SUPPLEMENTAL MATERIAL

Genetic Architecture of Abdominal Aortic Aneurysm in the Million Veteran Program

Derek Klarin et al.

Short Title: GWAS of AAA in MVP

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Expanded Methods

Genetic Data and Quality Control

DNA extracted from whole blood was genotyped in the Million Veteran Program (MVP) using a customized Affymetrix Axiom biobank array, the MVP 1.0 Genotyping Array[46]. Veterans (U.S. military personnel) of two mutually exclusive ethnic groups were identified for analysis: 1) non-Hispanic whites (European ancestry) for discovery and PRS analysis, and 2) non-Hispanic blacks (African ancestry) for PRS analysis. Prior to imputation, variants that were poorly called or that deviated from their expected allele frequency based on reference data from the 1000 Genomes Project[29] were excluded. After pre-phasing using EAGLE v2[47], genotypes from the 1000 Genomes Project[29] phase 3, version 5 reference panel were imputed into MVP participants via Minimac3 software[48]. Ethnicity-specific principal component analysis was performed using the EIGENSOFT v6 software[49]. Participants were then divided into two mutually exclusive ethnic groups based on self-identified race/ethnicity and admixture analysis using the ADMIXTURE v1.3 software[50]: 1) non-Hispanic whites (self-identified as "non-Hispanic," "white," and > 80% genetic European ancestry) and 2) non-Hispanic blacks (self-identified as "non-Hispanic," "black," and > 50% genetic African ancestry).

In MVP, sample and variant quality control was performed as previously described[31]. In brief, duplicate samples, those with more heterozygosity than expected, an excess (>2.5%) of missing genotype calls, or discordance between genetically inferred sex and phenotypic gender were excluded. In addition, one individual from each pair of related individuals (kinship > 0.0884 as measured by the KING 2.0 software[51]) were removed. In total, we identified 227,817 European participants for AAA discovery and PheWAS analysis from MVP release 2.1. For our PRS analysis, an additional, independent tranche of 1,656 AAA cases/44,908 controls of European ancestry and 718 AAA cases/46,380 controls of African ancestry were used after the above quality control metrics were applied.

Following imputation, variant level quality control was performed using the EasyQC R package (www.R-project.org), and exclusion metrics included: ancestry specific Hardy-Weinberg equilibrium P $<1x10^{-20}$, posterior call probability < 0.9, imputation quality < 0.3, minor allele frequency (MAF) < 0.0003, call rate < 97.5% for common variants (MAF > 1%), and call rate < 99% for rare variants (MAF < 1%). Variants were also excluded if they deviated > 10% from their expected allele frequency based on reference data from the 1000 Genomes Project. Following variant level quality control, we obtained 18.6 million DNA sequence variants for analysis.

We sought replication from either the 2016 AAA meta-analysis[7] or the combined HUNT/eMERGE datasets. In the 2016 AAA meta-analysis, lead DNA sequence variants were replicated *in silico* from the summary statistics generated at the time of analysis. Data were included only if the variant was available within each study in the overall meta-analysis. In HUNT, DNA from 71,860 samples was genotyped using one of three different Illumina HumanCoreExome arrays (HumanCoreExome12 v1.0, HumanCoreExome12 v1.1 and UM HUNT Biobank v1.0). We excluded samples that failed to reach a 99% call rate, had contamination > 2.5% as estimated with BAF Regress, large chromosomal copy number variants, lower call rate of a technical duplicate pair and twins, gonosomal constellations other than XX and XY, or whose

inferred sex contradicted the reported gender. Samples that passed quality control were analyzed in a second round of genotype calling following the Genome Studio quality control protocol. Genomic position, strand orientation and the reference allele of genotyped variants were determined by aligning their probe sequences against the human genome (build 37) using BLAT. PLINK v1.90[52] was then used to exclude variants if their probe sequences could not be perfectly mapped, cluster separation was < 0.3, Gentrain score < 0.15, showed deviations from Hardy Weinberg equilibrium in unrelated samples of European ancestry with p-value < 0.0001, had a call rate < 99%, or another assay with higher call rate genotyped the same variant. Ancestry of all samples was inferred by projecting all genotyped samples into the space of the principal components from 938 unrelated individuals of the Human Genome Diversity Project reference panel. The different arrays were harmonized by reducing to a set of overlapping variants and excluding variants that showed frequency differences > 15% between data sets, or that were monomorphic in one and had MAF > 1% in another data set. The resulting genotype data were phased using EAGLE v2[47] imputation was performed on samples of recent European ancestry using Minimac[48] and a merged reference panel that was constructed by combining the Haplotype Reference Consortium (HRC)[53] panel and a local reference panel based on 2,202 whole-genome sequenced HUNT study participants. We excluded variants with rsq < 0.3 resulting in over 24.9 million well-imputed variants for single variant association analysis. In eMERGE, genotyping, quality control, and HRC imputation have been previously described[54].

