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ORIGINAL RESEARCH ARTICLE

Epigenome-Wide Association Study of Abdominal Aortic Aneurysm Identifies Central Role for Inflammatory Pathways

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BACKGROUND: Epigenetic mechanisms underlying susceptibility to abdominal aortic aneurysm (AAA) remain poorly understood. Identifying causal DNA methylation markers for AAA can elucidate the regulatory processes that drive aneurysm formation and accelerate translational applications. We leveraged the Veterans Affairs (VA) Million Veteran Program to identify methylation biomarkers and delineate underlying pathways.

METHODS: We first conducted an epigenome-wide association study of incident AAA (1324 cases and 42 065 noncases), performed stratified analyses by population group and smoking status, and conducted Mendelian randomization to facilitate causal inference of the cytosine-phosphate-guanine (CpG)–AAA association. Chromatin state, island context, and transcription factor binding were implicated through functional annotation of identified CpGs. To identify genes impacted by change in methylation state, we aligned associations with transcriptional data obtained in blood, aorta, and liver. We performed expression quantitative trait methylation to capture CpG–gene-expression links across the genome. Network Mendelian randomization was used to test cardiometabolic and inflammatory mediation. Finally, we developed a methylation risk score for AAA using penalized regression and evaluated its performance against a clinical model in the Million Veteran Program and 2 external studies.

RESULTS: Epigenome-wide association study identified 1253 CpGs associated with incident AAA, and Mendelian randomization supported a putative causal role for 143 of these associations. Functional annotation pointed to predominantly distal, enhancer-centered regulation and enrichment of inflammatory transcription factor programs (eg, activator protein-1 and nuclear factor κB). This distal architecture was consistent with expression quantitative trait methylation results, which showed a larger number of trans associations. Among 143 CpGs associated with AAA in Mendelian randomization analyses, we identified 213 CpG–gene expression pairs with strong colocalization support, including *AGER, C2*, and *DDR1*. Multi-trait colocalization further prioritized 8 CpG–gene expression–AAA links with evidence of a shared genetic signal. Network Mendelian randomization identified 135 putative mediation pathways linking CpGs to AAA, including 58 via cardiometabolic traits and 77 via inflammation-related traits. Blood lipid traits mediated >40% of the associations between AAA risk and CpGs near *LDLR* (cg19305903) and *TRIB1* (cg14128479). Among inflammation-related mediators, circulating DAG1, NEXN, MMP9, IL1RN, and MMP13 were prioritized as potential intermediates. Additionally, a CpG selection process was used to develop a methylation risk score for AAA, which strongly associated with disease in both the discovery cohort and 2 external validation cohorts, even after adjustment for a full suite of clinical covariates.

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CONCLUSIONS: This study identified putative causal DNA methylation markers for AAA, and multi-omics analyses implicate activator protein-1–linked inflammatory transcriptional programs, blood lipids, and multiple inflammation-related proteins as key pathways underlying methylation-associated AAA risk.

Key Words: aortic aneurysm, abdominal ■ DNA methylation ■ epigenomics ■ inflammation

Clinical Perspective

What Is New?

- A large epigenome-wide association study identified 1253 blood DNA methylation sites associated with incident abdominal aortic aneurysm, with genetic analyses supporting a putative causal role for 143 sites.
- Multi-omic analyses implicated distal enhancer regulation, AP-1/NF-κB–centered inflammatory programs, lipid metabolism, and inflammation-related proteins as pathways linking DNA methylation to abdominal aortic aneurysm.
- A DNA methylation risk score was associated with abdominal aortic aneurysm independently of established clinical risk factors and showed consistent associations in 2 external cohorts.

What Are the Clinical Implications?

- Blood DNA methylation may provide molecular information about abdominal aortic aneurysm susceptibility beyond routinely measured clinical risk factors.
- The findings prioritize lipid-related and inflammatory pathways, including matrix metalloproteinase and interleukin-1 signaling, for mechanistic investigation and potential therapeutic evaluation.
- Although the methylation risk score improved disease discrimination, prospective validation is required before it can be used to refine screening or clinical risk stratification.

Nonstandard Abbreviations and Acronyms

AAA	abdominal aortic aneurysm
AP-1	activator protein-1
CHS	Cardiovascular Health Study
CpG	cytosine-phosphate-guanine
eQTL	expression quantitative trait locus
eQTM	expression quantitative trait methylation
EWAS	epigenome-wide association study
FDR	false discovery rate
GTEx	Genotype-Tissue Expression
GWAS	genome-wide association study
meQTL	methylation quantitative trait locus
MR	Mendelian randomization
MRS	methylation risk score
MVP	Million Veteran Program
NF-κB	nuclear factor κB
TF	transcription factor
VA	Veterans Affairs

Abdominal aortic aneurysm (AAA) is characterized by a focal dilation of the abdominal aorta of >3 cm in diameter. It remains clinically silent until rupture, which carries a mortality rate of >40% upon presentation.[1,2] Population screening studies estimate a prevalence of up to 8% in older adults, with incidence rising sharply after 65 years of age.[3–5] Although epidemiological investigations have identified major risk factors including male sex, advancing age, family history, and particularly cigarette smoking, no pharmacological therapy has been approved to halt aneurysm progression[6]; current medical management is limited to controlling cardiovascular risk factors (eg, antihypertensives and lipid-lowering agents) and surgical repair after the aneurysm reaches a threshold diameter.[7]

DNA methylation is a dynamic epigenetic mark that can record cumulative environmental exposures (eg, smoking, diet, pollution, and psychosocial stress) and thereby serves as a molecular integrator of external stimuli over time. These exposure-linked methylation changes can contribute to disease risk by altering gene regulation (modulating transcription factor [TF] binding, chromatin accessibility, and promoter/enhancer activity), leading to persistent shifts in gene expression and downstream inflammatory, metabolic, and vascular pathways.[8] However, the molecular drivers of AAA pathogenesis, especially epigenetic mechanisms, remain inadequately characterized.[9] An epigenome-wide association study (EWAS) offers an approach to identify differentially methylated regions (ie, cytosine-phosphate-guanine [CpGs]) associated with incident AAA. By mapping these CpGs to nearby genes and intersecting with gene-regulatory networks, EWAS can uncover novel biomarkers, elucidate pathogenic pathways, and prioritize candidate therapeutic targets.[10] Here, we conducted an EWAS integrating genomic data to identify potentially causal CpGs for AAA development, explore related biological pathways, and evaluate the potential utility of a methylation score for AAA risk prediction.

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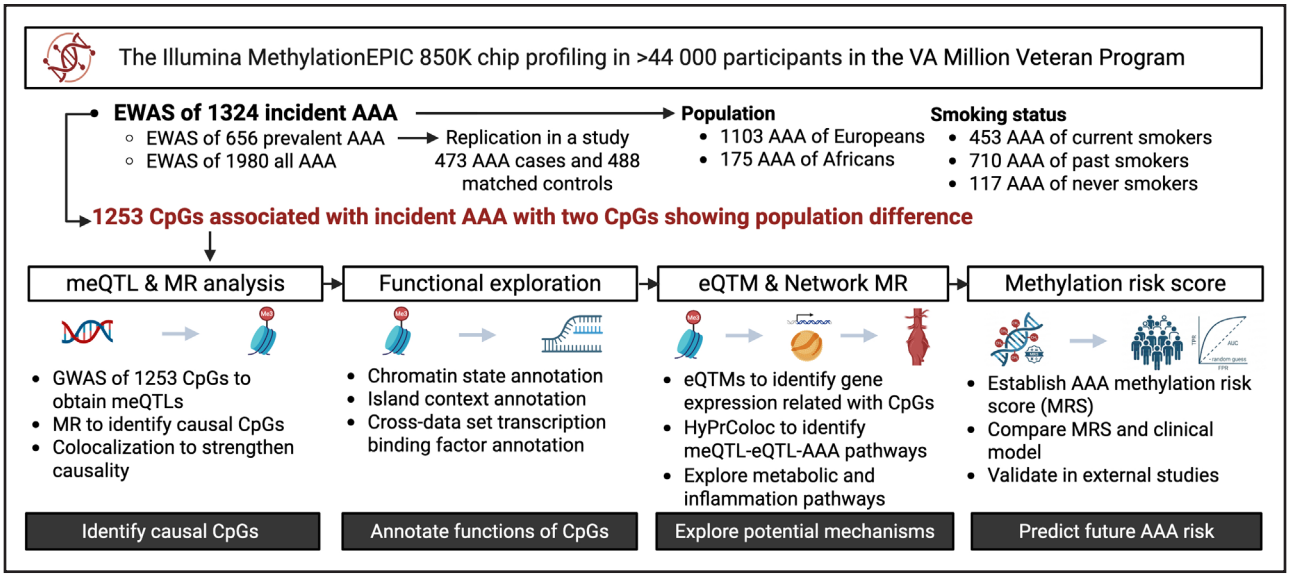


Figure 1. Study design overview.
AAA indicates abdominal aortic aneurysm; eQTM, expression quantitative trait methylation; EWAS, epigenome-wide association study; GWAS, genome-wide association study; meQTL, methylation quantitative trait locus; and MR, Mendelian randomization.

