Supplemental Online Content

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eMethods

Inclusion criteria

Strong Heart Study. For this study, one of the tribes declined to participate, leaving 3,517 potential participants. We excluded an additional 252 participants with prevalent cardiovascular disease, 429 participants missing urinary metals (as the study was funded in the context of metals and cardiovascular research), 44 participants missing data on other risk factors, and 469 lacking sufficient DNA samples; there were 2,347 participants eligible for blood DNAm analyses. After analysis, we removed 18 participants lacking a classical DNAm bimodal distribution and 8 individuals with low median intensity levels, leaving 2,321 participants for this study. These participants were similar to those eligible in sociodemographic and anthropometric characteristics (**eTable 1**, **eFigure 1**).

Women's Health Initiative. WHI enrolled 161,808 women starting in 1993 as part of randomized control trials that were continued as a prospective cohort study. A subset of 2,096 WHI participants free of cardiovascular disease had blood DNAm data. We excluded those missing data on traditional CHD risk factors (n=222), leaving 1,874 participants (869 non-Hispanic White (NHWs), 376 Hispanic, and 629 African American women, baseline mean age 64.3 years).

Framingham Heart Study. FHS recruited adults from Framingham, Massachusetts starting in 1948. Among 2,631 participants with blood DNAm data available in the FHS Offspring, we excluded those with prior CHD (n=279) and those missing information on CHD risk factors (n=224), leaving 2,128 NHWs participants (baseline mean age 65.6 years, 57.2% women).

Atherosclerosis Risk in Communities (ARIC) Study. Among 2,143 ARIC African-American participants with DNAm data and free of CHD at baseline, we excluded those missing CHD risk factors (n=29), leaving 2,114 participants (baseline mean age 56.5 years, 63.6% women); among 951 ARIC European-American participants with DNAm data and free of CHD at baseline, we excluded those missing CHD risk factors (n=20), leaving 931 participants (baseline mean age 59.6 years, 60.5% women).

Cardiovascular incidence and follow-up

Strong Heart Study. Incident cardiovascular end-points during follow-up were assessed by annual mortality and morbidity surveillance reviews of hospitalization and death records through 2017 and at two research clinic visits conducted in 1993–1995 and 1998–1999. Follow-up is 99.8% complete for mortality and 99.2% complete for morbid events. When possible events were identified, medical records were reviewed by mortality and morbidity review committees composed of physician reviewers who assigned cardiovascular events. Detailed definitions of fatal and nonfatal events have been described.[1,2] Incident CHD was defined as the first occurrence of definite fatal myocardial infarction, sudden death due to CHD, non-fatal myocardial infarction, or definite non-fatal CHD. Time to event was calculated as the difference between age at the date of the baseline examination and the age at the date of the cardiovascular event, age at the date of death, or age at 31 December 2017, whichever occurred first.

Women's Health Initiative. CHD included hospitalized myocardial infarction, definite silent myocardial infarction, and coronary death.[3] Women participating in the clinical trials were followed up through regularly scheduled examinations while the women participating in the observational study were contacted annually by mail to collect updates on their health. If a participant reported any cardiovascular event, additional documents were requested from the physician or the hospital. Only hospitalized cases were included as outcomes in WHI. A local adjudicator reviewed all the documents and recorded the cardiovascular outcome. CHD events were centrally adjudicated by the Cardiovascular Central Adjudication Committee. The participants of WHI were followed from baseline (1992) to 2016 with an average follow-up time of 12.2 years (range: 0.003 years – 21.3 years).

Framingham Heart Study. Incident CHD included coronary death, myocardial infarction, coronary insufficiency, and angina.[4] Medical histories, physical examinations during study visits, hospitalization records and personal physician records were used to identify any possible cardiovascular event. A panel of 3 experienced investigators reviewed the medical records of suspected new events and made final decision about each event. The participants of exam 8 (2005-2008) of FHS offspring cohort were followed through 2014 (average follow-up of 7.7 years; range: 0.04 years – 9.8 years).

Atherosclerosis Risk in Communities (ARIC) Study. Incident CHD was validated by physician review using standardized protocols and was defined as a definite or probable myocardial infarction, a silent MI between examinations by electrocardiography, or a definite CHD death.[5] In this analysis, participants were followed from the time of DNA collection at Visit 2 (1990-1992) or Visit 3 (1993-1995) through Dec. 31, 2018, or through December 31, 2017 for participants in Jackson, Mississippi.

Microarray DNA methylation measurement

Strong Heart Study. White blood cell DNA from fasting blood samples was extracted and stored at the MedStar Health Research Institute under a strict quality control system. In 2015, blood DNA was shipped to Texas Biomedical Research Institute for DNA methylation analysis. DNA was bisulfite-converted with the EZ DNA methylation kit (Zymo Research, Irvine, CA). Bisulfite converted DNA was measured using the MethylationEPIC BeadChip (Illumina 850K), which provides a measure of DNAm at a single nucleotide resolution at >850,000 CpG sites. Samples were randomized across and within plates to remove batch artifacts and confounding effects, and replicate and across-plate control samples were included on every plate. Data were read in six batches of ~ 400 individuals each and combined using the R package *minfi*. Individuals with no bimodal DNAm distribution were excluded (N=18). Methylation sites with a p-detection value greater than 0.01 in more than 5% of the individuals (N= 6159) were removed. Single sample snoob normalization was conducted using the 'preprocessNoob' function in R package *minfi*,[6,7] which includes a background correction with dye-bias normalization for Illumina Infinium methylation arrays. To account for probe type bias, regression on correlated probes (RCP) normalization was conducted after snoob using the R package ENmix.[8] Cross-hybridizing probes, sex chromosomes and SNP probes with minor allele frequency > 0.05[9,10] were removed as well as 307 probes declared as failures by Illumina (Infinium MethylationEPIC v1.0 B4 Manifest File Release Notes).[11] Following these preprocessing preliminary analyses, we had data from 2,321 individuals and 788,368 CpGs. Quality checks, data normalization, statistical preprocessing and beta-value calculation, which ranges from 0 to 1 and represents the proportion of cytosines (Cs) in bisulfite-converted DNA at specific locations were performed using the R package *minfi*.[6] We estimated Houseman cell proportions using the *minfi* R package (CD8T, CD4T, NK, B cells, monocytes and granulocytes). These estimations were used as adjustment variables in regression models. We corrected for potential batch effects by sample plate, sample row, and DNA isolation time with the combat function (*sva* R package).

Women's Health Initiative. In the WHI, DNAm was measured among a sub-sample of the original cohort who provided consent to be included in the genetic study. The selected participants were free of CVD during baseline and had genotyping data. Details about DNAm measurement and quality control have been published.[12] In brief, standard procedures of 450K Illumina assay were used to measure DNAm in peripheral blood. During quality control, we excluded probes with a missing rate > 5% at detection P value < 0.01, SNPs within 10 base pairs of targeted CpGs, and location on X or Y chromosomes. Finally, 434,113 CpGs and 2,096 individuals were available for analysis. Among the 2,096 individuals, we excluded those with missing information on traditional risk factors of CHD (n=222) and included 1,874 participants in our final analyses.

