381, rs517533, rs518027, rs6667104, rs667226, rs67607949, rs79140708, rs911245	87	10	603	0.36	8.2E-05	19.06	0.27
cg15860624	-4.68	1.58	0.003	0.25	6.27	0.97	-0.02	0.02	0.56	3	rs10410543, rs12459781, rs8111014	24	21	29	0.02	4.3E-05	1.74	0.42
cg17501210	0.58	1.07	0.59							3	rs239934, rs57794755, rs7757549	39	15	72	0.05	6.7E-06	1.27	0.53
cg17901584	-0.67	1.60	0.68							1	rs12732413	35	35	35	0.02	7.4E-07		
cg18120259	-1.49	0.68	0.03	-0.34	1.28	0.81	-0.01	0.01	0.40	4	rs2759270, rs67798973, rs9472155, rs9472159	53	14	151	0.13	3.5E-05	3.29	0.35
cg19016694	0.22	0.78	0.78							8	rs12601746, rs4986127, rs4986139, rs756072, rs78056681, rs78483419, rs8064531, rs8076137	37	12	164	0.13	1.9E-05	4.27	0.75
cg21429551	-2.37	1.09	0.03							1	rs6462211	34	34	34	0.01	2.0E-05		
cg27243685	0.15	2.02	0.94							2	rs748319, rs77704026	35	32	38	0.03	2.7E-06	0.62	0.43

The inverse variance weighted (IVW) approach was implemented for all CpGs and the MR-Egger method was implemented for CpGs with three or more instrumental variables (IVs) and significant using IVW. MR=Mendelian randomization. FHS=Framingham Heart Study. CpG=DNA methylation site. SNP=single nucleotide polymorphism. SE=standard error.