Supplementary Online Content

Aslibekyan S, Agha G, Colicino E, et al. Association of methylation signals with incident coronary heart disease in an epigenome-wide assessment of circulating tumor necrosis factor α. *JAMA Cardiol.* Published online April 4, 2018. doi:10.1001/jamacardio.2018.0510.

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This supplementary material has been provided by the authors to give readers additional information about their work.

eMethods 1. Descriptions of participating studies.

Framingham Heart Study (FHS)

The FHS offspring cohort is a community-based cohort recruited in 1971 and included the offspring (and their spouses) of the FHS original cohort. The eligible sample for this investigation (n=1875) was drawn from participants in the FHS offspring cohort who gave consent for genomic studies and had TNFα assays completed on plasma samples collected at the seventh examination cycle (1998-2001) and DNA methylation assays completed on whole blood samples collected at the eighth examination cycle (2005-2008). The mean (SD) time between TNFα and DNA methylation measurements was 6.5 (0.7) years. At each examination, participants provided fasting blood samples and had a standardized medical examination, including obtaining smoking history, current medication use, and height and weight. Details are available at http://www.framinghamheartstudy.org/. Participants (n=125) who reported having an auto-immune disease or taking immune modulating agents (oral corticosteroids, hormone replacement therapy, TNFα blockers, etc.) were excluded. After exclusions and assay quality control filtering, the final sample size of 1730 was used in this analysis. The FHS protocols were approved by the Institutional Review Board of Boston University School of Medicine and written informed consents were obtained from all participants. Participant-level phenotype and genotype data from the Framingham Heart Study are accessible from the U.S. National Center for Biotechnology Information (NCBI) database of Genotypes and Phenotypes (dbGaP) at https://dbgap.ncbi.nlm.nih.gov/ to approved scientific investigators pursuing research questions that are consistent with the informed consent agreements provided by individual research participants. The FHS methylation data are available at dbGaP under the accession number phs000724.v2.p9 and gene expression data at accession number phs000363.v3.p6. Participants were not renumerated in any way.

Genetics of Lipid Lowering Drugs and Diet Network (GOLDN)

In 2002-2004, GOLDN recruited self-identified European American participants from three-generational families previously participating in the National Heart, Lung, and Blood Institute Family Heart Study at the Minneapolis and Salt Lake City sites. Participants were screened and excluded if they met the following criteria: extreme hypertriglyceridemia (>1500 mg/dL), chronic disease (e.g. cardiovascular, renal, or hepatic conditions), pregnancy/ breastfeeding/ not using contraception. Additionally, participants were excluded if they were acutely sick (e.g. with a cold), had a chronic autoimmune disease, or were using immunomodulating drugs. DNA methylation, TNFα, and all relevant covariates were measured at baseline, during the same examination. The final sample size included 970 individuals who provided informed consent to their use of DNA in research and had the requisite exposure, outcome, and covariate information. All were approved by the Institutional Review Boards of University of Minnesota, University of Utah, University of Alabama at Birmingham, and Tufts University/New England Medical Center; all participants provided written informed consent. Participants were paid $50 per each study visit. Methylation data on GOLDN participants are available at dbGap under the accession number phs000741.v2.p1.

Helsinki Birth Cohort Study (HBCS)

The HBCS comprises 13345 individuals (6370 women and 6975 men), born as singletons between 1934 and 1944 in one of the two main maternity hospitals in Helsinki and who were living in Finland in 1971 when a unique personal identification number was allocated to each member of the Finnish population. The HBCS has been approved by the Ethics Committee of the National Public Health Institute. Register data were linked with permission from the Finnish Ministry of Social Affairs and Health and the Finnish National Archives. In 2001–2004 at an average age of 61.5 years (SD=2.9 and range=56.7–69.8 years), a randomly selected subsample of the cohort comprising 2003 individuals (1075 women and 928 men) was invited to a clinical examination including collection of a blood sample for (epi)genetic and biochemical studies, measures of weight and height, and survey on health behaviors, such as smoking, personal characteristics and well-being. Of this sample, assaying TNFα was successful in 1989 participants. For 269 participants, extraction of DNA was not successful, or DNA showed gender discrepancy or close relatedness. The excluded and the included participants did not differ from each other in any of the study variables (P>0.13). From the remaining sample of 1720 individuals, 115 women and 97 men had been evacuated to Sweden and Denmark during World War II according to the Finnish National Archives' register. Methylation profiles were measured in 80 evacuated men and 79 non-evacuated controls matched for sex, birth year and father's occupational status in childhood. In this study, the analyses are based on 78 evacuated men and 76 non-evacuated controls with TNFα levels within 4SD. All participants provided written informed consent, they were not renumerated in any way, and their data are not publicly available.

Invecchiare in Chianti (InCHIANTI)
The InCHIANTI study is a population-based epidemiological study aimed at evaluating the factors that influence mobility in the older population living in the Chianti region in Tuscany, Italy. Briefly, 1616 residents were selected from the population registry of Greve in Chianti (a rural area: 11709 residents with 19.3% of the population greater than 65 years of age), and Bagno a Ripoli (Antella village near Florence; 4,704 inhabitants, with 20.3% greater than 65 years of age). The participation rate was 90% (n=1453), and the age of the participants ranged between 21 and 102 years. The study protocol was approved by the Italian National Institute of Research and Care of Aging Institutional Review and Medstar Research Institute (Baltimore, MD). Information on smoking behavior and BMI measurements were collected at the same time as blood samples used to quantify DNA methylation. This analysis was restricted to 498 subjects with data on DNA methylation and TNFα measures. All participants provided written informed consent, and their data are not publicly available.