METHODS

Data Availability

The Million Veteran Program (MVP) individual-level data are open to Veterans Affairs (VA)–affiliated researchers via application (https://www.mvp.va.gov/pwa/mvp-data-available-research). The EWAS results and summary genome-wide association study (GWAS) statistics for CpG probes are publicly available at https://genhub.va.gov/public/sumstats/dashboard/studies/vas558818/analyses.

Study Overview

Figure 1 outlines the study. We first conducted an EWAS of incident AAA, evaluated population-specific effects, assessed smoking, and contrasted incident versus prevalent associations. To strengthen evidence of causality, we ran GWASs of AAA-associated CpG sites and used the results in 2-sample Mendelian randomization (MR) and colocalization experiments. Then, we functionally annotated putative causal CpGs and integrated methylation with gene expression and cardiometabolic and immune/inflammation-related traits to map pathways. Finally, we derived and tested a methylation-based score for predicting AAA and evaluated its performance in external studies.

Study Population

We performed this study using data from the VA MVP, a large, national cohort of US Veterans established by the Department of Veterans Affairs to elucidate genetic and environmental contributors to human disease.[11] This study analyzed MVP participants with available DNA methylation data. Genome-wide methylation was assayed using the Illumina Infinium Methylation EPIC BeadChip array v1 (≈850K probes).[12] Raw intensity data were processed centrally in R using the SeSAMe Bioconductor package for both probe- and sample-level quality control.[13] Rigorous quality controls were performed (Supplemental Methods).

Age, sex, smoking status (current, former, and never), and AAA diagnoses were ascertained from electronic health records at the time of analysis. Participants with AAA were identified by at least 2 records of the relevant diagnostic codes (*International Classification of Diseases, 9th revision* [*ICD-9*]: 441.3 or 441.4; *ICD-10*: I71.3 or I71.4).[14] Comparator participants without AAA lacked these codes and were also excluded if they possessed any related vascular disease codes (*ICD-9*: 440–448; *ICD-10*: I71–I75 and I77–I79, K55). AAA occurrence was categorized according to the timing of the first diagnosis recorded in the electronic health record relative to blood sample collection at enrollment: prevalent AAA was defined as a diagnosis before enrollment, whereas incident AAA was defined as a diagnosis after enrollment. Among prevalent AAA cases, the median interval between diagnosis and enrollment was 3.86 years (interquartile range, 1.69–7.32 years) before enrollment; among incident AAA cases, the median interval between enrollment and diagnosis was 2.91 years (interquartile range, 1.69–4.49 years) after enrollment. In the primary EWAS analysis, we excluded prevalent AAA cases to minimize the potential for reverse causation. Participants were classified into population groups based on similarity to 1000 Genomes superpopulation reference panels,[14] which was calculated by plink2.[15] Additional covariates comprised array chip type, scanner ID, storage time (days between blood draw and array hybridization), Houseman-estimated blood cell proportions, and top 10 technical principal components (PCs), and top 10 genomic PCs. Diagnosis of hyperlipidemia, hypertension, coronary artery disease, peripheral artery disease, and stroke were defined by self-reported questionnaires and *ICD* codes in electronic health records, and concentrations of high-density lipoprotein and low-density lipoprotein cholesterol, triglycerides, and blood pressure were measured at enrollment.[16]

Institutional Approval

The MVP study was approved by the US Department of Veterans Affairs central institutional review board. All participants provided written informed consent before enrollment. Analyses of

data from the Framingham Heart Study, Cardiovascular Health Study, and the New Zealand Abdominal Aortic Aneurysm Study were conducted under the institutional approvals and consent procedures established for each respective cohort. The study was approved by the VA central institutional review board (No. 19-06).

Statistical Analyses

EWAS and Stratified Analyses

Our primary EWAS of incident AAA was performed using logistic regression; covariate specifications are detailed in the Supplemental Methods. In brief, age, sex, array chip type, scanner ID, storage time, Houseman-estimated blood cell (B cells, CD4 T cells, CD8 T cells, eosinophils, monocytes, neutrophils, and natural killer cells) proportions,[17] top 10 technical PCs, and top 10 genetic PCs were considered as covariates in the primary analysis model. To reduce reverse causation, we repeated the primary EWAS excluding incident AAA cases diagnosed within 12 months after enrollment. We also performed Cox proportional hazards regression as a sensitivity analysis to assess the consistency of the associations across different statistical models (Supplemental Methods). To control for statistical inflation, we applied the BACON method[18]; multiple testing was corrected by the Bonferroni procedure, with corrected $P<0.05$ deemed significant. For CpG–AAA associations identified in this analysis, we evaluated heterogeneity between participants genetically similar to European and African reference populations. Between-population heterogeneity of CpG–AAA associations was assessed using the Cochran Q statistic and I^2, defining significant heterogeneity as Bonferroni-adjusted Q $P<0.05$ and $I^2>75\%$. To aid interpretation of the African population–stratified results, we estimated statistical power using European population effect estimates as the assumed true effects, African population standard errors as the target precision, and a Bonferroni-corrected 2-sided z-test, defining CpGs with ≥80% power as adequately powered.

As internal validation, we tested these CpGs against prevalent AAA and contrasted effect estimates with those for incident AAA. To assess external validity, we compared our prevalent AAA EWAS results with those from an independent New Zealand case-control study.[19]

Given the substantial impact cigarette smoking has on DNA methylation and AAA risk, we analyzed data using 3 models to examine its effects with adjustment for: (1) self-reported smoking behavior; (2) DNA methylation levels at cg05575921 (*AHRR*); and (3) a methylation score for smoking based on 1255 CpGs.[20] We also conducted stratified analyses by self-reported smoking status.

GWAS of DNA Methylation Biomarkers and MR Analysis

To strengthen the evidence for causal associations between CpG methylation and AAA, we conducted 2-sample MR, a causal inference method that employs genetic variants as instrumental variables.[21] To mitigate population structure bias (as the AAA outcome GWAS primarily comprises data from individuals genetically similar to European reference populations), MR analyses were conducted among individuals genetically similar to the 1000G EUR superpopulation.

Instrumental Variable Selection

We performed GWAS of the EWAS-identified CpGs among individuals genetically similar to the 1000G EUR superpopulation in the VA MVP study to obtain genetic instruments. Detailed information on GWAS is presented in Supplemental Methods. To reduce horizontal pleiotropy, only cis methylation quantitative trait loci (cis-meQTL), defined as variants located within ±250 kb of the CpG site, were selected as instrumental variables at $P<5\times10^{-8}$ and clumped at $r^2<0.01$. F statistics were computed to assess instrument strength and instrumental variables with $F<10$ were removed.[22]

Outcome Source

AAA GWAS summary statistics were obtained from the AAAGen consortium meta-analysis, which included 14 studies comprising 37 214 individuals with AAA and 997 776 controls without AAA.[23]

MR Analysis

Data were harmonized using TwoSampleMR R package.[24] Because of partial sample overlap, we estimated potential bias and type I error assuming an observational bias of 0, 0.2, 0.4, 0.6, 0.8, or 1.[22] CpG–AAA MR associations were estimated using the Wald ratio for CpGs instrumented by a single nucleotide polymorphism inverse variant weighted method with fixed-effects for CpGs instrumented by 2 single nucleotide polymorphisms, and inverse variant weighted for CpGs instrumented by >2 single nucleotide polymorphisms, with fixed-effects models used when there was no evidence of heterogeneity, and random-effects models used when heterogeneity was present according to the Cochran Q test. The weighted median, MR-Egger, and MR-PRESSO methods were used as sensitivity analyses.[25]

Functional Annotations and Enrichment Analyses

To investigate regulatory functions of AAA-related CpGs, we performed functional annotation across chromatin state, CpG-island context, and TF binding. We defined 3 CpG sets: ALL (all EPIC probes used as the array background), EWAS (EWAS-significant CpGs), and MR (MR-significant CpGs). Chromatin states were assigned from Roadmap Epigenomics ChromHMM "mnemonics" tracks for blood, aorta, and liver.[26] CpG-island context followed the EPIC manifest. To assess TF involvement, we analyzed MR-significant CpGs by expanding the significant CpG sites to ±100 bp windows. TF binding enrichment was then evaluated by (1) TF ChIP-seq peak overlap using LOLA,[27] and (2) sequence-motif enrichment using JASPAR 2022 CORE (vertebrates) position-weight matrices with TFBSTools/motifmatchr[28] (Supplemental Methods). We used Fisher exact tests to examine differences and controlled the false discovery rate (FDR) Benjamini-Hochberg method for multiple comparisons.