Framingham Heart Study. In the FHS, buffy coat preparations were obtained from whole-blood samples (Gentra Puregene Blood Kit-Qiagen, Venlo, Netherlands) collected during the eighth examination of the Framingham Offspring Study (2005-2008). DNAm was quantified in the bisulfite converted genomic DNA (EZ DNA Methylation Kit-Zymo Research, Irvine, CA) using Illumina Infinium HumanMethylation450K Beadchip array. DASEN methodology in wateRmelon package 10 was used to conduct within laboratory batch normalization of raw data. The exclusion criteria for samples were a missing rate > 1% at detection P-value <0.01, poor matching to the 65 single nucleotide polymorphism (SNP) control probe locations, and identification as outliers using multi-dimensional scaling techniques. In addition, the exclusion criteria for the probes were missing rate > 20% at detection P-value< 0.01, previously identified to map multiple locations, underlying SNP (minor allele frequency > 5% in European ancestry 1000 genomes project data) at the CpG or <10 bp of the single base extension, and location in sex chromosomes. Finally, 408,254 CpGs and 2,631 participants were available for analysis. Among those 2,631 participants, we excluded those with a history of CHD (n=279) and those missing information on traditional risk factors of CHD (n=224) and finally included 2,128 participants for our analyses.

Atherosclerosis Risk in Communities (ARIC) Study. DNA methylation data was obtained on 2853 African-Americans from the Jackson, MS and Forsyth County, NC study sites and on a subsample of 1,104 European Americans from Forsyth County, Minneapolis, or Washington County with cerebral magnetic resonance imaging data. All included provided informed consent and had available DNA at visit 2 or visit 3. Bisulfite converted DNA

extracted from peripheral blood leukocytes was hybridized to the Illumina HumanMethylation450 BeadChip (Illumina Inc., San Diego, CA), following the Illumina HD Methylation protocol (Illumina Inc., San Diego, CA). Individuals were excluded from the analyses if the pass rate for the DNA sample for the participant was < 99% (probes with a detection p-value <0.01/all probes on the array). CpG sites were not analyzed in this study if more than 5% of the samples showed a detection p-value > 0.01. Methylation values were processed with normal-exponential out-of-band (NOOB) method for background subtraction and then normalized using the Beta MIxture Quantile dilation (BMIQ) method for type I/type II bias correction. Among 2143 Black participants with DNAm data available and free of cardiovascular disease, we excluded those with missing CHD risk factors (n=29), leaving 2114 participants (baseline mean age 56.5 years, 63.6% women); among 951 White participants with DNAm data available and free of cardiovascular disease, we excluded those with missing CHD risk factors (n=20), leaving 931 participants (baseline mean age 59.6 years, 60.5% women).

Statistical methods

High-dimensional models for differentially methylated positions (DMPs) in the SHS. We used GLMnet penalized regression (elastic-net) applied to survival time (R package *glmnet*) to account for the complex interrelationships across CpGs. Elastic-net is a mix between Ridge and Lasso regression[13] that can successfully model high-dimensional DNAm data.[14] To test all the CpG sites simultaneously, the algorithm fits a Cox regression model using the coordinate descent algorithm with penalty controlled by the α parameter. The α parameter can range from 0 –corresponding to Ridge regression, which can introduce more than one predictor from a correlated set, to 1 –corresponding to Lasso regression, which generally selects only one of the correlated predictors. We selected α=0.05, a common choice for methylation data,[14] based on the performance of the model after testing different values in the range between 0 and 1. The regularization path is computed for the selected penalty at a set of values as specified by the regularization parameter λ[15] so that the minimum mean squared error is achieved, which was selected using 10-fold cross-validation in our study.

The predictive ability of the selected CpGs was estimated comparing the C-statistic[16] in an elastic-net model with traditional risk factors for the SHS (sex, smoking status, BMI, LDL cholesterol, HDL cholesterol, hypertension treatment, systolic blood pressure, type 2 diabetes, and albuminuria status) and center (Arizona, Oklahoma, and North/South Dakota), to the elastic-net model further adding the 788,368 CpGs, as well as blood cell counts and five genetic PCs. Because few cardiovascular studies have 850K data, we also conducted the analysis restricted to CpGs present in the 450K array.

In sensitivity analyses, we conducted elastic-net models including the CpGs selected by the overall model in models with events before and after 1995 separately, to check if DNAm status was more likely to preferentially predict CHD events closer vs. more distal to the time when blood was collected, and in models in each of the study regions separately.

Standard models for differentially methylated positions (DMPs) in the SHS. For comparison with previous studies, and to estimate hazard ratios for the CpGs selected by elastic-net, we ran Cox proportional hazards models for the association of incident CHD with methylation at each CpG as the independent variable using a loop and a parallel backend (R package *survival*). Beta-values of DNAm were used as predictors in Cox regression models with age as time scale and individual entry times (age at baseline) treated as staggered entries. Models were adjusted for sex, smoking status, BMI, LDL cholesterol, HDL cholesterol, hypertension treatment, systolic blood pressure, type 2 diabetes, albuminuria status, center, blood cell counts and five genetic PCs. Multiple comparisons were accounted for with the Benjamini and Hochberg method to control for false discovery rates (FDR). As genomic inflation can lead to inflated p-values and false positives,[17] we calculated genomic inflation factors for p-values.

Targeted and untargeted approaches were implemented to evaluate DMPs associated with CHD. In the targeted approach, we selected 248 CpG sites related to atherosclerotic cardiovascular disease (all cardiovascular disease, CHD, or stroke) in the scientific literature,[18,19] to replicate signals previously associated with cardiovascular disease in our study population at a nominal p-value of 0.05. In the untargeted approach, we modeled all CpGs available individually as it has been typically done in previous EWAS.[18] We annotated the DMPs that passed FDR threshold to the nearest gene according to the Infinium MethylationEPIC Manifest File (Infinium MethylationEPIC Product Files, b4 version).[11] In addition to these standard Cox regressions, for the 248 CpG sites previously related to atherosclerotic cardiovascular disease, we also ran an elastic-net model including the 248 CpGs simultaneously.

DMPs analyses in WHI, FHS and ARIC. We conducted three analyses in FHS, WHI and ARIC selecting three sets of CpGs from the SHS analyses, restricted to the ones in the 450K platform. First, we selected the CpGs identified as informative for prediction by the elastic-net model. Second, we selected the individual CpGs with FDR p-value <0.1 in the Cox models. Last, we conducted an epigenome-wide elastic-net analysis in each of the cohorts separately. Elastic-net and Cox regression models were implemented using the same analytical strategies and adjustments as described above for SHS.