Kooperative Gesundheitsforschung in der Region Augsburg (KORA)
The Cooperative Health Research in the Region of Augsburg (KORA) study is a series of independent surveys and follow-up studies from the general population living in the region of Augsburg, Southern Germany. All participants are residents of German nationality identified through the local registration offices. The study was approved by the ethics committee of the Bavarian Medical Association, and informed written consent was obtained from all participants. For the present study, methylation measurements were performed on whole blood samples from the KORA F4 study, conducted in 2006-2008. DNA methylation and TNFα data were available in 845 subjects in F4. For the present analysis, 43 subjects taking anti-inflammatory agents and 2 subjects with outlying (>4SD from the mean) values of TNFα were excluded, producing the final sample size of 800. Participants were not renumerated in any way. KORA transcriptomics are publicly available at E-MTAB-1708.

Lothian Birth Cohort 1921 (LBC1921)
LBC1921 is a longitudinal study of aging, derived from the Scottish Mental Survey of 1932. Survivors of the Survey living in the Lothian area of Scotland were recruited at the mean age of 79 (n=550). Follow-up has taken place at ages 79, 83, 87, 90, and 92 years. The 87 year visit was used in the following analysis. Collected data include genetic and epigenetic information, numerous blood biomarkers, anthropomorphic and lifestyle measures. One individual was removed from the analysis due to their outlying TNFα reading (>4SD from the mean). Post quality control, DNA methylation and TNFα data were available for 165 LBC1921 participants. Study protocol was approved by the appropriate Institutional Review Board (LREC1702/98/4/183). Written informed consent was obtained for all participants. Participants were not renumerated in any way. LBC methylation data have been submitted to the European Genome-phenome Archive –accession number EGAS00001000910.

Normative Aging Study (NAS)
The US Department of Veterans Affairs NAS is an ongoing longitudinal cohort of aging men established in 1963. Participants were 21-80 years of age and free of known chronic medical conditions at enrollment. Clinical health data and demographic factors were collected at 3-5 year intervals and supplemented with periodic medical examinations. DNA samples for the methylation analysis were collected from the 675 active participants in 1999-2007. Participants were excluded if they were not of European descent or had missing information on TNFα or covariates, leaving a total of 631 participants. NAS was approved by the Institutional Review Boards (IRBs) of the participating institutions. Participants have provided written informed consent at each visit. The authors (EC) are not aware of any renumerations. NAS data are partially available on dbGaP under the accession numbers phs000853.v1.p1.

Northern Finland Birth Cohort (NFBC)
NFBC 1966 is a prospective follow-up study of children from the two northernmost provinces of Finland. 96% of all women in this region with expected delivery dates in 1966 were recruited though maternity health centers (12,058 live births). All individuals still living in northern Finland or the Helsinki area (n=8,463) were contacted and invited for clinical examination. A total of 6007 participants attended the clinical examination, when blood samples for measurement of DNA methylation and TNFα measurements were obtained. This subset is representative of the original cohort in terms the major environmental and social factors known to influence the tested trait. DNA methylation was measured for 807 randomly selected subjects that attended the clinical examination and completed the questionnaire. Written informed consent for the use of the data including DNA

was obtained from all subjects. Participants were not renumerated in any way, and data are not publicly accessible.

Rotterdam Study (RS)

The RS is a prospective population-based cohort study in the well-defined area of Rotterdam, the Netherlands. The present study is restricted to the random subset of participants of the third RS cohort (aged 45 years and older), who had measurements of whole blood DNA methylation, gene expression, and genotype (n=750). Blood samples were obtained during the first visit of the third cohort between February 2006 and December 2008. During the research center visit, anthropometric measures including height and weight were obtained. Body mass index was calculated as weight in kilogram divided by height in meters squared. Smoking behavior (current, former and never) was assessed during home interview by trained research assistants. White blood cells counts (monocytes, granulocytes and lymphocytes) were measured immediately at the research center using a standard hematology analyzer (Beckman Coulter). TNFα was not measured in RS participants, and therefore these data were only used for methylation vs. expression and methylation vs. sequence variation analyses. The RS protocols were approved by the Medical Ethics Committee of the Erasmus MC and by the Ministry of Health, Welfare and Sport of the Netherlands as per the "Wet Bevolkingsonderzoek: ERGO (Population Studies Act: Rotterdam Study)." All participants provided written informed consent. Participants were not renumerated in any way. RS methylation and genetic data are not publicly available. The expression data are available in the GEO repository, GSE33828.

eMethods 2. Methods for gene expression measurement and analysis.

Measurements

Framingham Heart Study (FHS)

Expression data in FHS were obtained from whole blood samples collected in PaxGene™ tubes (BD Biosciences), collected at the same time as the samples for quantification of DNA methylation, and assayed using the Affymetrix Human Exon Array ST-1.0. The FHS gene expression data are available on dbGAP (accession number: phs000363.v15.p10). The association of DNA methylation with gene expression was examined in 2262 participants. Study-specific covariates were the family structure covariance and technical covariates were row, chip, and column.