Cell-Type–Specific Methylation Analysis

To identify blood cell types potentially driving AAA-associated methylation signals, we performed CellDMC analysis[29] restricted to EWAS-identified CpGs. CellDMC models whole-blood methylation beta values using AAA status, estimated blood cell proportions, and AAA-by-cell-type proportion interaction terms. Blood cell proportions were estimated using the Houseman method.[17] Models were adjusted for age, sex, array chip type, scanner ID, sample storage time, the top 10 technical

PCs, and the top 10 genetic PCs. To characterize their regulatory context, we applied the previously described LOLA TF binding-site enrichment and JASPAR motif enrichment analyses to the CellDMC-significant CpGs within each leukocyte subtype. All CpGs tested in the CellDMC analysis were used as the background. Statistical significance was assessed using one-sided Fisher's exact tests, with multiple testing controlled using the Benjamini–Hochberg FDR method.

Genetic Colocalization and HyPrColoc Analyses

We linked MR-significant AAA-associated CpGs (±500 kb) to variation associated with cis-gene expression (ie, expression quantitative trait loci [eQTLs]) using colocalization.[30] We then applied HyPrColoc to identify shared meQTL–eQTL–AAA signals,[31] in which posterior probability of hypothesis 4 (PPH4) is the shared signal hypothesis. The eQTL data were drawn from whole blood (GTEx v8 and eQTLGen), aorta (GTEx v8), and liver (GTEx v8).[32,33] Full model settings are provided in the Supplemental Methods. We defined strong colocalization as PPH4≥0.7. We considered HyPrColoc clusters to show colocalization when the posterior probability of full colocalization (PPFC) was ≥0.7 and the regional probability was ≥0.8. Clusters with PPFC 0.5 to <0.7 and regional probability ≥0.8 were considered suggestive.

Expression Quantitative Trait Methylation Analysis

An expression quantitative trait methylation (eQTM) is a CpG site for which DNA methylation level is associated with the expression level of a nearby (cis) or distant (trans) gene, linking epigenetic variation to transcriptional regulation. Because colocalization targets only cis effects, we performed an eQTM analysis to capture both cis and trans CpG–gene links. The analysis was performed for MR-significant AAA-associated CpGs. Using whole-blood data from 2115 Framingham Heart Study participants (Supplemental Methods),[34] we tested genome-wide associations between AAA-associated CpGs and genome-wide transcript levels. Multiple testing was controlled by Bonferroni correction.

Two-Stage Network MR Analysis

We used network MR to test whether cardiometabolic and inflammatory traits mediate CpG–AAA associations.[35] In the first step, we estimated the effects of genetically predicted CpGs on cardiometabolic and inflammatory traits ($\beta_{CpG\rightarrow mediator}$) using cis meQTLs only, defined as variants located within ±250 kb of the CpG site, were selected as instrumental variables at $P<5\times10^{-8}$ and clumped at $r^2<0.01$. In the second step, we estimated the effects of genetically predicted mediators on AAA ($\beta_{mediator\rightarrow AAA}$) using standard 2-sample MR; for nonprotein mediators, genome-wide significant variants were selected, whereas for protein mediators, only cis pQTLs were used (Supplemental Methods). The total effect was assessed in CpG–AAA MR ($\beta_{CpG\rightarrow AAA}$). Mediated pathways were evaluated when the associations for all 3 components ($\beta_{CpG\rightarrow mediator}$, $\beta_{mediator\rightarrow AAA}$, and $\beta_{CpG\rightarrow AAA}$) were each nominally significant ($P<0.05$). Pathways were considered supported when the total effect ($\beta_{CpG\rightarrow AAA}$) and the indirect effect ($\beta_{CpG\rightarrow mediator}\times\beta_{mediator\rightarrow AAA}$) were directionally concordant. The proportion of the CpG–AAA association mediated by cardiometabolic and inflammatory traits was estimated as, βCpG→mediator × βmediator →AAAβCpG→AAA,

and standard errors for the mediated effects were calculated using the delta (error-propagation) method.[36] The FDR Benjamini-Hochberg method was used for multiple testing.

Methylation Score Establishment and Risk Prediction

We developed a DNA methylation risk score (MRS) for AAA using a 3-stage data partitioning strategy. A schematic is shown in the Supplemental Methods. Participants with incident or prevalent AAA as well as those without AAA were randomly divided into training, recalibration, and testing sets in an 80:10:10 ratio with preservation of the relative proportions of prevalent and incident AAA rates across partitions. In the training set, we first performed a logistic regression EWAS including all 757 266 autosomal CpG sites for combined incident or prevalent AAA, with BACON correction for test-statistic inflation. CpG sites associated with AAA and surviving Bonferroni correction were carried forward into a least-absolute shrinkage and selection operator regression.[37] Methylation values were standardized before fitting, and ten-fold cross-validation was used for regularization parameter selection with area under the curve as the objective function. To favor parsimony, we selected the largest regularization parameter with cross-validated area under the curve within one standard error of the estimate at the maximum area under the curve. CpG sites with non-zero coefficients were retained in the MRS with their least-absolute shrinkage and selection operator coefficients used as weights. To facilitate comparison with external data sets, we also constructed an MRS compatible with 450K arrays by restricting the discovery methylation data to CpG sites available on the 450K chip.

To determine the strength of the association between the MRS and AAA, the trained MRS was used to compute AAA probabilities for the non-testing set participants (20% of total population), which were then Z-score standardized. Logistic regression was used to determine the strength of association between the MRS and combined incident and prevalent AAA, both without and with adjustment for clinical covariates. In MVP, the basic covariate model included age, sex, and smoking status; the fully adjusted model additionally included ancestry group, peripheral artery disease, coronary artery disease, stroke, hypertension, hyperlipidemia, body mass index, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, systolic blood pressure, and diastolic blood pressure. In the Cardiovascular Health Study (CHS), the same full covariate set was available and included age, sex, smoking status, ancestry group, peripheral artery disease, coronary artery disease, stroke, hypertension, hyperlipidemia, body mass index, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, systolic blood pressure, and diastolic blood pressure. In the New Zealand case-control study, available covariates included age, smoking status, hypertension, hyperlipidemia, coronary artery disease, and body mass index. Sex and ancestry were not included as covariates in the New Zealand analysis because all participants were male and of the same ancestry group. Data set–specific covariate adjustment sets are also indicated in the corresponding Figure legend. This analysis was repeated for both the 850K MRS and 450K MRS, as well as for prevalent-only disease by excluding incident cases.

We compared 4 models in the testing set, which comprised 10% of the total population: (1) the MRS alone; (2) a clinical model including age, sex, and smoking status that was developed in the 10% training set; (3) a combination clinical and MRS model recalibrated in the 10% recalibration set; and (4) a combination clinical and MRS model that was not recalibrated. Recalibration was performed by fitting a logistic regression

model of the predicted clinical probabilities and MRS probabilities for combined incident and prevalent AAA and then applying this model to the testing set. Models were evaluated for overall performance (via Brier score) as well as discrimination (via area under the receiver operating characteristic curve). CIs were obtained using a bootstrapping procedure with 1000 replicants. To permit comparison of the Brier scores between study populations, each with different population prevalence of disease, Brier skill scores were computed using a null model (ie, one predicting the population-specific disease prevalence) as the reference. A Bayesian framework was used to assess statistical and practical differences in both area under the curve and Brier skill score between the recalibrated and nonrecalibrated clinical plus MRS models and the clinical variables–only model. A relatively large region of practical equivalence of ±5% was selected, in part to reflect the high bar for incorporation of relatively costly methylation data into routine clinical practice. Further details of this analysis can be found in the Supplemental Methods.