Differentially Methylated Regions (DMRs). Testing differential methylation at the regional level can remove spatial redundancy, reducing the dimensionality of the data and increasing robustness.[20] DMRs might also be more biologically relevant than DMPs.[21,22] We assessed DMRs for CHD using *DMRcate*, which computes a kernel estimate against a null comparison using the coefficients and standard errors from the DMP Cox models to identify differentially methylated regions, and ranks the DMRs by Stouffer p-value. DMRs were annotated to the closest gene based on hg19 notation.[23]

Protein-protein interaction network. From the 450K CpGs reported in the SHS elastic-net model, a list of unique protein-coding genes was created. Protein interaction data were obtained from the STRING database v11.0.[24] The STRING database provides a confidence score (from 0 to 1) to indicate the estimated likelihood that the annotated interaction between a given pair of proteins is biologically meaningful, specific, and reproducible. The protein interaction network was analyzed and displayed using the yfiles Organic layout by Cytoscape v. 3.7.1.[25] In the resultant network, we kept connections obtained from experimental studies, publicly available databases, and text mining with a minimum confidence score of 0.5. Unconnected nodes were excluded from the network.

eTable 1. Descriptive characteristics for eligible Strong Heart Study participants versus finally selected participants

	Included (N=2321)	Eligible (N=2792)
Age, years	55 (49, 62)	55 (49, 62)
Sex (% male)	41.5	40.6
Smoking status		
% Current	38.4	37.6
% Former	32.2	33.0
BMI, kg/m^2	29.6 (26.2, 33.6)	29.7 (26.3, 33.7)
Education		
% High	58.6	59.2
% Medium	23.9	23.5

Data are median (25^{th}, 75^{th} percentile) or percentage.

eTable 2. Strong Heart Study baseline participants' characteristics by coronary heart disease (CHD) incidence status

	Incident CHD (N=749)	No Incident CHD (N=1572)	Total (N=2321)
Age (years), median (IQR)	56 (50.2, 63.0)	54.4 (48.9, 61.5)	55 (49.2, 62)
Sex, % Male	48.9	37.9	41.4
Smoking status, %			
Never	33.4	31.5	32.1
Former	27.1	30.6	29.5
Current	39.5	37.9	38.4
BMI, median (IQR)	30.1 (27.3, 34.2)	29.2 (25.6, 33.3)	29.6 (26.2, 33.6)
LDL cholesterol (mg/dL), median (IQR)	125 (103, 147)	116 (95, 137)	119 (98, 140)
HDL cholesterol (mg/dL), median (IQR)	42 (35, 49)	45 (38, 54)	44 (37, 53)
Systolic blood pressure, median (IQR)	126 (116, 139)	122 (111, 135)	124 (113, 136)
Hypertension, %	27.1	16.6	20
Diabetes, %	53.1	36.1	41.6
Albuminuria, %			
Microalbuminuria	18.4	14.3	15.6
Macroalbuminuria	10.0	5.8	7.2

Abbreviations: SD, standard deviation; IQR, interquartile range; LDL, low density lipoprotein; HDL, high density lipoprotein. Data are median (IQR) for continuous variables and percentages for categorical variables.

eTable 3. Hazard ratios (95% CIs) of CpGs available in the Illumina 450 000 platform initially associated with CHD by elastic-net in the Strong Heart Study (SHS), subsequently associated in the Women's Health Initiative (WHI), the Framingham Heart Study (FHS) and the Atherosclerosis Risk in Communities (ARIC) Study, and not statistically significant in the meta-analysis

			SHS	WHI	FHS	ARIC-Black	ARIC-White	Meta-analysis	
CpG	chr	Gene	HR (95%CI)[a]	HR (95%CI)[a]	HR (95%CI)[a]	HR (95%CI)[a]	HR (95%CI)[a]	Pooled HR (95%CI)	P-value
cg04037707	7	*PAXIP1-AS2*	0.72 (0.57, 0.90)	1.22 (0.95, 1.56)	0.63 (0.38, 1.05)	0.81 (0.61 1.07)	0.70 (0.44 1.12)	0.80 (0.60, 1.07)	0.13
cg18171855	10	*LINC00701*	0.71 (0.60, 0.85)	0.70 (0.55, 0.88)	1.33 (0.78, 2.26)	1.05 (0.79 1.41)	0.57 (0.39 0.83)	0.81 (0.64, 1.02)	0.08
cg04280397	22	*PLA2G6*	0.71 (0.58, 0.88)	0.99 (0.78, 1.27)	0.55 (0.29, 1.03)	0.80 (0.57 1.11)	0.81 (0.49 1.35)	0.84 (0.71, 1.00)	0.05
cg27071405	7	*VIPR2*	0.74 (0.64, 0.87)	1.03 (0.88, 1.22)	1.46 (0.91, 2.33)	0.72 (0.56 0.91)	0.58 (0.39 0.88)	0.85 (0.68, 1.07)	0.16
cg00695391	1	*MMEL1*	0.89 (0.70, 1.12)	0.82 (0.61, 1.09)	0.81 (0.41, 1.61)	0.91 (0.65 1.28)	0.84 (0.44 1.58)	0.87 (0.75, 1.01)	0.06
cg25586410	2	*GPR155*	0.78 (0.63, 0.96)	0.98 (0.76, 1.27)	0.64 (0.35, 1.16)	0.90 (0.66 1.24)	0.99 (0.59 1.65)	0.88 (0.78, 1.00)	0.05
cg06513733	19	*REXO1*	0.81 (0.68, 0.96)	1.02 (0.83, 1.24)	1.15 (0.69, 1.94)	1.01 (0.82 1.25)	0.77 (0.63 0.96)	0.88 (0.73, 1.07)	0.22
cg08054038	19	*PVRL2*	0.74 (0.62, 0.89)	1.05 (0.83, 1.31)	0.56 (0.32, 0.98)	1.13 (0.90 1.41)	0.83 (0.52 1.34)	0.88 (0.71, 1.10)	0.26
cg21443773	2	*FASTKD2*	0.72 (0.60, 0.86)	1.13 (0.94, 1.37)	0.53 (0.33, 0.87)	1.09 (0.89 1.34)	0.79 (0.48 1.29)	0.90 (0.76, 1.06)	0.19
cg04232681	9	*NAALADL2*	0.82 (0.70, 0.96)	1.03 (0.85, 1.24)	0.66 (0.44, 1.01)	1.09 (0.85 1.40)	0.84 (0.68 1.03)	0.92 (0.82, 1.03)	0.14
cg06481800	13	*SOX1*	0.81 (0.69, 0.95)	1.21 (0.95, 1.53)	0.75 (0.45, 1.23)	0.79 (0.62 1.01)	1.01 (0.72 1.41)	0.92 (0.79, 1.07)	0.28
cg18635064	2	*IL1B*	1.31 (1.08, 1.60)	0.84 (0.69, 1.02)	0.59 (0.37, 0.94)	0.94 (0.85 1.04)	0.89 (0.80 0.99)	0.95 (0.85, 1.06)	0.37
cg17959722	9	*MRPL41*	0.70 (0.58, 0.84)	1.07 (0.83, 1.37)	0.70 (0.40, 1.22)	1.10 (0.86 1.41)	1.41 (0.86 2.31)	0.95 (0.75, 1.20)	0.66
cg22453911	19	*SPPL2B*	0.75 (0.63, 0.90)	0.84 (0.67, 1.04)	1.74 (1.01, 2.99)	1.21 (0.97 1.51)	0.85 (0.71 1.03)	0.96 (0.82, 1.13)	0.63
cg17683593	11	*SYT7*	0.77 (0.63, 0.93)	1.14 (0.92, 1.40)	1.08 (0.70, 1.69)	1.12 (0.96 1.30)	0.70 (0.41 1.18)	0.96 (0.83, 1.12)	0.64
cg26306154	3	*ZFYVE20*	1.32 (1.11, 1.59)	0.90 (0.81, 1.00)	0.52 (0.31, 0.87)	1.02 (0.97 1.08)	0.92 (0.82 1.03)	0.99 (0.94, 1.06)	0.86
cg09080788	17	*CDRT15P*	1.03 (1.01, 1.05)	0.96 (0.92, 1.00)	1.05 (0.91, 1.22)	0.95 (0.90 1.00)	0.95 (0.89 1.01)	1.00 (0.98, 1.02)	0.70
cg04250451	1	*RUNX3**	1.37 (1.15, 1.64)	0.97 (0.88, 1.08)	0.66 (0.42, 1.03)	0.99 (0.73 1.34)	1.88 (1.08 3.27)	1.04 (0.95, 1.15)	0.39
cg11202345	17	*LGALS3BP*	1.18 (0.97, 1.43)	0.96 (0.77, 1.21)	1.05 (0.61, 1.81)	1.28 (1.02 1.61)	0.55 (0.32 0.94)	1.04 (0.85, 1.27)	0.72
cg01311360	5	*PCDHGA4*	1.16 (1.01, 1.33)	0.77 (0.61, 0.97)	1.28 (0.86, 1.92)	1.07 (0.85 1.35)	1.20 (0.75 1.92)	1.05 (0.91, 1.22)	0.49
cg08178991	16	*ITGAX**	1.44 (1.19, 1.74)	1.10 (0.90, 1.33)	1.56 (0.89, 2.70)	0.86 (0.77 0.96)	1.25 (0.75 2.07)	1.07 (0.95, 1.20)	0.24
cg14531668	4	*PPARGC1A*	1.32 (1.10, 1.59)	1.03 (0.83, 1.28)	1.21 (0.75, 1.97)	0.83 (0.65 1.05)	0.99 (0.59 1.66)	1.07 (0.85, 1.34)	0.58
cg18234115	1	*KIF26B*	1.22 (1.00, 1.49)	1.29 (1.01, 1.64)	0.50 (0.31, 0.80)	1.11 (0.84 1.48)	1.13 (0.67 1.91)	1.08 (0.82, 1.41)	0.58