Kooperative Gesundheitsforschung in der Region Augsburg (KORA)

Whole blood was collected in PAXgene™ tubes (BD Biosciences, Germany) and frozen at −80°C. RNA was extracted using the whole blood RNA System Kit (Qiagen, Venlo, Netherlands) and mRNA expression profiling was assessed using Illumina HT-12 v3 platform, which contains more than 47,000 probes targeting the expression of ~20,000 genes. Quantile normalization and L2T was performed in R using the *lumi* package from the Bioconductor open source software (http://www.bioconductor.org/). The methylation-expression associations were examined in 726 participants that had complete measurements that passed quality control. Technical covariates included RNA amplification batch, RNA integrity number (RIN), and sample storage time.

Rotterdam Study (RS)

Whole-blood was collected (PAXGene™ tubes, BD Biosciences) and total RNA was isolated (PAXGene™ Blood RNA kits, Qiagen). To ensure a constant high quality of the RNA preparations, all RNA samples were analysed using the Labchip GX (Calliper) according to the manufacturer's instructions. Samples with an RNA Quality Score of 7 or higher were amplified and labeled (Ambion TotalPrep RNA), and hybridized to the Illumina HT-12 v4 Expression Beadchips as described by the manufacturer's protocol. Processing of the Rotterdam Study RNA samples was performed at the Genetic Laboratory of Internal Medicine, Erasmus University Medical Centre Rotterdam. The RS-III expression dataset is available at GEO (Gene Expression Omnibus) public repository under the accession number GSE33828. Illumina gene expression data was quantile-normalized to the median distribution and subsequently log2-transformed. The probe and sample means were centered to zero. Genes were declared significantly expressed when the detection P values calculated by GenomeStudio were less than 0.05 in more than 10% of all discovery samples, which added to a total number of 21,238 probes. Quality control was done using the eQTL-mapping pipeline. Only probes that uniquely mapped to hg 37 were analyzed. The expression analyses (n=750) considered genomic regions 500kb up- and downstream of the CpG of interest.

Statistical Analysis

All three cohorts conducted the expression-methylation association (eQTM) analyses in two stages. First, both DNA methylation β scores and gene expression values were residualized with adjustment for age, sex, imputed cell count proportions, as well as study-specific and technical covariates detailed in eMethods 2. Second, the eQTM linear regression models were fit with the gene expression residual as the dependent variable and the methylation residual as the independent variable. FHS models were additionally adjusted for 25 methylation surrogate variables (SVs) and 25 expression SVs to account for unmeasured technical and batch effects. Statistical significance was established using the Bonferroni threshold of 0.05/number of hypotheses tested, i.e. the number of CpG-gene transcript pairs.

eMethods 3. Methods for genotyping and methylation quantitative trait loci analysis.

Genotyping and Imputation

Genetics of Lipid Lowering Drugs and Diet Network (GOLDN)

GOLDN participants were genotyped at 906,600 loci using the Human SNP Array 6.0 (Affymetrix). SNPs were excluded from subsequent analyses if they were monomorphic, had a call rate of < 96%, or exhibited Mendel errors as follows: 3+ families with errors if minor allele frequency (MAF) of a given SNP > 20%, 2+ families with errors if 20% ≥ MAF>10%, 1+ family with errors and 10% ≥ MAF>5%, or SNPs with MAF < 5% and Mendel errors in any family. Of 718,452 SNPs that passed quality control procedures described above, only 12 had a Hardy-Weinberg $P < 10^{-6}$. Variants with MAF < 1% were excluded, leaving 654,634 SNPs for imputation. Phase 1 release of 1000 Genomes reference panel was used for imputation, performed with MACH (pre-phasing) and Minimac (imputation) software (Abecasis Lab, Ann Arbor, MI). After merging the typed and imputed data and removing SNPs with imputation $r^2 < 0.3$ or MAF <1%, 9,432,837 variants were available for the analysis. 690 participants passed quality control and were included in the methylation quantitative trait loci analysis.

The Rotterdam Study (RS)

Participants from the RS third cohort (n=3,540) were genotyped using the Human610-Quad BeadChip (Illumina). After excluding samples with the call rate of <97.5% and SNPs with a call rate <95% and Hardy-Weinberg $P < 10^{-6}$, 543,360 SNPs and 3,504 participants remained. To obtain imputed data, more restrictive SNP filters (namely minimum minor allele frequency of 0.01 and SNP call rate of 0.98%) were applied and 514,073 SNPs passed the filters. 2,543,887 SNPs were imputed using phased haplotypes of 1000 Genomes Phase 1 in MACH software. 731 participants with both methylation and genotype data that passed quality control were included in the methylation quantitative trait loci analysis.

Statistical Analysis

In the discovery phase, we created residuals by regressing inverse-normal transformed methylation β scores on the first ten methylation principal components and up to the first ten ancestry principal components. The residuals then served as the dependent variables in a linear regression model with SNPs as predictors. SNPs with low imputation quality ($r^2 < 0.4$) and low frequency variants (MAF < 0.01) were removed from the analyses. SNPs with the FDR < 0.05 were carried forward to replication. In the replication phase, we fit linear mixed models with the methylation β scores as outcomes, adjusted for age, sex, study site, four CD4+ T-cell purity principal components (fixed effects) and family relatedness (random effect). Due to limited evidence of population stratification in GOLDN, we did not adjust for ancestry.

eMethods 4. Methods for the DNA methylation vs. incident coronary heart disease meta-analysis.

Study Populations

Four of the six cohorts included in the circulating TNFα meta-analysis also participated in the incident coronary heart disease (CHD) meta-analysis: FHS, InCHIANTI, KORA, and NAS. Other cohorts included Atherosclerosis Risk in Communities Study (ARIC), Cardiovascular Health Study (CHS), Long-term Follow-up of Antithrombotic Management Patterns in Acute Coronary Syndrome (EPICOR), and Women's Health Initiative (WHI). The WHI cohort included two independent studies: Epigenetic Mechanisms of Particulate Matter-Mediated Cardiovascular Disease (WHI-EMPC) and the Integrative Genomics and the Risk of CHD and Related Phenotypes in the Women's Health Initiative (WHI-BAA23).