We assessed external generalizability of the clinical plus MRS models in 2 independent data sets: the CHS,[38] which utilized an 850K array and included only prevalent AAA, and a New Zealand AAA case-control study,[19] which utilized a 450K array and also included only prevalent disease. Each cohort was divided into a 20% recalibration set and an 80% testing set. Strength of association analysis was performed using all participants and all available shared covariates. Detailed information on these analyses is provided in the Supplemental Methods.

RESULTS

EWAS of Incident AAA

After quality controls, 44 045 of the 48 298 MVP participants with available DNA methylation data were included. Further removing 656 veterans with prevalent AAA, the EWAS in MVP comprised 1324 veterans with incident AAA and 42 065 veterans without incident AAA, testing 757 266 autosomal CpG sites. The analysis included individuals genetically similar to African (n=11 406), European (n=26 291), and other superpopulation (n=6348) reference panels. After BACON correction, the genomic inflation factor was λ=1.16, indicating minimal residual inflation. After Bonferroni adjustment, 1253 CpGs were significantly associated with AAA risk (Figure 2A) after stringent adjustment for age, sex, population structure, and technical PCs; of these, hypermethylation of 545 CpGs and hypomethylation of 708 CpGs were associated with increased risk (Figure 2B; Table S1). The associations remained consistent in the analysis excluding incident AAA cases diagnosed within 12 months after enrollment (Figure S1) and in Cox regression (Table S2).

Population-Based Differences

Overall, we did not observe substantial heterogeneity between analyses in European and African population groups: among 1253 CpGs, only 2 CpG–AAA associations showed significant heterogeneity (Table S3; Figure 2C). Hypomethylation of cg22675726 near the body of the *MYOM1* gene and hypermethylation of cg00731338 near the body of the *AHRR* gene were associated with increased risk of AAA in participants genetically similar to European reference populations but not in those genetically similar to African reference populations (Figure 2D). Both CpGs had ≥80% estimated power, suggesting that the observed between-population differences were unlikely to be explained solely by limited statistical power.

Smoking Status Difference

Smoking is among the strongest risk factors for AAA and is known to have profound effects on DNA methylation.[20] Supporting this, the strongest AAA-associated CpG included cg05575921 (*AHRR*), cg14391737 (*PRSS23*), and cg03636183 (*F2RL3*), all of which have been shown to be associated with smoking. Among models with different covariates, QQ plots showed pronounced inflation in the unadjusted model and the model adjusted for self-reported smoking, whereas models adjusting for *AHRR* methylation or a smoking methylation score lay close to the null (Figure S2). We further evaluated the 1253 CpG–AAA associations separately in never, current, and former smokers. However, we found that the overall pattern of associations appeared similar between current and former smokers (Table S4); heterogeneity tests did not identify significant differences in effect estimates when comparing never versus current smokers (Table S5) or never versus former smokers (Table S6; Figure 2E). We additionally examined several top smoking-related CpGs across the 3 groups. The effect sizes were directionally consistent in never, current, and former smokers, with wider CIs in never smokers reflecting smaller sample size (Figure S3).

CpG Associations With Prevalent Versus Incident AAA

We tested whether the 1253 CpGs associated with incident AAA were also associated with prevalent AAA and whether effects differed by case definition. Most effects were concordant; however, 80 CpGs showed significant heterogeneity (Table S7), supporting stage-dependent methylation dynamics rather than a single cross-sectional signal. Notably, among the 80 CpGs with significant heterogeneity, 29 hypomethylated CpGs showed stronger associations with prevalent AAA, whereas 51 hypermethylated CpGs showed stronger associations with incident AAA. Pathway enrichment analysis of genes annotated to the 29 CpGs suggested involvement of inflammatory pathways, including interleukin-6 (IL-6)/JAK/STAT3 signaling in the MSigDB Hallmark 2020 database (P = 0.003; full results: https://maayanlab.cloud/Enrichr/enrich?dataset=af119717115044d8ecb5ce4cc681f853),

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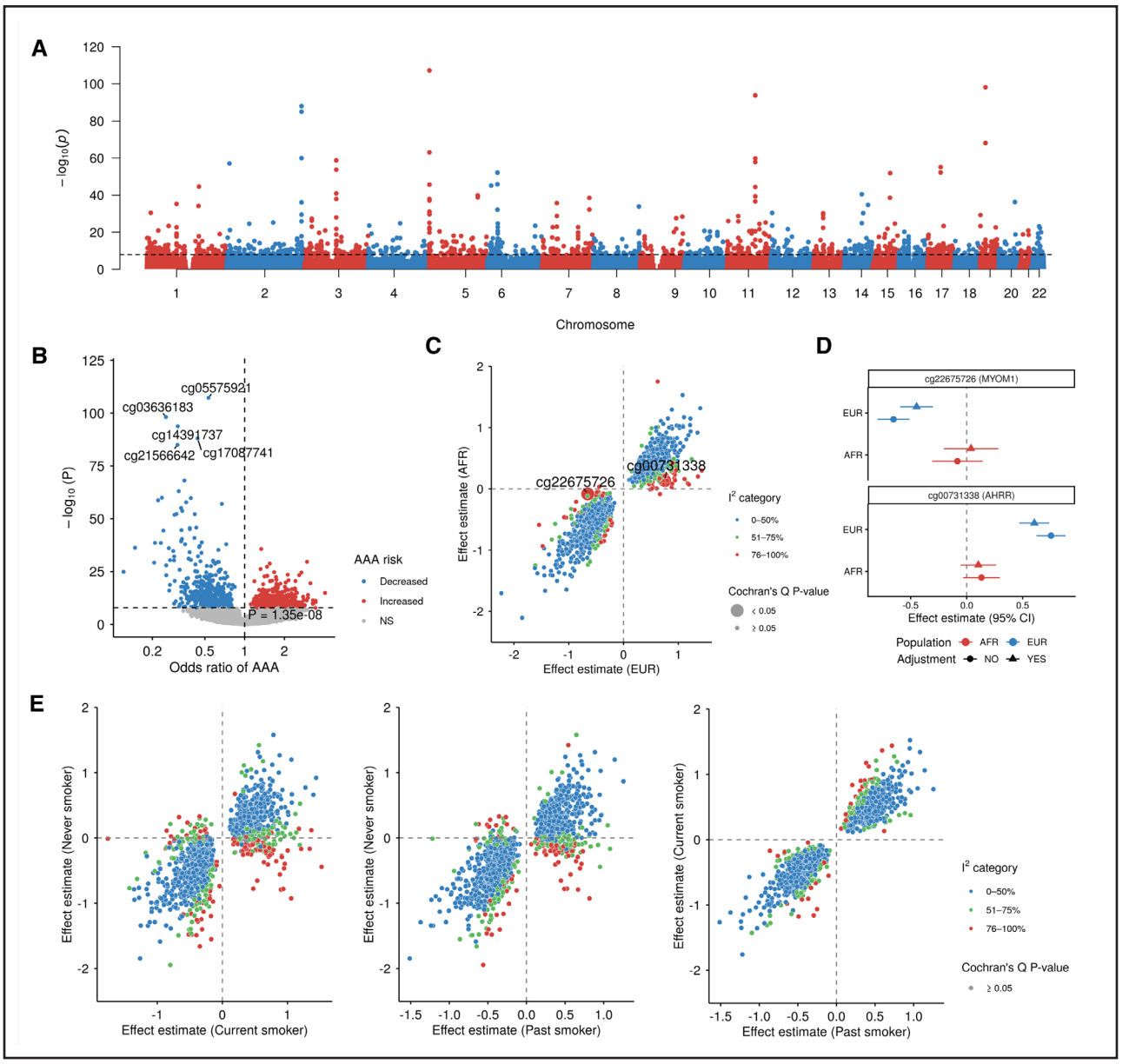


Figure 2. Epigenome-wide association analysis of incident abdominal aortic aneurysm (AAA).
A, Manhattan plot of EWAS of incident AAA among 1324 cases and 42 065 controls. The line indicates the significance threshold after Bonferroni correction. **B**, Volcano plot of CpG–AAA associations from the same analysis as in **A**. Top 5 CpGs by *P* value were labeled. **C**, Scatter plot of consistency of CpG–AAA associations between individuals of European (1103 incident AAA case and 24 692 non-AAA) and African (175 incident AAA case and 11 129 non-AAA) population (determined by genetically similarity to 1000Genomes reference population panels). Two associations (cg22675726-AAA and cg00731338-AAA) were observed with significant heterogeneity after Bonferroni correction. **D**, Forest plot of cg22675726–AAA and cg00731338–AAA associations with and without smoking status adjustment in individuals of European and African populations with the same number of participants described in **C**. **E**, Scatter plot of consistency of CpG–AAA associations across never (117 incident AAA case and 8793 non-AAA), past (710 incident AAA case and 20 210 non-AAA), and current (453 incident AAA case and 10 213 non-AAA) smokers. All these analyses were performed using multivariable logistic regression.

suggesting that methylation differences at inflammatory pathway-related loci may be more prominent in prevalent AAA.