[a] Hazard ratio (HR) and 95% CIs comparing the 90th vs the 10th percentiles of differentially methylated CpGs. HRs and p-values correspond to those estimated by Cox regression with that CpG entered in the model together with traditional risk factors (same adjustment as in Table 1, model 2) but without adjustment for other CpGs.

The CpGs are ordered based on the pooled hazard ratio. DMPs selected as predictor of incident CHD in the untargeted elastic-net in the SHS and subsequently by targeted elastic-net models in the four other cohorts are marked with *; all other DMPs were selected in the SHS and in 3 other cohorts.

eTable 4. Hazard ratios (95% confidence intervals) of incident coronary heart disease comparing the 90th vs 10th percentile of differentially methylated CpGs available in the Illumina 450 000 platform and selected by untargeted elastic-net in each of the study cohorts, replicated with targeted elastic-net models in three or four other cohorts, and statistically significant in the meta-analysis

			SHS		WHI		FHS		ARIC-Black		ARIC-White		Meta-analysis
CpG	chr	Gene	HR (95%CI)[a]	p	HR (95%CI)[a]	p	HR (95%CI)[a]	p	HR (95%CI)[a]	p	HR (95%CI)[a]	p	Pooled HR (95%CI)
Inverse pooled HR													
cg05575921	5	*AHRR*	0.82 (0.64, 1.06)	0.14	0.79 (0.60, 1.04)	0.10	0.84 (0.47, 1.51)	0.57	0.48 (0.33, 0.69)	<0.0001	0.75 (0.38, 1.46)	0.39	0.68 (0.54, 0.85)
cg03068497*	7	*GARS1*	0.90 (0.74, 1.11)	0.32	0.84 (0.66, 1.06)	0.14	0.58 (0.34, 0.99)	0.049	0.83 (0.63, 1.09)	0.17	0.45 (0.28, 0.75)	0.02	0.70 (0.53, 0.93)
cg23006040	4	*LOC339975*	0.88 (0.71, 1.08)	0.23	0.97 (0.75, 1.25)	0.80	0.72 (0.44, 1.20)	0.21	0.72 (0.55, 0.93)	0.01	0.46 (0.28, 0.78)	0.003	0.71 (0.53, 0.95)
cg03725309	1	*SARS1*	0.85 (0.66, 1.11)	0.24	0.61 (0.44, 0.85)	0.003	0.58 (0.30, 1.11)	0.10	0.78 (0.48, 1.27)	0.32	0.65 (0.36, 1.19)	0.17	0.76 (0.65, 0.88)
cg14491535	7	*JAZF1*	0.76 (0.61, 0.93)	0.009	0.71 (0.56, 0.90)	0.005	0.68 (0.40, 1.18)	0.17	0.91 (0.69, 1.20)	0.49	0.58 (0.30, 1.13)	0.11	0.76 (0.66, 0.89)
cg05228408*	1	*MTHFR*	0.88 (0.72, 1.09)	0.25	0.84 (0.67, 1.06)	0.14	0.59 (0.33, 1.06)	0.08	0.67 (0.50, 0.91)	0.01	0.63 (0.39, 1.03)	0.07	0.77 (0.65, 0.91)
cg23933602*	10	*RSU1*	0.84 (0.70, 1.00)	0.06	0.73 (0.57, 0.95)	0.02	0.44 (0.23, 0.84)	0.01	0.79 (0.65, 0.96)	0.02	0.73 (0.43, 1.22)	0.23	0.81 (0.74, 0.89)
cg21566642	2	*ALPG*	0.87 (0.68, 1.10)	0.25	0.82 (0.62, 1.10)	0.19	0.61 (0.34, 1.11)	0.11	0.77 (0.55, 1.09)	0.14	0.73 (0.40, 1.34)	0.32	0.82 (0.70, 0.95)
cg01620164	2	*FIGN*	0.89 (0.69, 1.13)	0.33	0.90 (0.70, 1.16)	0.42	1.17 (0.63, 2.16)	0.63	0.60 (0.41, 0.87)	0.007	0.66 (0.35, 1.21)	0.18	0.82 (0.68, 0.98)
cg12479512	3	*RBSN*	0.76 (0.64, 0.91)	0.002	0.86 (0.69, 1.06)	0.16	0.95 (0.61, 1.49)	0.83	0.67 (0.53, 0.83)	<0.0001	0.90 (0.78, 1.03)	0.13	0.85 (0.75, 0.95)
cg16604233*	6	*COL11A2*	0.89 (0.74, 1.06)	0.18	0.86 (0.67, 1.10)	0.22	0.54 (0.29, 1.00)	0.05	0.83 (0.65, 1.07)	0.15	0.71 (0.46, 1.10)	0.12	0.86 (0.78, 0.95)
cg22862003	21	*MX1*	0.95 (0.81, 1.10)	0.48	0.85 (0.70, 1.03)	0.09	0.75 (0.48, 1.18)	0.22	0.75 (0.62, 0.90)	0.02	1.04 (0.74, 1.46)	0.80	0.86 (0.78, 0.95)
cg08860619	16	*UBE2MP1*	0.92 (0.78, 1.09)	0.35	0.87 (0.72, 1.06)	0.17	0.47 (0.31, 0.71)	0.0005	0.85 (0.77, 0.94)	0.02	0.70 (0.46, 1.06)	0.09	0.87 (0.80, 0.95)
cg12303981	6	*GMDS*	0.91 (0.81, 1.03)	0.14	0.89 (0.71, 1.11)	0.30	0.60 (0.38, 0.95)	0.03	0.63 (0.50, 0.79)	<0.0001	0.93 (0.66, 1.29)	0.65	0.91 (0.85, 0.96)
cg10455785	3	*HDAC11*	0.91 (0.78, 1.07)	0.25	0.69 (0.55, 0.87)	0.002	0.52 (0.30, 0.90)	0.02	0.97 (0.80, 1.17)	0.77	0.53 (0.29, 0.96)	0.03	0.93 (0.89, 0.96)
cg15983626	2	*GPC1*	0.94 (0.80, 1.11)	0.47	0.79 (0.64, 0.99)	0.04	0.70 (0.55, 0.89)	0.005	0.96 (0.78, 1.18)	0.70	0.94 (0.87, 1.02)	0.12	0.93 (0.87, 0.99)
Positive pooled HR													
cg07964553	4	*NEUROG2*	1.14 (1.05, 1.25)	0.002	1.18 (1.01, 1.38)	0.04	1.18 (0.92, 1.51)	0.19	1.11 (0.91, 1.36)	0.29	1.21 (0.92, 1.60)	0.17	1.11 (1.06, 1.17)
cg22293458	3	*VPS8*	1.22 (1.06, 1.40)	0.005	1.09 (0.88, 1.34)	0.42	0.82 (0.49, 1.38)	0.46	1.17 (0.99, 1.38)	0.07	1.38 (0.96, 1.97)	0.08	1.11 (1.02, 1.21)