DNA Methylation Measurements and Quality Control

All cohorts measured DNA methylation using the Infinium HumanMethylation450 Beadchip (Illumina) on whole blood samples. Quality control procedures were conducted separately using cohort-specific filtering criteria, e.g. non-significant detection P for a high percentage of probes, low sample DNA, low bisulfate conversion efficiency, gender or genotype mismatch, high percentage of probes with missing values, or other quality control diagnostics. Normalization procedures were performed separately in each cohort and methylation was quantified using β scores.

Outcome Definition

Incident CHD outcomes were harmonized across all cohorts as a composite outcome including myocardial infarction (MI, defined as diagnostic ECG changes and/or cardiac markers of MI) coronary insufficiency, coronary revascularization, and coronary death. In EPICOR, InCHIANTI, and KORA, the CHD definition was restricted to MI. Hospitalization and death records were screened, and events were adjudicated by a panel of 3 physicians. Individuals who had prevalent CHD at the time of the DNA methylation assay were not included in the analysis.

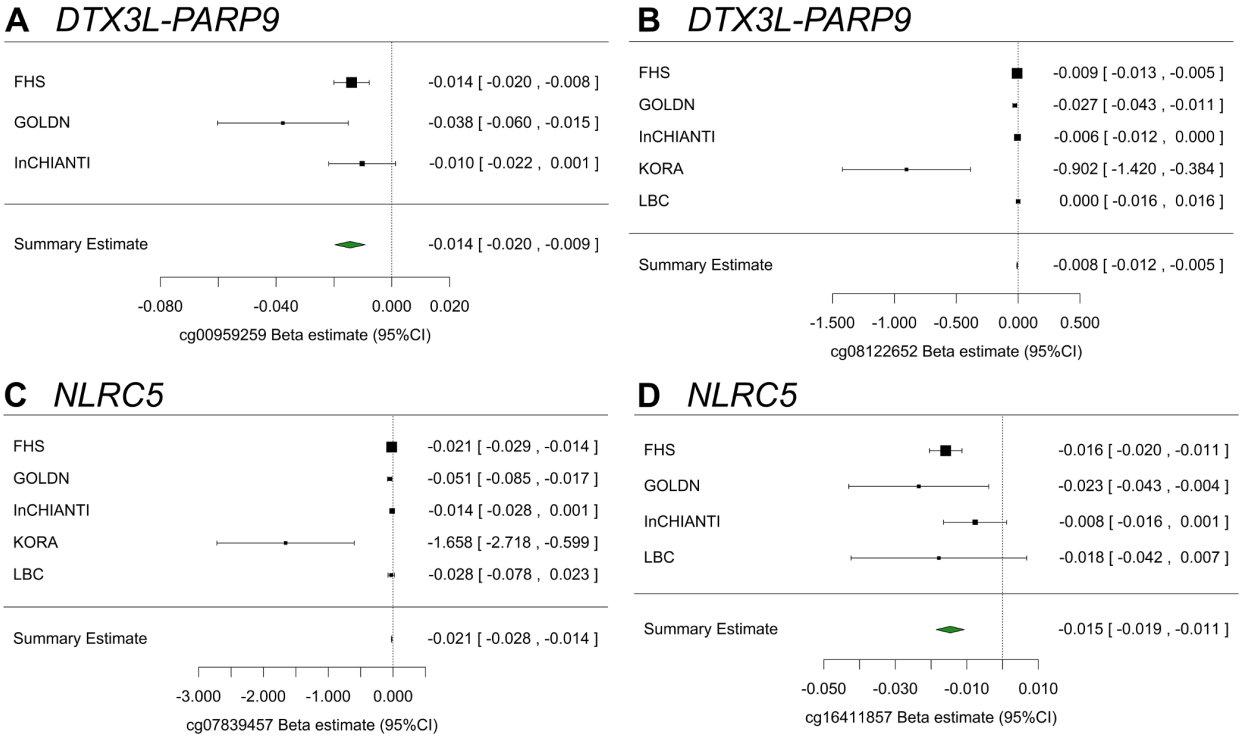
Cohort-Specific Statistical Analyses

The majority of cohorts (6 of 8) used a Cox proportional hazards model (or penalized Cox regression if the number of events is low) to analyze baseline DNA methylation at each CpG site in relation to incident CHD. EPICOR conducted incident density sampling within a nested case-control design and implemented logistic regression analyses, which provide odds ratios that approximate the hazards ratio due to the incident sampling strategy. WHI-BAA23 profiled prospectively adjudicated CHD cases and controls that occurred during follow-up, therefore penalized logistic regression models were used. Five cohorts only recruited individuals of European ancestry (FHS, KORA, EPICOR, InCHianti, NAS), while four cohorts (ARIC, CHS, WHI-EMPC, and WHI-BAA23) contributed data from both European and African American participants. All cohorts performed race-specific regression analyses, adjusting for age, sex, smoking status (current / former / never), education (either as years of education or categorical), body mass index (kg/m2), differential cell count (either directly measured or imputed using the Houseman method), and batch-related technical variables appropriate for their analyses.