Replication of Associations for Prevalence AAA

We compared the EWAS of prevalent AAA in MVP with results from an external New Zealand male case-control study (473 cases and 488 matched controls). Of the 316 CpG–AAA associations available in both data sets, 287 (90.8%) showed a concordant direction of effect (Table S8). In binomial testing, 262 (82.9%) associations showed concordant significance. After Bonferroni correction, 177 (56.0%) showed both concordant directionality and statistical significance. Among the remaining 110 associations with concordant directionality but

without Bonferroni-corrected significance, 69 (62.7%) were estimated to have <80% statistical power.

Mendelian Randomization

To obtain genetic instrumental variables, GWASs were performed for the 1253 CpGs associated with incident AAA in up to 25 987 individuals genetically similar to European reference populations. Genetic instruments were available for 1181 CpGs. Because of MVP contributions to the AAAgen consortium, this 2-sample MR involved limited sample overlap (<5.3%). To minimize the potential for weak instrument bias, we excluded 66 CpGs with weak genetic instruments (*F* statistic <10), leaving 1115 CpGs for the MR analysis (Figure 3A). Assuming sample overlap of 5.3% and observational bias of 0, 0.2, 0.4, 0.6, 0.8, or 1, limited bias attributable to sample overlap was found (bias <0.001; type I error <0.05).

Genetically predicted methylation (M-values) at 143 CpGs were associated with AAA at nominal significance (Table S9), far exceeding the 5% expected by chance (exact one-sided binomial test $P<2.2\times10^{-16}$). After FDR correction, 17 CpGs remained significant (Figure 3B). Effect sizes spanned from an odds ratio of 0.29 (95% CI, 0.14–0.59) for hypermethylation at cg02657160 (*CPOX*; body) to an odds ratio of 12.81 (95% CI, 4.70–34.95) for hypermethylation at cg05215605 (*CDK6*; body; Figure 3C). Ten CpGs showed strong colocalization with AAA, supporting shared causal variants (Figure 3D; Table S10). Several lay within or near established AAA risk loci[23] (lead variant position±250kb) encompassing previously prioritized AAA genes, including lipid-related genes (*LDLR* and *TRIB1*), immune/inflammation-related gens (*CDK6* and *PLAUR*), and a non-clear cluster (*ODF3* and *ZNF827*; Figure S4).

Functional Annotation and Pathway Enrichment

Across aorta, blood, and liver, CpGs associated with AAA (by EWAS or MR) were consistently enriched in enhancer chromatin and depleted at promoters relative to the EPIC array background (Figure 3E). By island context, both EWAS and MR sets were depleted in CpG islands and enriched in shelves (Figure 3F). For TF binding, LOLA ChIP–seq overlap analysis highlighted activator protein-1 (AP-1)–related binding as the strongest enrichment signal, led by c-Jun and supported by additional FOS/FOSL1 and BATF signals (Figure 3G). JASPAR motif enrichment analysis identified broader enrichment of inflammatory and immune-regulatory motifs, including FOS, SPIC, RELA, JUN-related, GATA/TAL1, FOX, and REL motifs, with many reaching FDR significance (Figure 3H). These results support involvement of AP-1/nuclear factor κB (NF-κB)–related inflammatory regulation and hematopoietic/immune-cell transcriptional programs in AAA-associated methylation signals.

Cell-Type–Specific Methylation Analysis

CellDMC analysis suggested that AAA-associated methylation signals were not evenly distributed across blood cell types. The largest number of cell type–specific CpGs was observed in CD8 T cells, followed by neutrophils (Figure S5). The largest numbers of significant CpGs were observed in CD8+ T cells and neutrophils, and both cell types were characterized predominantly by hypomethylated CpGs. In contrast, eosinophils showed fewer significant CpGs but a greater proportion of hypermethylated changes (Table S11). TF pathway enrichment showed that CellDMC-significant CpGs from multiple cell types were enriched for overlapping immune-inflammatory regulatory programs, including NF-κB/REL, AP-1/JUN, STAT, BATF/IRF, GATA/NFAT, and p300-associated signatures (Figure S6).

Gene Expression Pathways Linking CpG-AAA

Colocalization analysis of 143 AAA-associated CpGs with gene expression identified 213 meQTL-eQTL pairs with strong support for colocalization, involving 67 unique CpGs and 152 unique genes across blood, aorta, and liver (Table S12). HyPrColoc identified one meQTL-eQTL-AAA cluster with strong colocalization support and 7 with suggestive support (Table S13). Of the 10 AAA-associated CpGs supported by both MR and colocalization evidence, 7 colocalized with gene expression in blood or liver, including *AGER*, *C2,* and *DDR1* (Figure 4A), and cg26242531-*KLC1*-AAA in liver tissue showed strong multi-trait colocalization support (Figure 4B).

Genome-Wide Links Between AAA CpGs and Gene Expression

eQTM analyses were performed to estimate the correlations between the AAA-associated CpGs and gene expression across the genome in 2115 Framingham Heart Study participants. These analyses revealed that although only a small proportion of the AAA-associated CpGs (0.4%) map to transcripts in cis (within the same genomic region), the majority demonstrated a linkage to a larger number of genes in trans (at distant genomic locations; Figure 4C; Table S14). Several trans-linked genes (*CDKN1A, DAB2IP, EPHX2, IFIH1, LOXL1, MAP3K20, MCTP2, NFKB1, NICN1, SCARB1, SH3RF3, SMAD3,* and *SPSB1*) have been previously identified in a large-scale AAA GWAS[23] (Table S14).

Cardiometabolic and Immune Signaling Pathways Linking CpGs to AAA

Because AAA is driven by pathways shared with atherosclerotic vascular disease and immune-mediated vascular remodeling, we performed 2-stage network MR to