Diagnosis Codes and Definitions for AAA Phenotype Definition
AAA cases were defined as the presence of two instances of any of the following ICD-9/10 codes in a participant's EHR:
ICD-9 441.3 - Abdominal aortic aneurysm, ruptured
ICD-9 441.4 - Abdominal aortic aneurysm, without rupture
ICD-10 I71.3 - Abdominal aortic aneurysm, ruptured
ICD-10 I71.4 - Abdominal aortic aneurysm, without rupture

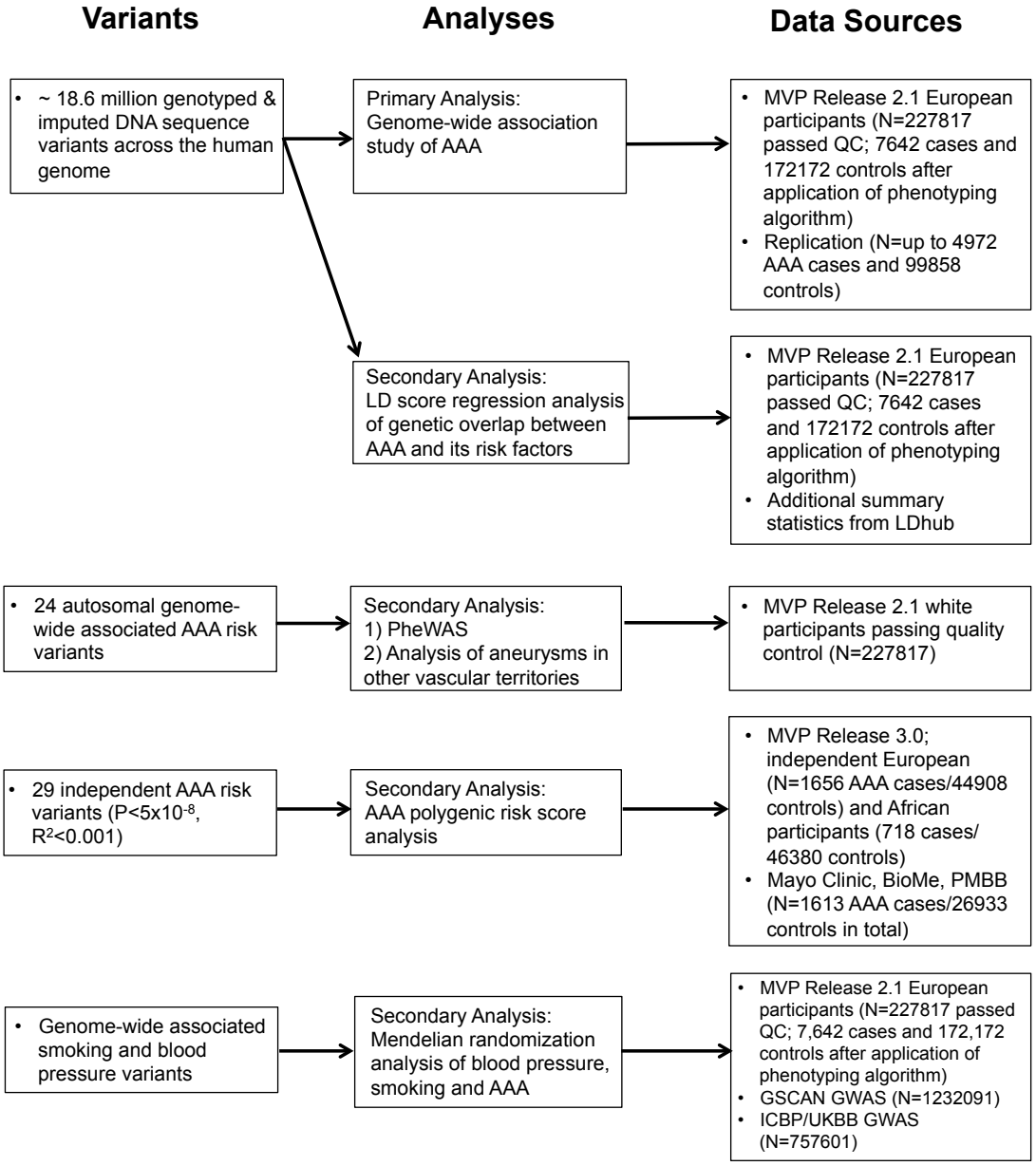
Controls were defined as possessing zero occurrences of the aforementioned ICD codes, as well as zero occurrences of:

ICD-9 440 - Atherosclerosis
ICD-9 441 - Aortic aneurysm and dissection
ICD-9 442 - Other aneurysm
ICD-9 443 - Other peripheral vascular disease
ICD-9 444 - Arterial embolism and thrombosis
ICD-9 445 - Atheroembolism
ICD-9 446 - Polyarteritis nodosa and allied conditions
ICD-9 447 - Other disorders of arteries and arterioles
ICD-9 448 - Diseases of capillaries

ICD-10 I71 - Aortic aneurysm and dissection
ICD-10 I72 - Other aneurysm
ICD-10 I73 - Other peripheral vascular disease
ICD-10 I74 - Arterial embolism and thrombosis
ICD-10 I75 - Atheroembolism
ICD-10 I77 - Other disorders of arteries and arterioles
ICD-10 I78 - Diseases of capillaries
ICD-10 I79 - Disorders of arteries, arterioles and capillaries in diseases classified elsewhere

ICD-10 K55 - Vascular disorders of intestine

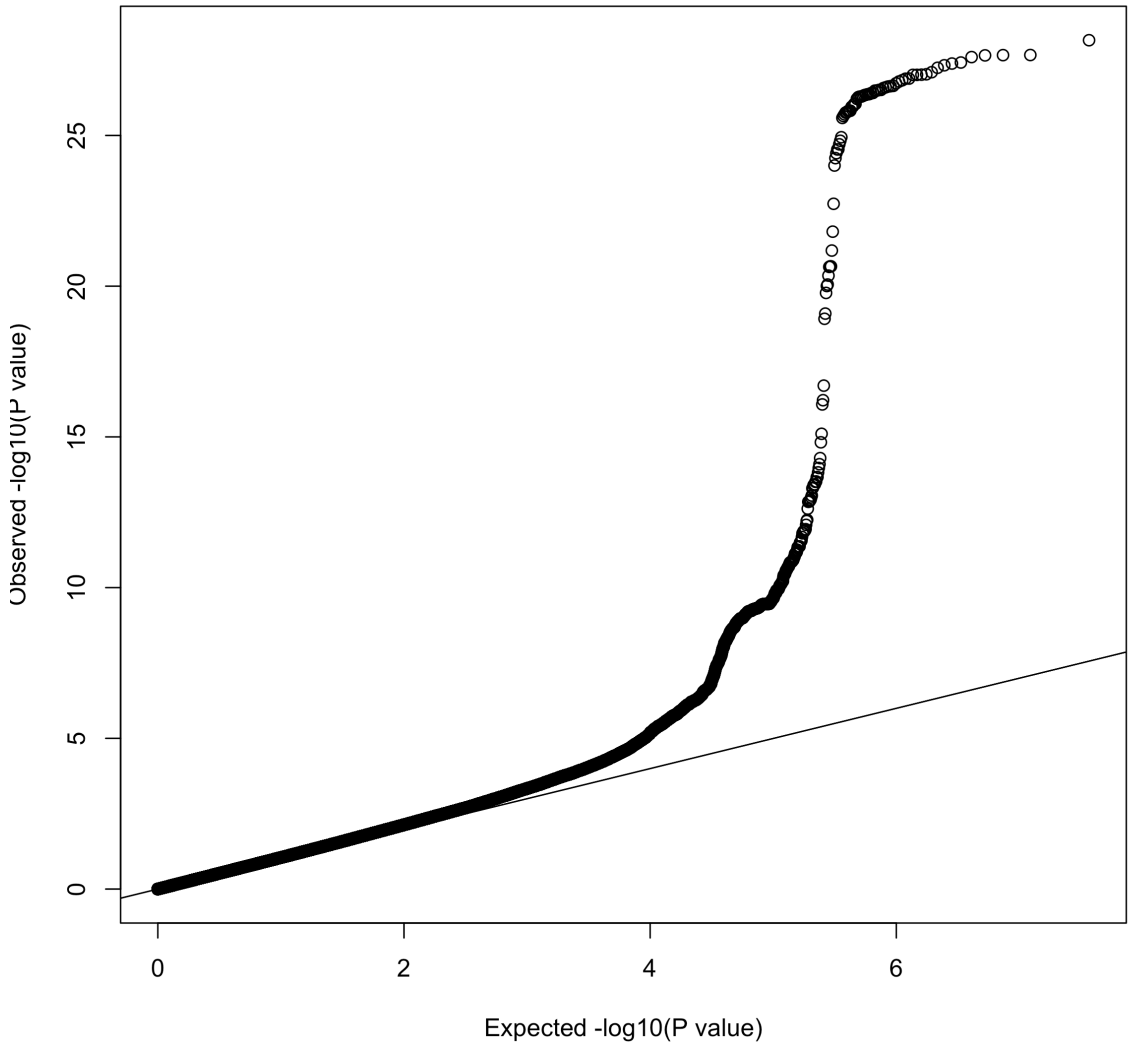
Supplementary Figure I - Overall Study Design