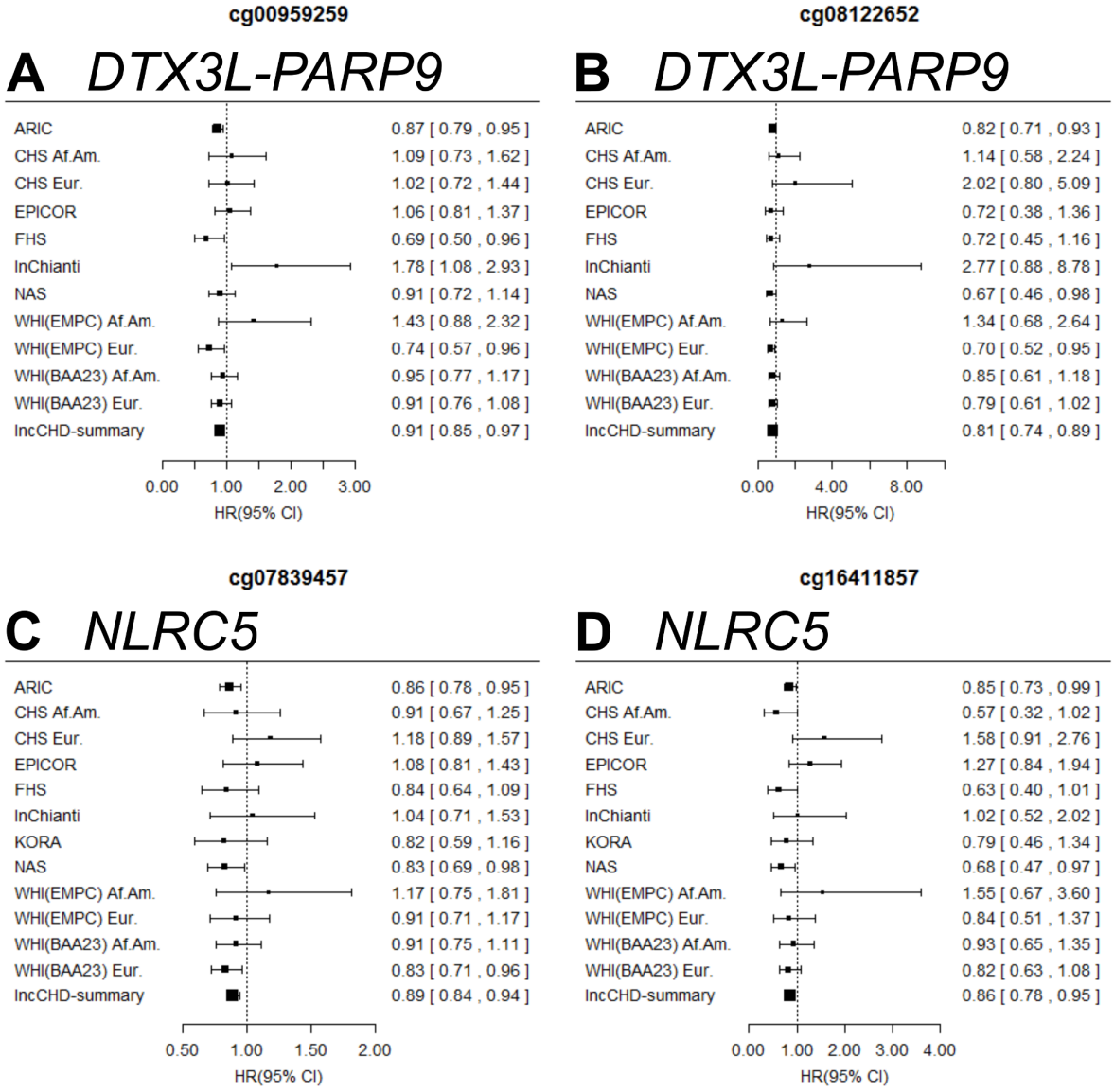
Meta-Analysis

Prior to the meta-analysis, we conducted a second round of quality control and further ensured that non-CpG probes, probes mapping to non-autosomal chromosomes, and cross-hybridizing probes were excluded. We further restricted the meta-analysis to probes that had non-missing methylation data for at least three of the included cohorts. We performed inverse-variance-weighted fixed-effects meta-analysis using the *metafor* package in R. We excluded polymorphic CpG sites, considered to be those where a SNP in the 1000 Genomes Project with a minor allele frequency > 0.01 resided at the target CpG or within 10 bp of the probed CpG, using the *Illumina450ProbeVariants.db* bioconductor package in R. We further excluded sites that had significant inter-study heterogeneity, assessed with the Cochran's Q test. Only four CpG sites that emerged as replicated hits in the circulating TNFα meta-analysis were included in this lookup.