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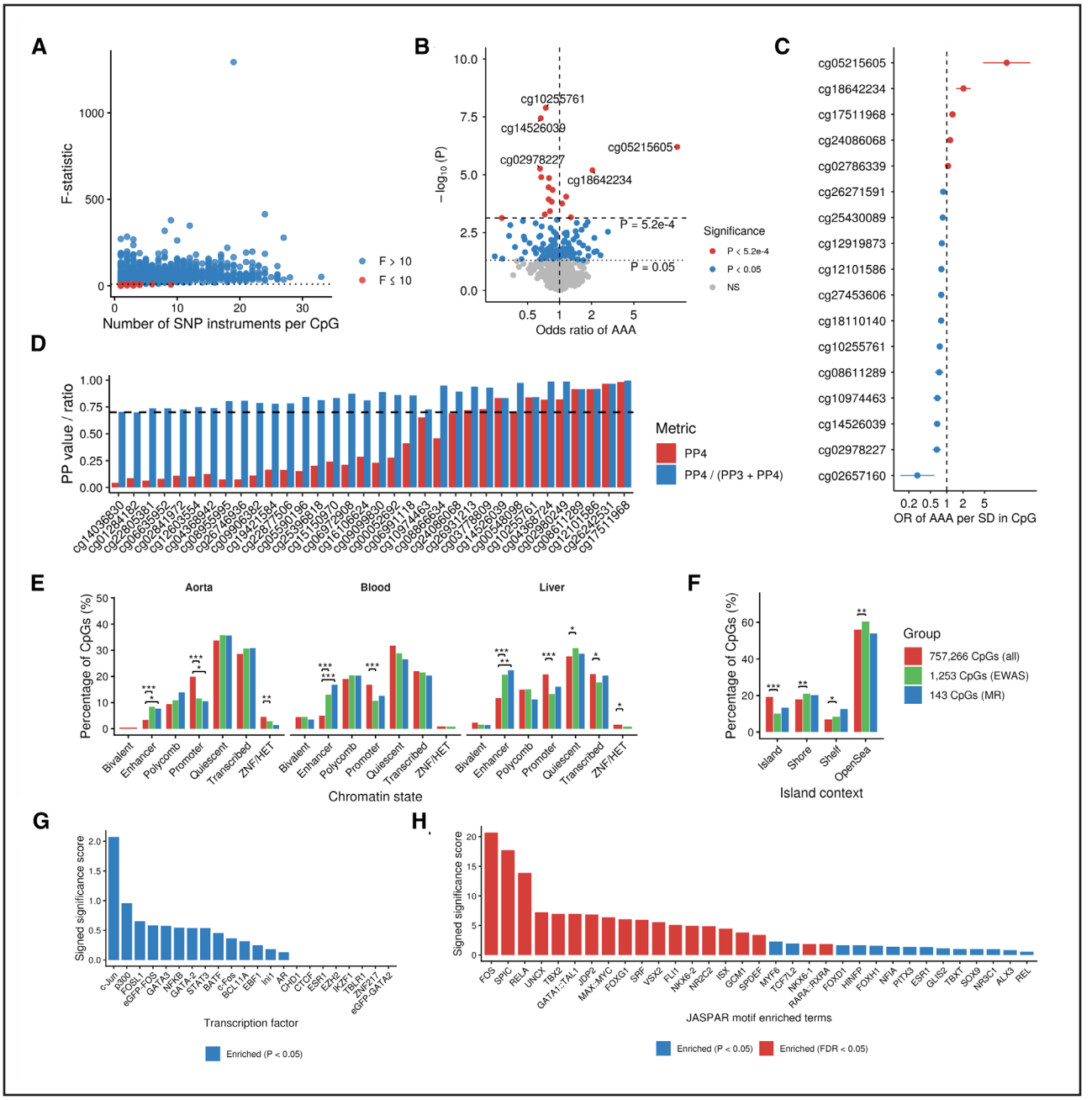


Figure 3. Causal DNA methylation biomarkers associated with AAA and their functional annotations.
A, Genetic instruments and their strength in Mendelian randomization (MR) analysis. **B**, Volcano plot of MR associations between genetically predicted levels of CpGs and abdominal aortic aneurysm (AAA) risk. Associations were estimated using the Wald ratio or inverse variant weighted method among 37 214 individuals with AAA and 997 776 controls without AAA. **C**, Forest plot of significant CpG–AAA MR associations after false discovery rate (FDR) correction. Associations were obtained from the same analyses described in **A**. **D**, Colocalization results obtained from Bayesian approximate Bayes factor analysis in the same study population described in **A**. **E**, Chromatin state annotation of AAA-associated CpGs against the background CpGs from EpicV2 array. **F**, Island context annotation of AAA-associated CpGs against the background CpGs from EpicV2 array. **G**, Roadmap transcription factor binding annotation of causal CpGs. **H**, JASPAR transcription factor binding annotation of causal CpGs.

evaluate whether cardiometabolic traits and immune/inflammatory signaling proteins mediate CpG-AAA associations. After FDR correction, 12 genetically proxied cardiometabolic traits and 26 genetically proxied inflammatory traits (platelet count and 25 blood proteins) were associated with genetic liability AAA and were considered in the network MR analyses (Table S15). Among 143 AAA-associated CpGs in MR, genetically predicted methylation of 115 CpGs was associated with at least one of 12 genetically proxied cardiometabolic traits, yielding 349 CpG–cardiometabolic trait pairs (Table S16). For inflammatory traits, genetically predicted methylation of

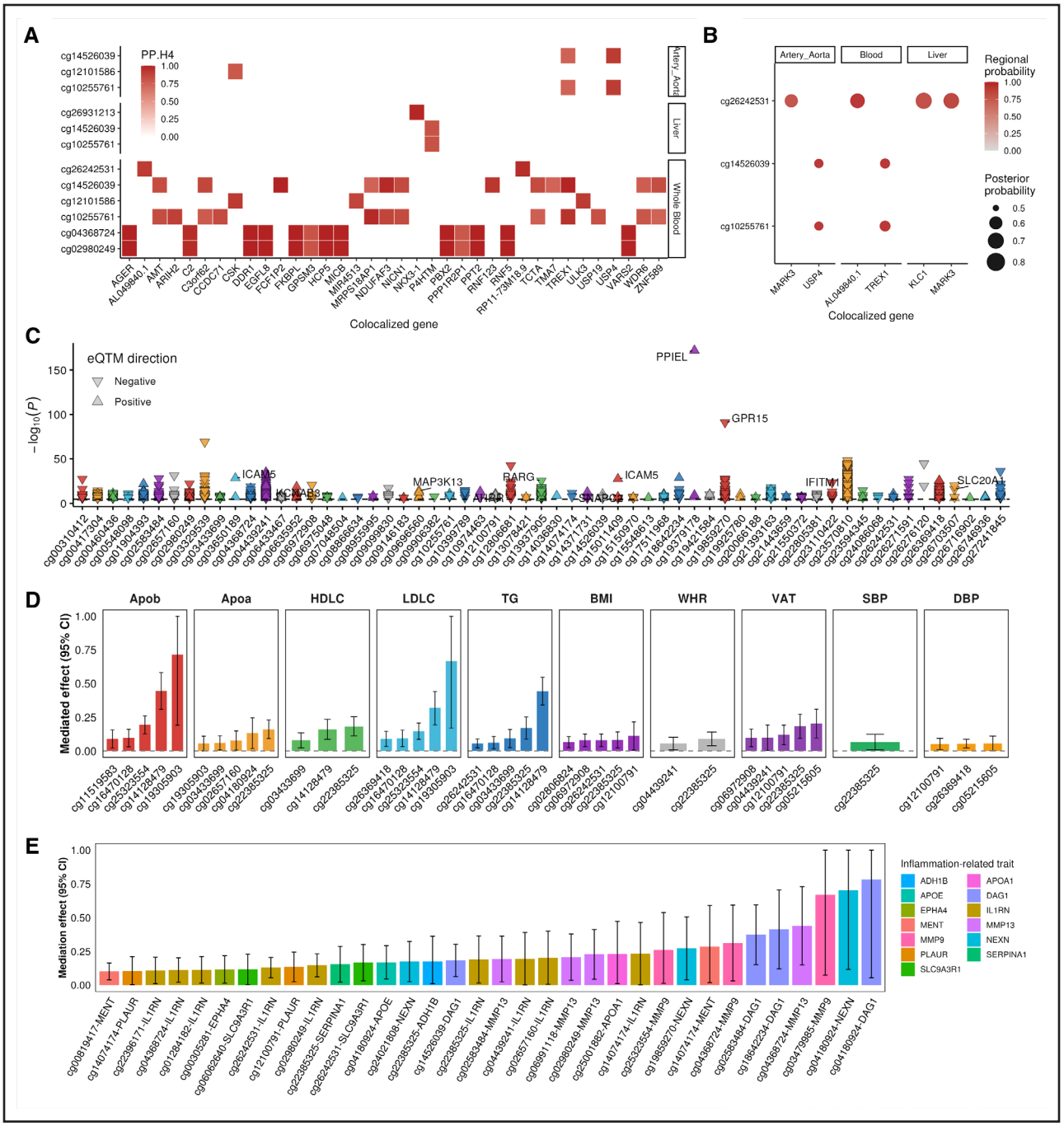


Figure 4. Gene expression and cardiometabolic pathways supporting CpG–AAA links.
A, Gene expression (expression quantitative trait loci [eQTL]) colocalized with abdominal aortic aneurysm (AAA)–associated methylation quantitative trait loci (meQTLs) at the cis region in blood (GTEX v8 N=670; eQTLGen N=31 684), artery aorta (N=387), or liver (N=208) tissues. Estimates were obtained from genetic colocalization analysis. **B**, meQTL-eQTL–AAA associations prioritized by Hyprcoloc analysis in blood, artery aorta, or liver tissues. The sample size was the same as that described **A**. **C**, Gene expression associated with AAA-associated CpGs based on eQTM data set. The analysis was performed using linear mixed-effects regression among 2115 Framingham Heart Study participants. **D**, Cardiometabolic pathways linking CpGs to AAA and their mediation magnitude. **E**, Immune/inflammation-related pathways linking CpGs to AAA and their mediation magnitude. Sample sizes for the mediator traits shown in **D** and **E** are provided in the supporting information.