cg12671121	6	*STK19*	1.09 (0.93, 1.28)	0.27	1.23 (1.00, 1.51)	0.048	0.72 (0.43, 1.19)	0.20	1.36 (1.03, 1.80)	0.03	1.67 (1.21, 2.31)	0.002	1.13 (1.01, 1.26)
cg26955383	10	*CALHM1*	1.22 (1.01, 1.47)	0.04	1.12 (0.91, 1.38)	0.29	1.26 (0.81, 1.95)	0.30	1.13 (0.91, 1.41)	0.27	1.31 (0.71, 2.42)	0.38	1.17 (1.05, 1.30)
cg02628823*	4	*MGAT4D*	1.23 (1.06, 1.43)	0.02	0.92 (0.74, 1.15)	0.46	1.21 (0.94, 1.57)	0.15	1.11 (0.91, 1.35)	0.30	1.60 (1.22, 2.11)	0.0007	1.17 (1.01, 1.36)
cg22454769*	2	*FHL2*	1.00 (0.81, 1.23)	0.98	1.17 (0.89, 1.53)	0.25	1.73 (0.90, 3.33)	0.10	1.41 (1.04, 1.92)	0.03	1.24 (0.73, 2.12)	0.43	1.17 (1.01, 1.36)
cg03129964	9	*BARX1*	1.13 (1.00, 1.28)	0.06	1.05 (0.89, 1.22)	0.58	1.35 (1.04, 1.76)	0.03	1.38 (1.17, 1.62)	0.0001	1.33 (0.95, 1.85)	0.09	1.18 (1.06, 1.33)
cg06223172	16	*CBLN1*	1.12 (0.95, 1.34)	0.18	1.06 (0.85, 1.32)	0.60	1.12 (0.69, 1.80)	0.66	1.32 (1.06, 1.65)	0.01	1.65 (0.99, 2.75)	0.05	1.19 (1.05, 1.34)
cg01297357	12	*ASIC1*	1.27 (1.08, 1.49)	0.005	1.02 (0.83, 1.26)	0.86	1.17 (0.87, 1.57)	0.32	1.61 (1.25, 2.08)	0.0002	1.50 (0.88, 2.54)	0.13	1.21 (1.04, 1.40)
cg25950235	15	*MIR9-3*	1.00 (0.82, 1.21)	0.99	1.37 (1.13, 1.67)	0.001	1.15 (0.81, 1.62)	0.44	1.17 (0.91, 1.51)	0.21	1.51 (0.99, 2.32)	0.06	1.21 (1.04, 1.41)
cg09926486	15	*FRMD5*	1.29 (1.08, 1.53)	0.004	1.11 (0.90, 1.38)	0.32	0.88 (0.56, 1.39)	0.59	1.39 (1.12, 1.73)	0.002	1.24 (0.84, 1.82)	0.27	1.23 (1.08, 1.39)
cg08622677	12	*PRMT8*	1.25 (1.03, 1.51)	0.02	1.06 (0.86, 1.32)	0.58	1.23 (0.77, 1.97)	0.39	1.32 (1.07, 1.63)	0.009	1.76 (1.10, 2.82)	0.02	1.23 (1.07, 1.42)
cg05190790*	11	*SOX1*	1.05 (0.89, 1.25)	0.55	1.29 (1.02, 1.63)	0.03	1.27 (0.78, 2.09)	0.34	1.58 (1.19, 2.10)	0.001	1.23 (0.77, 1.95)	0.39	1.26 (1.06, 1.49)
cg09476997*	16	*SLC9A3R2*	1.39 (1.13, 1.70)	0.003	1.16 (0.92, 1.45)	0.21	1.93 (1.05, 3.54)	0.04	1.41 (1.03, 1.93)	0.03	1.56 (0.96, 2.52)	0.07	1.28 (1.14, 1.44)
cg12920180	14	*COCH*	1.12 (0.91, 1.40)	0.29	1.26 (0.99, 1.59)	0.06	0.89 (0.53, 1.49)	0.66	1.31 (0.96, 1.80)	0.09	1.99 (1.18, 3.36)	0.01	1.28 (1.10, 1.49)
cg13543355*	14	*LRRC74A*	1.13 (0.95, 1.35)	0.16	1.17 (0.95, 1.44)	0.15	1.16 (0.74, 1.83)	0.52	1.97 (1.60, 2.43)	<0.0001	1.94 (1.01, 3.73)	0.047	1.28 (1.05, 1.57)
cg03312958	5	*IRX2*	1.31 (1.07, 1.60)	0.009	1.09 (0.86, 1.39)	0.48	2.15 (1.36, 3.38)	0.001	1.34 (1.01, 1.78)	0.04	1.17 (0.76, 1.82)	0.47	1.32 (1.10, 1.59)
cg02003183	14	*CDC42BPB*	1.15 (0.94, 1.41)	0.16	1.41 (1.12, 1.78)	0.004	1.00 (0.63, 1.58)	0.98	1.20 (0.97, 1.50)	0.10	1.54 (0.97, 2.42)	0.06	1.33 (1.10, 1.60)
cg07600636	11	*RASSF10*	1.20 (0.97, 1.47)	0.09	1.15 (0.93, 1.41)	0.21	1.09 (0.66, 1.81)	0.73	2.07 (1.55, 2.76)	<0.0001	1.93 (1.39, 2.69)	<0.0001	1.45 (1.10, 1.90)