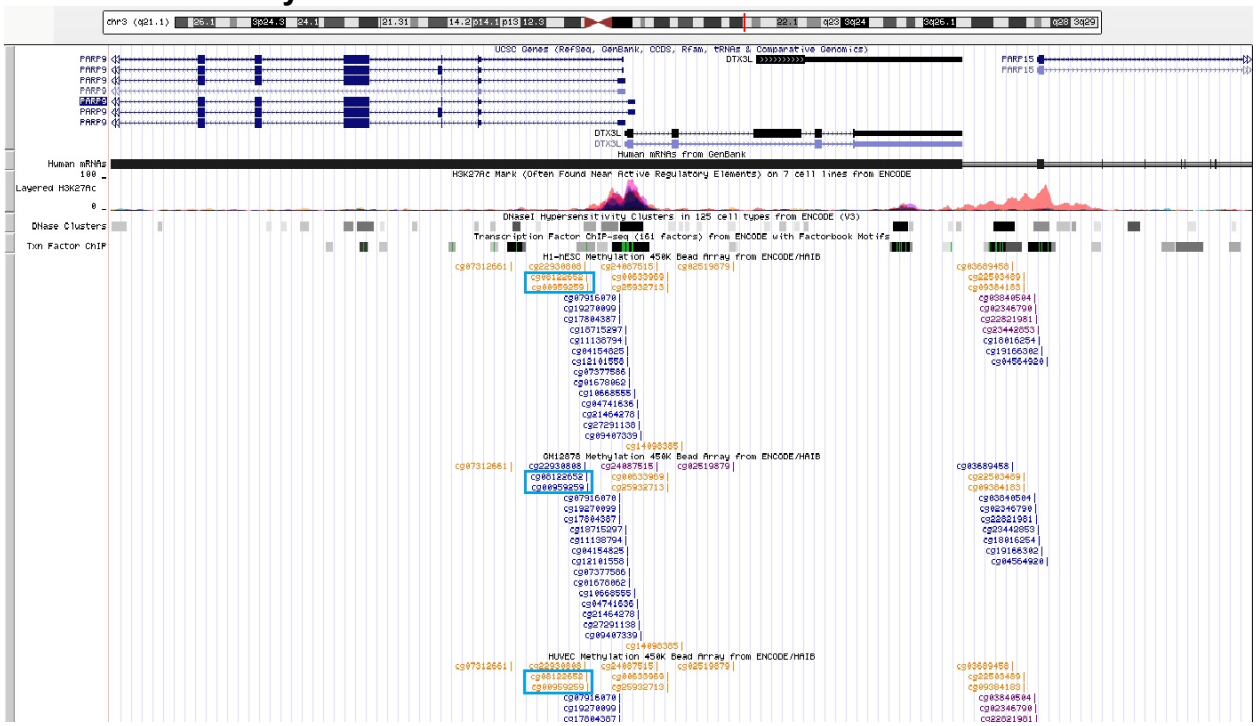
eFigure 1. Forest plots for the associations between top *NLRC5* and *DTX3L-PARP9* loci and circulating TNFα. Panels A-D represent cg00959259, cg08122652, cg07839457, and cg16411857, respectively.



eFigure 2. Forest plots for the associations between top *NLRC5* and *DTX3L-PARP9* loci and the risk of incident CHD. Panels A-D represent cg00959259, cg08122652, cg07839457, and cg16411857, respectively.

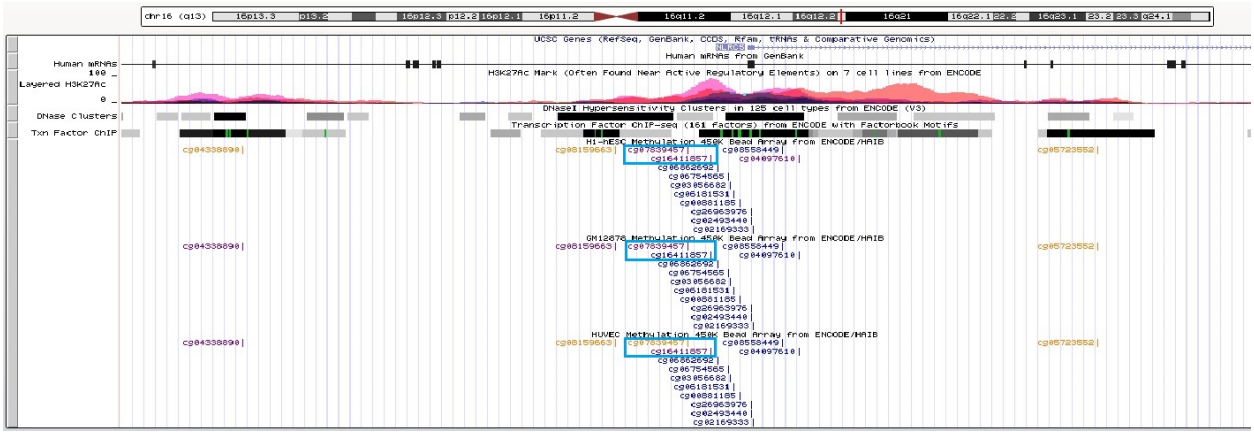


eFigure 3. Bioinformatic annotation of the genomic region containing the top *DTXL3/PARP9* methylation loci.



The top CpG sites associated with TNFα are framed in blue; their methylation status (orange= methylated, purple= partially methylated, blue= unmethylated) is displayed by human cell type (top= embryonic stem cells, middle= B-lymphocytes, bottom= umbilical vein endothelial cells). Other overlapping features include, from top to bottom: known genes, messenger RNAs, H3K27Ac histone peaks indicating proximity to regulatory elements, DNase I hypersensitivity clusters, and transcription factors. Figure generated with UCSC Genome Browser.

eFigure 4. Bioinformatic annotation of the genomic region containing the top *NLRC5* methylation loci.



The top CpG sites associated with TNFα are framed in blue; their methylation status (orange= methylated, purple= partially methylated, blue= unmethylated) is displayed by human cell type (top= embryonic stem cells, middle= B-lymphocytes, bottom= umbilical vein endothelial cells). Other overlapping features include, from top to bottom: known genes, messenger RNAs, H3K27Ac histone peaks indicating proximity to regulatory elements, DNase I hypersensitivity clusters, and transcription factors. Figure generated with UCSC Genome Browser.

eTable 1. Quality control procedures for Illumina Infinium Human Methylation450K BeadChip data across cohorts.