135 CpGs was associated with genetically predicted levels of 25 inflammatory markers, yielding 431 CpG–inflammation-related trait pairs (Table S17). We identified 135 putative mediation pathways with directionally concordant total and indirect effects and a mediation proportion ≥5%. Among these, 58 involved cardiometabolic mediators (Table S18) and 77 involved immune- and inflammation-related mediators (Table S19). Among cardiometabolic traits, blood lipids including apolipoprotein B, low-density lipoprotein cholesterol, and triglycerides

mediated substantial proportions (≥40%) of the association for several CpGs (ie, cg19305903 at the body of *LDLR* gene, cg14128479 near *TRIB1* gene; Figure 4D). Visceral adipose tissue percentage mediated CpGs in *CDK6* in relation to AAA, albeit with smaller effects (Figure 4D). For inflammation-related pathways, DAG1, NEXN, MMP9, IL1RN, and MMP13 proteins mediated several CpG–AAA associations (Figure 4E).

Methylation Risk Score for AAA

We randomly assigned 1557 participants with incident or prevalent AAA as well as 32 592 participants without AAA to the training set and used regularized regression to develop methylation risk scores (MRS) compatible with both 850K and 450K array data. Model coefficients are shown in Table S20, and a total of 196 and 168 CpG sites survived regularization in the 850K and 450K models, respectively. Amongst the remaining 385 participants with disease and 8153 participants without disease, both versions of the MRS positively associated with combined incident and prevalent AAA, even after correction for a full suite of clinical covariates (OR 1.35 per SD for 850K model, 95% CI 1.23-1.47; OR 1.32 per SD for 450K model, 95% CI 1.21-1.44; Figure 5). Strength of association was similar when considering only prevalent disease (OR 1.39 per SD for 850K model, 95% CI 1.22-1.58; OR 1.30 per SD for 450K model, 95% CI 1.15-1.47; Figure 5). The MRS also strongly associated with disease in two external cohorts even after correction for available clinical covariates (OR 1.48 per SD for CHS, 95% CI 1.27-1.72; OR 3.09 per SD for NZ, 95% CI 2.20-4.45; Figure 5). Full results are listed in Table S21. Combining the MRS with the basic set of available clinical covariates currently used to inform screening (age, sex, and smoking) generally improved discrimination of AAA across the MVP, CHS, and NZ cohorts compared to age, sex, and smoking alone; however overall predictive performance only practically improved when the population prevalence of AAA was high (Figure S7, Figure S8, Table S22, and Table S23).

DISCUSSION

This large-scale EWAS identified 1253 CpGs associated with AAA in observational analyses and obtained genetic support for causality for more than 100 CpG–AAA associations. Population-stratified analyses showed minimal heterogeneity in CpG–AAA associations between individuals genetically similar to European and African reference populations. Functional annotation implicated predominantly distal, enhancer-centered regulation and highlighted AP-1/NF-κB inflammatory TF programs. Mediation analyses indicated that several key gene expression, blood lipids, and several inflammation-related traits (proteins DAG1, NEXN, MMP9, IL1RN, and MMP13) partially explain the CpG–AAA relationships. We additionally demonstrated that an MRS independently associated with disease and generally enhanced discrimination of AAA over clinical variables alone. Collectively, these findings elucidate epigenetic mechanisms underlying AAA, provide

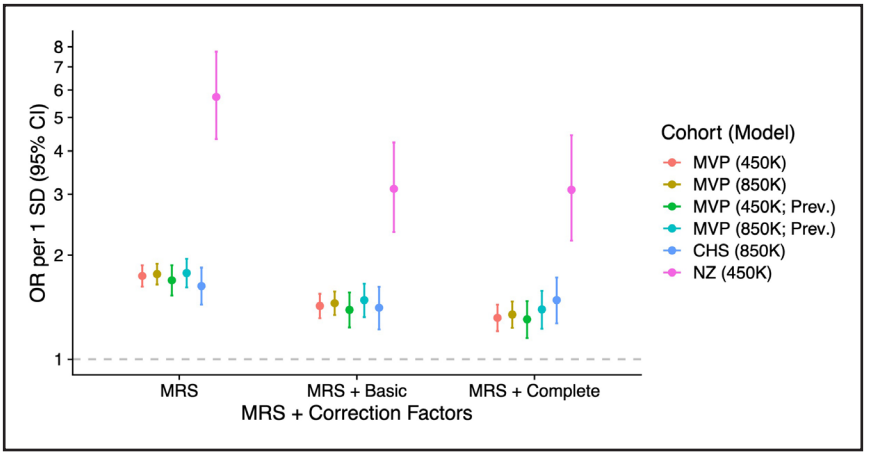


Figure 5. Methylation risk score in relation to AAA risk.
Strength of association between the MRS and AAA is reported as the odds ratio (OR) per standard deviation (SD) increase in score, with and without adjustment for available clinical covariates. In MVP, the "basic" covariate model included age, sex, and smoking status; the "complete" covariate model additionally included ancestry group, peripheral artery disease, coronary artery disease, stroke, hypertension, hyperlipidemia, body mass index, triglycerides, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol, systolic blood pressure, and diastolic blood pressure. The same complete covariate set was available in the Cardiovascular Health Study (CHS). In the New Zealand case-control study, available covariates included age, smoking status, hypertension, hyperlipidemia, coronary artery disease, and body mass index. Sex and ancestry were not included in the New Zealand models because all participants were male and of the same ancestry group. These associations were obtained from logistic regression in the MVP internal test set (195 individuals with AAA and 4074 non-AAA), the Cardiovascular Health Study (106 individuals with AAA and 2615 non-AAA), and the New Zealand study (470 individuals with AAA and 478 non-AAA).

deeper insight into disease etiology, and highlight opportunities for improved risk stratification.

Smoking, a major risk factor for AAA, is also a dominant driver of DNA methylation.[20] Adjustment for AHRR methylation or a smoking methylation score markedly attenuated CpG–AAA associations, indicating that much of the signal lies on a smoking-related methylation axis and is consistent with partial mediation. In contrast, stratified analyses by self-reported smoking status showed no strong interaction, with broadly similar CpG–AAA effect estimates across never, former, and current smokers. Together, these findings suggest that smoking-related methylation underpins a substantial proportion of the CpG–AAA signal, whereas the relative CpG–AAA association is similar across smoking categories and not fully captured by crude self-report. Accordingly, CpG profiles generally appear to record a durable smoking-related epigenetic imprint that contributes to AAA risk and may serve as an integrated biomarker of vascular risk.

Our pathway analyses implicated lipid metabolism as key cardiometabolic axes linking CpG methylation to AAA. This conclusion was supported by 2 independent lines of evidence. First, several lipid-related genes (*LDLR* and *TRIB1*) mapped in cis to AAA-associated CpGs. Second, MR-based mediation analyses showed that methylation at cg19305903 and cg14128479 in these loci was associated with AAA, with much of the effect mediated through apolipoprotein B, low-density lipoprotein cholesterol, and triglycerides. These findings strengthen the case for a causal contribution of selected CpGs, prioritize druggable lipid pathways for mechanistic follow-up. In turn, they underscore lipids as a primary cardiometabolic driver of AAA, with direct implications for risk stratification and for targeting lipid-lowering interventions in AAA prevention.

Integrating functional annotation with eQTM analyses suggested that multiple AAA-associated CpGs preferentially localize to distal regulatory elements and align with immune- and inflammation-related transcriptional programs. TF and motif enrichment analyses highlighted AP-1–related regulators, including c-Jun and FOS, together with additional enrichment of NF-κB/RELA and other immune-regulatory TFs.[39] CellDMC analyses further showed that these methylation signals were most prominent and predominantly hypomethylated in CD8+ T-cell and neutrophil signatures, whereas eosinophil-associated CpGs were fewer and predominantly hypermethylated; however, regulatory enrichment was broadly shared across leukocyte subsets rather than clearly cell type–specific. AP-1, composed of FOS/JUN family members, is a canonical stress- and cytokine-responsive TF complex downstream of MAPK signaling and regulates genes involved in leukocyte activation, cytokine production, and matrix remodeling, processes central to aneurysm formation in experimental models.[40,41] Consistent with a role for distal regulatory control, eQTM analyses showed a predominance of trans CpG–gene associations. Together, these findings suggest that AAA-associated methylation changes may mark enhancer-like regulatory elements involved in AP-1/NF-κB–centered inflammatory transcriptional programs. Although these data do not establish direct effects on TF binding or enhancer activity, they nominate inflammatory enhancer regulation as a candidate mechanism linking methylation changes to downstream gene-expression programs in AAA. Further functional studies will be required to determine whether modulation of AP-1/NF-κB–linked signaling or enhancer-regulatory mechanisms can attenuate pathogenic transcriptional programs in AAA.