[a] Hazard ratios and p-values correspond to those estimated by Cox regression with that CpG entered in the model together with traditional risk factors (similar to models in Table 1, model 2) but without adjustment for other CpGs and ordered based on the pooled HR. HRs shown in italics correspond to the cohort that initially identified that DMP in the untargeted elastic-net model.
*DMPs selected by elastic-net models as predictive of incident CHD in all five cohorts.

eTable 5: Targeted approach for the association of differentially methylated CpGs associated with atherosclerotic cardiovascular disease in previous studies[18,19,26,27] and their association with incident CHD in the Strong Heart Study

CpG	Chr	Gene	Function	Hazard ratio (95% CI) [a]	P-value
cg13980719	2	*TNP1*	Spermatids elongation	0.77 (0.63, 0.94)	0.009
cg02491017	12	*HOXC4*	Transcription factor involved in developmental anterior-posterior axis regulatory system	1.29 (1.07, 1.57)	0.009

cg26215428	4	*PKD2*	Kidney protein (before birth) unclear function	1.27 (1.05, 1.52)	0.012
cg14216068	7	*HOXA3*	Gene expression, morphogenesis, differentiation	1.22 (1.05, 1.43)	0.012
cg25196881*	15	*THBS1*	Uncharacterized	1.22 (1.04, 1.43)	0.013
cg20163085	1	*APITD1*	Apoptosis-inducing gene	1.23 (1.03, 1.47)	0.022
cg08732950	16	*CBFA2T3*	Transcriptional co-repressor. Glycolysis and mitochondrial respiration functions.	1.23 (1.03, 1.48)	0.024
cg07899076	7	*RNF216*	Ring finger protein. Promotes TNF and RIP mediated apoptosis	1.26 (1.03, 1.55)	0.025
cg06639874*	2	*MLPH*	Melanin producer	0.83 (0.70, 0.98)	0.026
cg15006881	8	*GDF6*	Controls proliferation and cellular differentiation in the retina and bone formation	0.82 (0.69, 0.98)	0.026
cg05861567	22	*MLC1*	Astrocyte response to hypo-osmosis	1.29 (1.03, 1.61)	0.028
cg11573170	10	*DIP2C*	Related to autism spectrum disorder	1.20 (1.01, 1.42)	0.034
cg09591524	7	*HOXA3*	Gene expression, morphogenesis, differentiation	1.19 (1.01, 1.40)	0.036
cg23245316*	2	*TSSC1*	Tumor suppressor gene	1.21 (1.01, 1.46)	0.037
cg16374343	17	*ABR*	Regulatory activities small GTP-binding proteins	1.34 (1.01, 1.77)	0.041
cg12935350	2	*MRPS9*	Mitochondria protein synthesis	1.15 (1.00, 1.33)	0.048

NMDA: N-methyl-D-aspartate receptor. TNF: tumor necrosis factor. RIP: receptor interacting protein.

Models adjusted for sex, smoking status (never, former, current), BMI, LDL cholesterol, HDL cholesterol, hypertension treatment (yes / no), type 2 diabetes (yes / no), systolic blood pressure, albuminuria status (microalbuminuria, normal, macroalbuminuria), center (Arizona, Oklahoma, North and South Dakota), blood cell counts (CD8T, CD4T, NK cells, Monocytes, Granulocytes, and B cells) and five genetic PCs. Age was the time scale with age at baseline treated as staggered entries.

* CpGs associated with CVD in Agha et al 2019 (effect estimates for cg25196881 and cg23245316 are in opposite direction in SHS and Agha et al; effect estimate for cg06639874 is in the same direction).[18]

eTable 6: Untargeted approach (EWAS) for coronary heart disease in the Strong Heart Study and replication in the Framingham Heart Study and the Women's Health Initiative

			Strong Heart Study		Women's Health Initiative		Framingham Heart Study		Atherosclerosis Risk in Communities (Black)		Atherosclerosis Risk in Communities (White)	
CpG	Chr	Gene	Hazard ratio (95% CI) [a]	FDR	Hazard ratio (95%CI)	p-value	Hazard ratio (95%CI)	p-value	Hazard ratio (95%CI)	p-value	Hazard ratio (95%CI)	p-value
cg10812236	2	*PLEK*	2.24 (1.66, 3.02)	0.08	0.95 (0.67, 1.36)	0.79	0.47 (0.18, 1.24)	0.13	1.18 (0.70, 1.99)	0.52	0.84 (0.33, 2.12)	0.71
cg22512011	13	*KCTD12*	0.47 (0.36, 0.63)	0.09	--	--			--			
cg24914185	2	*FTCDNL1*	1.84 (1.45, 2.33)	0.09	0.99 (0.77, 1.27)	0.92	0.79 (0.40, 1.51)	0.47	1.75 (1.21, 2.54)	0.003	0.84 (0.47, 1.50)	0.56
cg17651972	12	*NXPH4*	2.12 (1.58, 2.85)	0.09	0.99 (0.72, 1.37)	0.97	0.50 (0.20, 1.21)	0.13	0.97 (0.55, 1.73)	0.93	1.43 (0.73, 2.69)	0.29
cg11498967	1	*C1orf198*	0.62 (0.51, 0.75)	0.09	--	--			--		--	
cg10307212	4	*TEC*	1.95 (1.49, 2.54)	0.09	--	--			--		--	
cg06897548	22	*TAB1*	1.79 (1.42, 2.26)	0.09	--	--			--		--	
cg13958199	9	*NEK6*	1.99 (1.51, 2.62)	0.09	0.95 (0.72, 1.25)	0.72	0.69 (0.34, 1.41)	0.31	1.21 (0.74, 1.97)	0.44	0.79 (0.58, 1.09)	0.15
cg05710777	2	*LINC02245*	0.59 (0.48, 0.73)	0.09	--	--			--		--	
cg02079181	1	*RHEX*	0.42 (0.30, 0.60)	0.09	--	--			--		--	
cg04872689	2	*PLEK*	1.95 (1.49, 2.56)	0.09	0.99 (0.73, 1.36)	0.97	0.43 (0.19, 0.98)	0.05	2.21 (1.34, 3.64)	0.002	0.96 (0.48, 1.94)	0.91

FDR: false discovery rate (for FHS, WHI and ARIC the p-values are nominal).

The hazard ratios (95% confidence intervals) for coronary heart disease compare the 90th vs. 10th percentiles of DNA methylation for each CpG entered individually in models adjusted for sex, smoking status (never, former, current), BMI, LDL cholesterol, HDL cholesterol, hypertension treatment (yes / no), type 2 diabetes (yes / no), systolic blood pressure, albuminuria status (microalbuminuria, normal, macroalbuminuria), center (Arizona, Oklahoma, North and South Dakota), blood cell counts (CD8T, CD4T, NK cells, Monocytes, Granulocytes, and B cells) and five genetic PCs.