Study	Tissue	Background Correction	N Bead Filtering	Detection P[a]	Probe Call %	Sample Call %	Outliers Removal	Sex Mismatch	Genotype Mismatch	Normalization
FHS	Whole blood	Yes	No	0.01	80	99	Yes	Yes	Yes	DASEN
GOLDN	CD4+ T-cells	No	Yes (<3)	0.01	90	98.5	No	Yes	No	ComBat
HBCS	Whole blood	No	No	0.01	87	97	Yes	Yes	No	Functional normalization (*minfi*)
InCHIANTI	Whole blood	Yes	Yes (<3)	0.01	95	95	Yes	Yes	Yes	DASEN
KORA	Whole blood	Yes	Yes (<3)	0.01	95	80	No	No	No	BMIQ
LBC1921	Whole blood	Yes	No	0.01	95	93	No	Yes	Yes	--
NAS	Whole blood	Yes	Yes (<3)	0.05	95	95	No	Yes	Yes	BMIQ
NFBC	Whole blood	Yes	No	10^{-16}	95	95	Yes	Yes	No	Functional normalization (*minfi*)
RS	Whole blood	Yes	No	0.01	99	99	No	Yes	No	DASEN

FHS, Framingham Heart Study; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; HBCS, Helsinki Birth Cohort Study; InCHIANTI, Invecchiare in Chianti Study; KORA, Kooperative Gesundheitsforschung in der Region Augsburg Study; LBC1921, Lothian Birth Cohort 1921; NAS, Normative Aging Study; NFBC, Northern Finland Birth Cohort; RS, Rotterdam Study

[a]In all studies, detection P values were estimated by proprietary Illumina software (Genome Studio) and defined as the probability that the total observed signal fell within the background intensity. The ratio of the methylated probe intensity to the overall signal intensity (the β score), estimated by Illumina Genome Studio software, was used as the quantitative measure of DNA methylation.

eTable 2. Analyses of Illumina Infinium Human Methylation450K BeadChip data across cohorts.

Study	Batch Effect Adjustment	White Blood Cell Counts	Additional Covariates
FHS	Chip, row, column	Estimated (Houseman method)	Family, 2 methylation PCs
GOLDN	Plate, position on plate	--	Pedigree, 4 cell purity PCs
HBCS	Column	Estimated (Houseman method)	--
InCHIANTI	Plate	Measured	--
KORA	Position on plate	Estimated (Houseman method)	--
LBC1921	Plate, chip, position on chip, hybridization date	Measured	--
NAS	Plate, chip, row, column	Measured	--
NFBC	Array, row	Estimated (Houseman method)	--
RS	Chip, row, column	Measured	N/A (only participated in methylation vs. expression/genotype analyses)

FHS, Framingham Heart Study; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; HBCS, Helsinki Birth Cohort Study; InCHIANTI, Invecchiare in Chianti Study; KORA, Kooperative Gesundheitsforschung in der Region Augsburg Study; LBC1921, Lothian Birth Cohort 1921; NAS, Normative Aging Study; NFBC, Northern Finland Birth Cohort; PCs, principal components; RS, Rotterdam Study

eTable 3. Associations of methylation loci and circulating TNFα in the model adjusted for age, sex, family and technical artifacts (where needed) with FDR<0.05 in the discovery stage.

CpG site	Chr	Position[a]	β ± SE	P	Gene
cg16411857	16	57023191	-0.01 ± 0.002	2.01×10^{-11}	*NLRC5*
cg00959259	3	122281975	-0.01 ± 0.002	3.42×10^{-8}	*DTX3L;PARP9*
cg07839457	16	57023022	-0.02 ± 0.004	5.40×10^{-8}	*NLRC5*
cg08122652	3	122281939	-0.009 ± 0.002	1.26×10^{-7}	*DTX3L;PARP9*
cg13683939	9	136152547	0.04 ± 0.007	1.29×10^{-7}	intergenic; proximal to *ABO*
cg22930808	3	122281881	-0.01 ± 0.002	1.95×10^{-7}	*DTX3L;PARP9*

[a]hg19

Chr, chromosome; CpG, cytosine-phosphate-guanine; TNFα, tumor necrosis factor α

eTable 4. Associations of methylation loci and circulating TNFα in the model adjusted for age, sex, family, technical artifacts (where needed), and blood cell counts with FDR<0.05 in the discovery stage.

CpG site	Chr	Position[a]	β ± SE	P	Gene
cg16411857	16	57023191	-0.01 ± 0.002	2.75×10^{-12}	*NLRC5*
cg07839457	16	57023022	-0.02 ± 0.003	1.83×10^{-8}	*NLRC5*
cg00959259	3	122281975	-0.01 ± 0.003	1.61×10^{-7}	*DTX3L;PARP9*
cg22930808	3	122281881	-0.01 ± 0.002	2.14×10^{-7}	*DTX3L;PARP9*
cg24267699	9	136151359	-0.009 ± 0.002	2.38×10^{-7}	*ABO*
cg13683939	9	136152547	0.04 ± 0.007	3.23×10^{-7}	intergenic; proximal to *ABO*
cg06298346	5	10565679	-0.006 ± 0.001	4.21×10^{-7}	*ANKRD33B*
cg08122652	3	122281939	-0.008 ± 0.002	8.10×10^{-7}	*DTX3L;PARP9*

[a]hg19
Chr, chromosome; CpG, cytosine-phosphate-guanine; TNFα, tumor necrosis factor α

eTable 5. Associations of methylation loci and circulating TNFα in the model adjusted for age, sex, family, technical artifacts (where needed), blood cell counts, body mass index, and smoking with FDR<0.05 in the discovery stage, including participants from the Normative Aging Study removed from the main analysis due to extreme TNFα values.