In the immune/inflammation arm of the network MR, several plausible mediators of epigenetic risk for AAA emerged, including DAG1, NEXN, MMP9, IL1RN, and MMP13. These proteins connect methylation signals to core features of AAA biology, including inflammatory activation, extracellular matrix degradation, and vascular smooth muscle cell structural remodeling. MMP9 and MMP13 are particularly consistent with aneurysm pathobiology,[42,43] given the established role of matrix metalloproteinases in elastin and collagen degradation, inflammatory cell recruitment, and aortic wall weakening. IL1RN implicates IL-1–mediated inflammatory signaling, a pathway shown in experimental models to promote aneurysm inflammation and progression and to be attenuated by IL-1β/IL-1R blockade.[44] DAG1 and NEXN may reflect complementary mechanisms related to extracellular matrix–cytoskeletal coupling and smooth muscle actin organization.[45] Collectively, these results suggest that AAA-associated methylation changes may influence disease risk through inflammatory proteolysis and impaired aortic wall structural integrity.

Tissue-level gene expression data were interrogated to explore mechanisms. Although >100 genes showed cis colocalization with AAA-related CpGs in relevant tissues, relatively few overlapped established AAA GWAS signals[23] likely reflecting limited power or imperfect gene prioritization at loci identified in GWAS, or regulation driven by both genetic and environmental inputs. Notably, 2 AAA-colocalized CpGs (cg02980249 and cg04368724) colocalized with AAA-related genes within the *MHC* class III region (*AGER* [also known as *RAGE*], *C2*, and *RNF5*) which also have experimental or human-tissue support in AAA and are linked to inflammatory pathways.[46–48] Multi-trait colocalization linked cg2624253 methylation, *MARK3* expression, and AAA risk, suggesting a shared regulatory mechanism at this locus. Given the role of *MARK3* in cytoskeletal organization and microtubule dynamics,[49] this signal may implicate altered vascular smooth muscle cell structure or mechanotransduction in AAA susceptibility. Functional validation is needed.

Our data elucidate multiple lines of converging evidence that point to the therapeutic potential for immune

modulation in treating AAA. Clinical trials of immune modulatory drugs that act on the inflammatory programs downstream of AP-1, including IL-1β and the NLRP3 inflammasome (canakinumab in CANTOS,[50] colchicine in COLCOT,[51] and LoDoCo2[52]); and IL-6 (ziltivekimab in RESCUE[53] and the ongoing outcomes trial ZEUS[54]) have demonstrated or are testing efficacy in treating atherosclerotic cardiovascular disease. Combined with preclinical data,[55,56] our findings lend human epigenetic and genetic support for assessing these treatments in the setting of AAA. Of note, the COIN trial is currently evaluating whether low-dose colchicine (0.5 mg/day) can slow AAA progression.[57]

Previous studies in a mouse AAA model and cultured human smooth muscle cells have implicated promoter methylation of genes such as *IL6R* and *SMYD2* in AAA.[58,59] In our data, methylation at CpG sites located in the promoter or gene body of these loci was associated with incident AAA at nominal levels of significance, although none surpassed the epigenome-wide multiple testing threshold. A likely explanation is tissue context: earlier studies profiled vascular smooth muscle cells, whereas our analyses were conducted in whole blood.

We developed a methylation-based risk score for AAA and found robust associations with disease even after adjustment for a full suite of clinical covariates. We found a stronger association in the New Zealand study than in MVP or CHS. The greater strength of association observed in the New Zealand study may partly reflect stronger phenotypic contrast, as those without AAA were stringently screened to have aortic diameters <26 mm whereas those with AAA had relatively large aneurysms on average, likely accentuating methylation differences between groups. Combining the methylation risk score with basic clinical covariates generally improved discrimination of AAA in a statistically and practically significant manner compared with clinical covariates alone, with the single exception of the recalibrated clinical plus MRS score in the CHS population; this may be an artifact of the relatively low case numbers in the CHS cohort, with only ≈20 positive cases used for the recalibration procedure, which may have reduced the generalizability of the recalibrated score. Despite improvement in discrimination, the overall predictive performance of the combined model was only practically superior to the clinical model when the population was enriched in positive cases. Taken together, these results demonstrate that methylation data provide discriminatory information beyond that of clinical variables alone and highlight their potential to improve AAA risk stratification particularly in groups with a high population prevalence of disease.

Several caveats warrant consideration. First, our EWAS was based on whole-blood DNA methylation, which may not fully reflect methylation changes in aortic or other vascular tissues directly involved in AAA pathogenesis. Although blood is relevant to systemic inflammation, immune activation, and circulating vascular processes in AAA, future studies in disease-relevant vascular tissues are needed to clarify tissue-specific mechanisms. Second, the VA MVP cohort is predominantly male veterans with unique exposures and health profiles; residual confounding may affect internal validity, and selection mechanisms may limit external generalizability. We mitigated bias by prespecifying covariates, controlling test-statistic inflation, and applying MR to reduce confounding. We also observed concordance with external case-control studies. In addition, because the MVP methylation cohort included very few female incident AAA cases, sex-stratified analyses in women were severely underpowered, and the applicability of these findings to women requires confirmation in independent cohorts with larger female case numbers. Third, MR was conducted in individuals genetically similar to European reference populations because of data availability, although stratified EWAS suggested broadly similar CpG–AAA associations in those similar to African reference populations; population-diverse MR will be needed. In addition, a small degree of sample overlap between the CpG instrument derivation set and the MVP component of AAAGen may have introduced modest bias, although this is unlikely to have materially affected the results given the limited overlap and the use of strong instruments.[22] Fourth, the eQTM analyses utilized the older Infinium HumanMethylation450 (450k) array,[34] which resulted in coverage that did not include all CpGs identified in our analysis; consequently, eQTM data were unavailable for a subset of our CpGs. Fifth, although we have undertaken efforts to externally validate the MRS performance, it should be noted that the external cohorts are relatively small in size and that one cohort (New Zealand) is comprised exclusively of European men; further replication efforts should focus on larger, more diverse, or more pragmatic populations of interest. Sixth, although the clinical-epigenetic score provided statistically improved discrimination beyond clinical variables alone, the magnitude of improvement was fairly modest, which may limit its immediate clinical utility. Finally, all evidence here is generated from in-silico analyses; orthogonal validation in experimental models and prospective clinical studies is required to establish mechanisms and translational utility.

In summary, this large-scale EWAS, integrated with genetic and multi-omic analyses, identifies blood DNA methylation markers for incident AAA with supportive causal evidence. Mechanistic analyses implicate distal, enhancer-centric, inflammation-linked regulation and point to several gene-expression, lipid, and inflammation-related pathways as mediators. We also introduce a methylation-based risk score that complements clinical factors, although its clinical utility requires prospective validation. Collectively, these findings establish an epigenetic framework for refining AAA risk prediction

and prioritizing molecular targets for prevention and therapy.

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Author Contributions

S.Y. and S.M.D. conceived and designed the study. S.Y., G.S., T.D., A.V., J.A.L., S.P., D.L., R.J., K.C., J.A.B., J.C.B., B.M.P., P.S.T., G.T.J., S.M.D. contributed to data collection. S.Y., M.G.C., J.C.B., and G.T.J. undertook statistical analyses. S.Y. and M.G.C. created the data visualizations. S.Y. authored the initial draft of the manuscript. S.Y., M.G.C., G.S., M.G.L., K.H., R.J., T.D., A.V., J.A.L., S.J., D.L., R.J., K.C., J.A.B., J.C.B., B.M.P., B.F.V., P.S.T., G.T.J., and S.M.D. contributed to data interpretation, offered significant intellectual insights to the manuscript, and approved its final version.

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