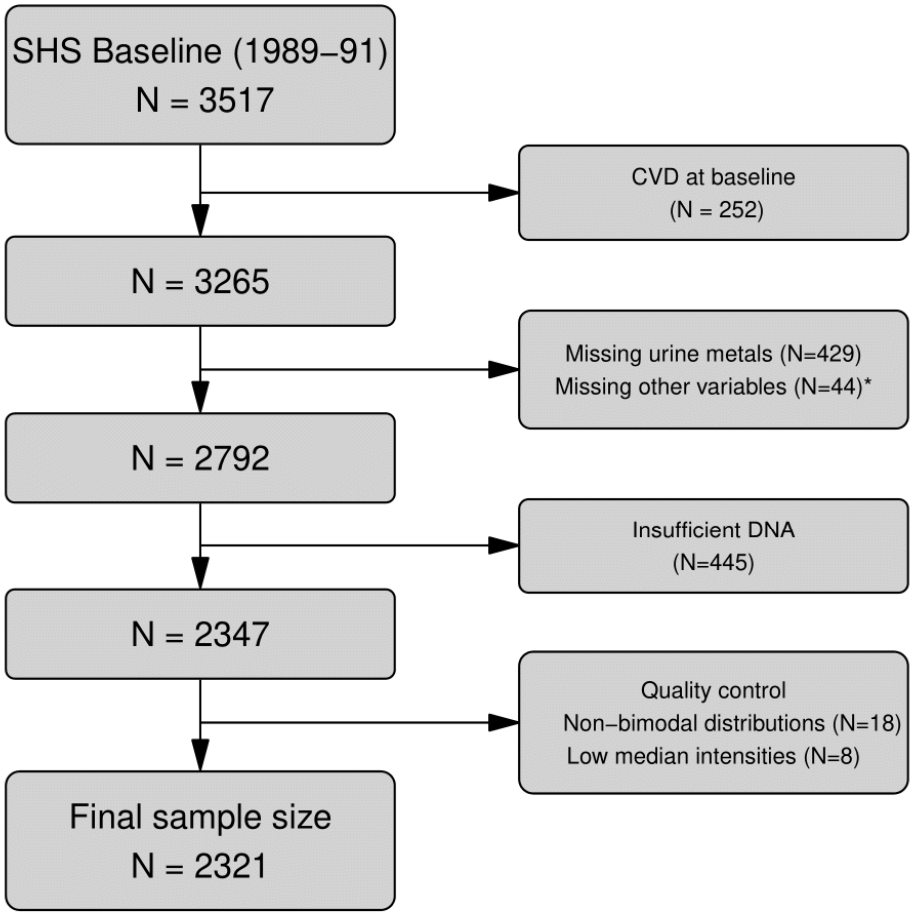
For FHS, WHI and ARIC only CpG sites that are present in 450k are shown

eTable 7: Differentially methylated regions (DMRs) associated with incident coronary heart disease (CHD) in the Strong Heart Study

Region	Width	N CpGs	Stouffer p-value	Overlapping promoters	Nearest gene	Gene function
chr15:78009566-78010791	1226	7	0.051	-	*LINGO1*	Neuronal precursor cell motility
chr13:31019673-31020468	796	5	0.076	-	*HMGB1*	Transcriptional regulation, inflammation, cell differentiation, platelet activation and tumor cell migration
chr6:15400462-15401067	606	6	0.081	JARID2-202	*JARID2*	Transcriptional repressor. Regulates gene expression during embryonic development
chr7:97755534-97755792	259	6	0.090	LMTK2-003	*LMTK2*	Nerve growth factor, signaling and endosomal membrane trafficking
chr3:196351327-196352455	1129	6	0.092	-	*NRROS*	Microglia function in the nervous system

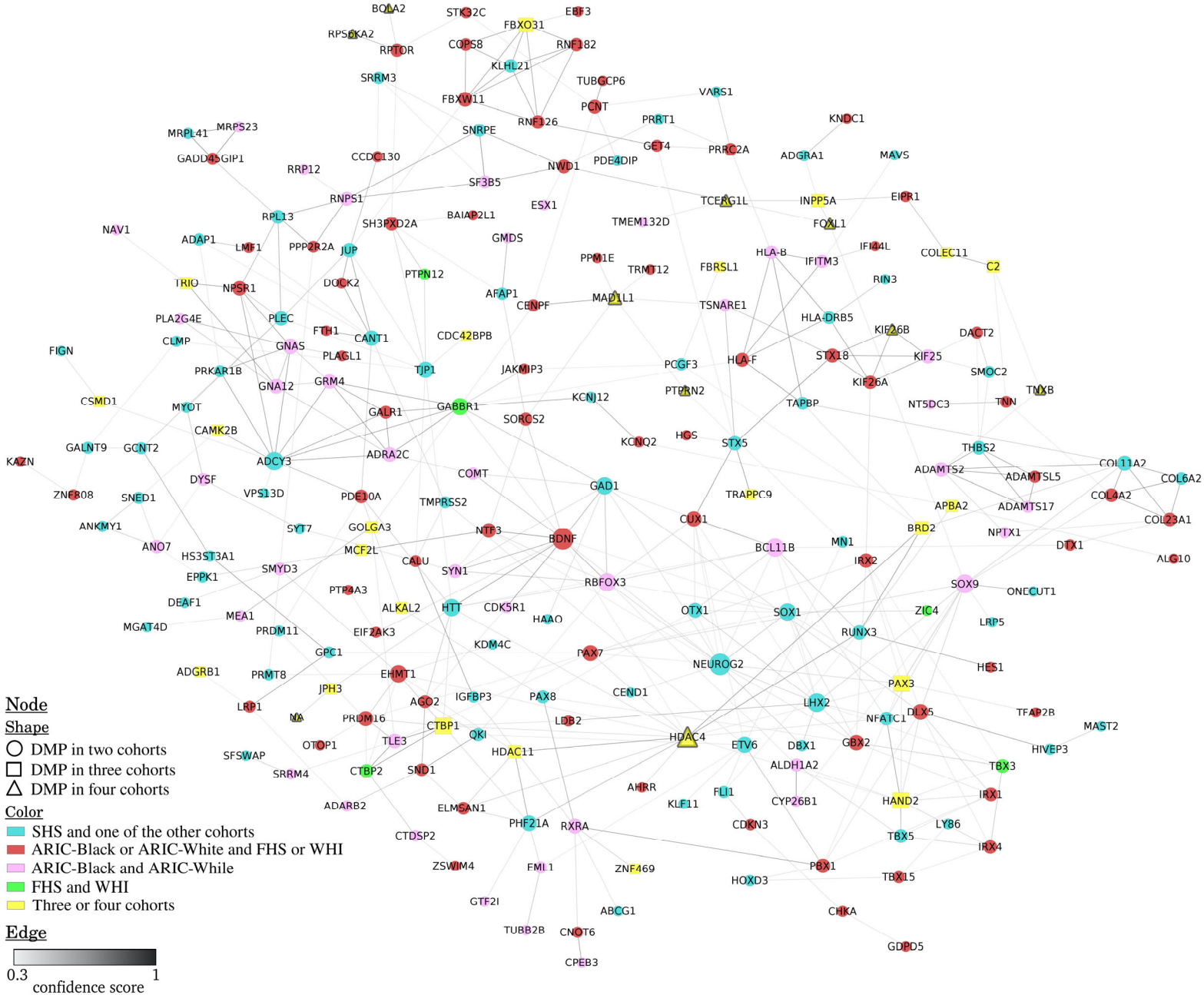
Models adjusted for sex, smoking status (never, former, current), BMI, LDL cholesterol, HDL cholesterol, hypertension treatment (yes / no), type 2 diabetes (yes / no), systolic blood pressure, albuminuria status (microalbuminuria, normal, macroalbuminuria), center (Arizona, Oklahoma, North and South Dakota), blood cell counts (CD8T, CD4T, NK cells, Monocytes, Granulocytes, and B cells) and five genetic PCs.