CpG site	Chr	Position[a]	β ± SE	P	Gene
cg16411857	16	57023191	-0.009 ± 0.001	2.95×10^{-10}	*NLRC5*
cg13683939	9	136152547	0.04 ± 0.008	1.31×10^{-8}	intergenic; proximal to *ABO*
cg07839457	16	57023022	-0.01 ± 0.002	2.49×10^{-8}	*NLRC5*
cg21549285	21	42799141	-0.01 ± 0.002	6.09×10^{-8}	*MX1*
cg11601443	12	113415930	-0.004 ± 0.0008	2.78×10^{-7}	*OAS2*
cg00959259	3	122281975	-0.01 ± 0.002	4.39×10^{-7}	*DTX3L;PARP9*
cg08122652	3	122281939	-0.006 ± 0.001	4.75×10^{-7}	*DTX3L;PARP9*
cg22107533	15	45028083	-0.006 ± 0.001	9.13×10^{-7}	*TRIM69*
cg05439368	15	45028098	-0.006 ± 0.001	1.02×10^{-6}	*TRIM69*
cg22930808	3	122281881	-0.008 ± 0.002	2.52×10^{-6}	*DTX3L;PARP9*

[a]hg19

Chr, chromosome; CpG, cytosine-phosphate-guanine; TNFα, tumor necrosis factor α

eTable 6. Associations of methylation loci and circulating TNFα in the model adjusted for age, sex, family, technical artifacts (where needed), blood cell counts, body mass index, and smoking with FDR<0.05 in the discovery stage, removing participants from the GOLDN study due to difference in sample (CD4+ T-cells in GOLDN vs. whole blood elsewhere).

CpG site	Chr	Position[a]	β ± SE	P	Gene
cg16411857	16	57023191	-0.009 ± 0.001	2.95×10^{-10}	*NLRC5*
cg13683939	9	136152547	0.04 ± 0.008	1.31×10^{-8}	intergenic; proximal to *ABO*
cg07839457	16	57023022	-0.01 ± 0.002	2.49×10^{-8}	*NLRC5*
cg21549285	21	42799141	-0.01 ± 0.002	6.09×10^{-8}	*MX1*
cg11601443	12	113415930	-0.004 ± 0.0008	2.78×10^{-7}	*OAS2*
cg00959259	3	122281975	-0.01 ± 0.002	4.39×10^{-7}	*DTX3L;PARP9*
cg08122652	3	122281939	-0.006 ± 0.001	4.75×10^{-7}	*DTX3L;PARP9*

[a]hg19

Chr, chromosome; CpG, cytosine-phosphate-guanine; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; TNFα, tumor necrosis factor α

eTable 7. Associations between methylation status of top TNFα CpG sites and neighboring DNA sequence variation (FDR < 0.05 in the discovery phase).

CpG site	Chr	Gene	SNP	MAF	Discovery (RS)		Replication (GOLDN)	
					β ± SE	P	β ± SE	P
cg07839457	16	*NLRC5*	rs17369768	0.30	-0.21 ± 0.05	5.70×10^{-6}	-0.73 ± 0.33	0.03
cg07839457	16	*NLRC5*	16:57042641:D	0.32	-0.20 ± 0.05	1.71×10^{-5}	-0.66 ± 0.33	0.05
cg07839457	16	*NLRC5*	rs1991515	0.46	-0.16 ± 0.04	0.0001	-0.36 ± 0.36	0.31
cg07839457	16	*NLRC5*	rs289752	0.41	0.14 ± 0.04	0.0003	0.39 ± 0.36	0.28
cg07839457	16	*NLRC5*	rs1566439	0.40	-0.14 ± 0.04	0.0004	-0.65 ± 0.38	0.09
cg07839457	16	*NLRC5*	rs12598522	0.39	-0.14 ± 0.04	0.0004	-0.67 ± 0.37	0.07
cg07839457	16	*NLRC5*	rs56315364	0.39	-0.14 ± 0.04	0.0004	-0.68 ± 0.37	0.07
cg07839457	16	*NLRC5*	rs12149572	0.39	-0.14 ± 0.04	0.0004	-0.67 ± 0.37	0.07
cg07839457	16	*NLRC5*	rs17369468	0.30	-0.16 ± 0.05	0.0006	-0.33 ± 0.33	0.32
cg07839457	16	*NLRC5*	rs60169561	0.29	-0.15 ± 0.05	0.001	-0.37 ± 0.34	0.27
cg07839457	16	*NLRC5*	16:57026785:D	0.29	-0.15 ± 0.05	0.001	-0.38 ± 0.33	0.25
cg16411857	16	*NLRC5*	rs144032256	0.01	-0.93 ± 0.25	0.0002	-0.003 ± 0.18	0.99
cg00959259	3	*DTX3L/PARP9*	rs117399974	0.01	-0.93 ± 0.25	0.0002	0.06 ± 0.06	0.35

Chr, chromosome; CpG, cytosine-phosphate-guanine; GOLDN, Genetics of Lipid Lowering Drugs and Diet Network; MAF, minor allele frequency; RS, Rotterdam Study; SNP, single-nucleotide polymorphism