eFigure 1. Flowchart of the data exclusion process of the Strong Heart Study

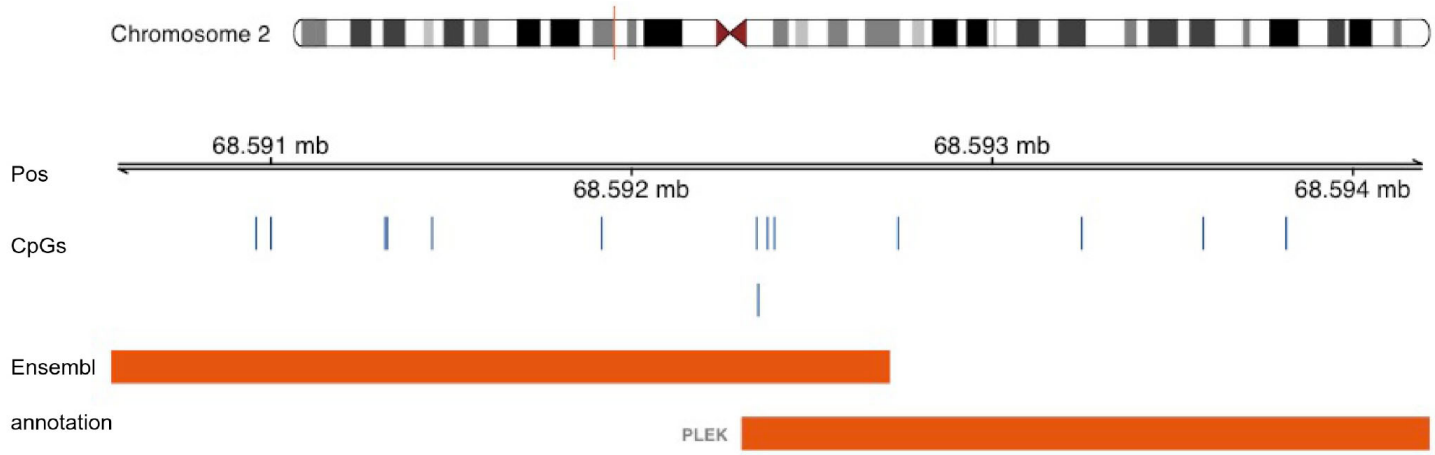


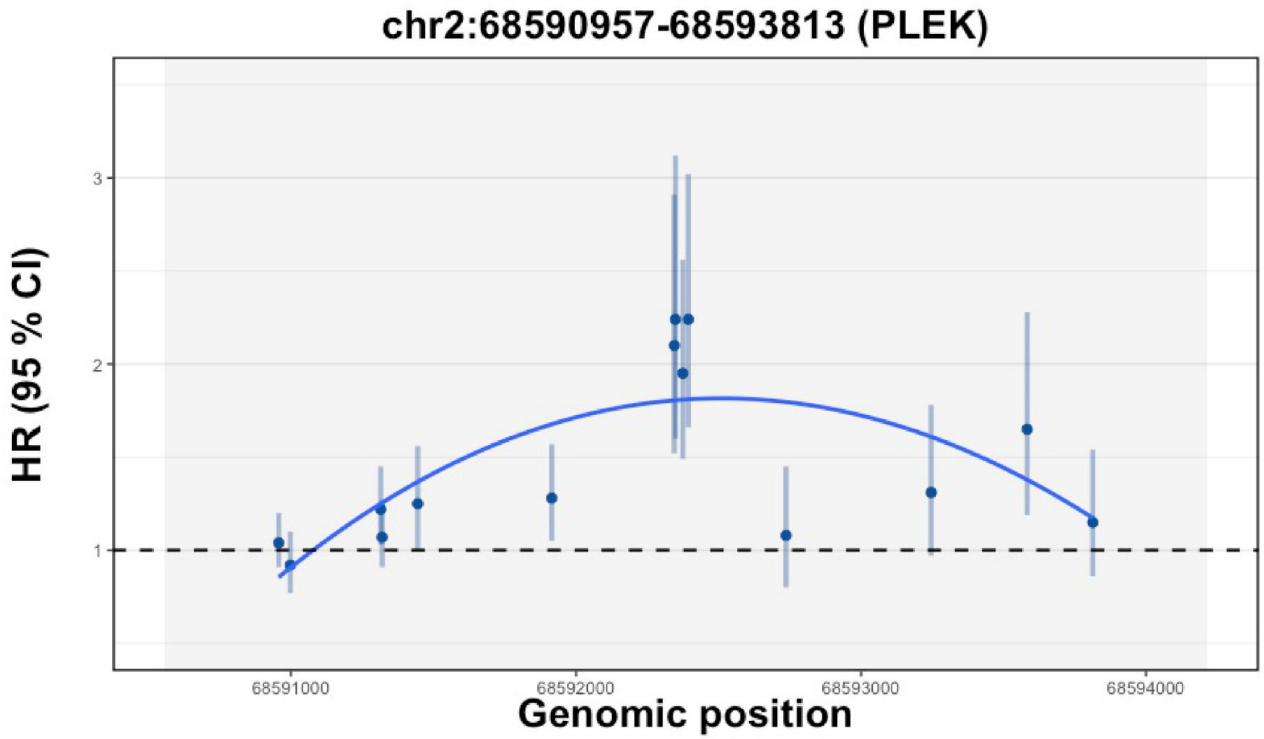
* 8 participants missing BMI, 9 diabetes, 11 systolic blood pressure, 16 cholesterol

eFigure 2. Protein-protein interaction network of genes annotated to DMPs selected by untargeted elastic-net models in two or more cohorts
The network includes 139 nodes. The size of the nodes is proportional to the number of connections. The edges indicate confidence score interaction (only confidence ≥0.5 were included).



eFigure 3: Hazard ratio (95% confidence intervals) for coronary heart disease and several hypermethylated CpGs annotated to *PLEK* in the Strong Heart Study. The effect estimates report the hazard ratios for incident coronary heart disease comparing the 90th to the 10th percentile of DNA methylation for 14 CpGs annotated to the *PLEK* gene on chromosome 2. Models adjusted for sex, smoking status (never, former, current), BMI, LDL cholesterol, HDL cholesterol, hypertension treatment (yes / no), type 2 diabetes (yes / no), systolic blood pressure, albuminuria status (microalbuminuria, normal, macroalbuminuria), center (Arizona, Oklahoma, North and South Dakota), blood cell counts (CD8T, CD4T, NK cells, monocytes, granulocytes, and B cells) and five genetic PCs.





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