Supplementary Figure I - The primary analysis consisted of a genome-wide association study to identify novel AAA risk variants. Secondary analyses included: an analysis of AAA and overlap with its risk factors, a closer examination of AAA risk variants through PheWAS and their association with aneurysms in other vascular territories, generation and analysis of AAA polygenic risk scores, and Mendelian randomization analyses of smoking, blood pressure, and AAA.

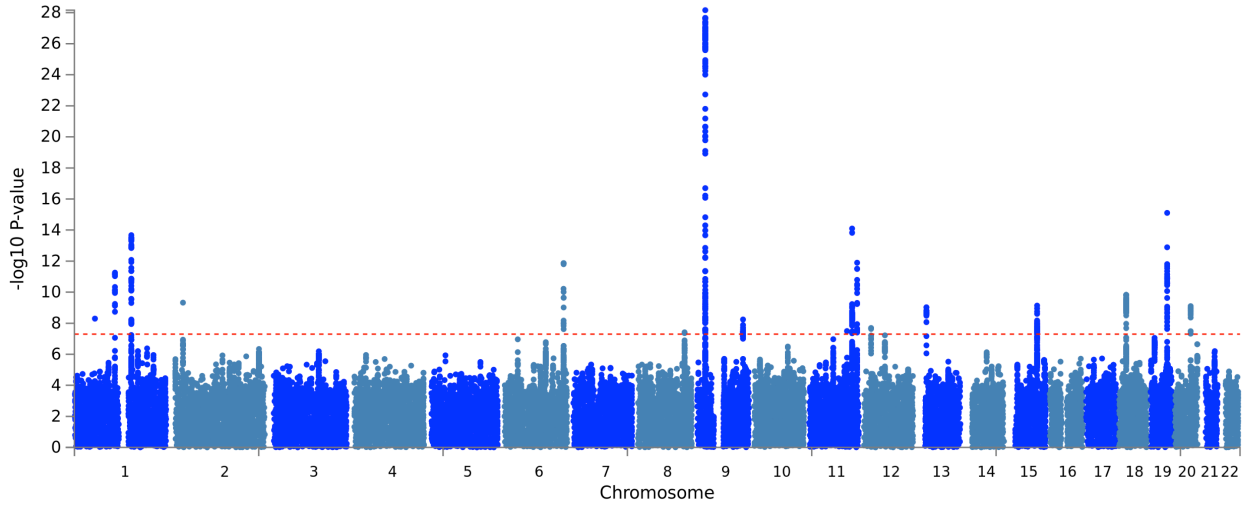
Abbreviations: AAA, Abdominal Aortic Aneurysm; MVP, Million Veteran Program; PheWAS, Phenome-wide Association Study; QC, Quality Control; LD, Linkage Disequilibrium; PMBB, Penn Medicine Biobank; GSCAN, GWAS & Sequencing Consortium of Alcohol and Nicotine use; ICBP, International Consortium of Blood Pressure; UKBB, UK Biobank; GWAS, Genome-wide Association Study

Supplementary Figure II - Quantile-quantile plot for the discovery AAA GWAS in MVP



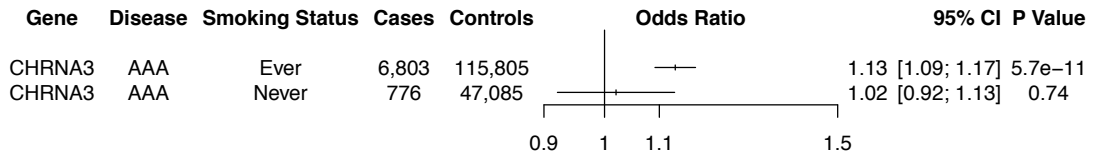
Supplementary Figure II - The expected logistic regression association P values versus the observed distribution of P values for AAA association are displayed. No systemic inflation was observed (λ_{GC} = 1.07). All P values were two-sided. Abbreviations: AAA Abdominal Aortic Aneurysm; GWAS, Genome-wide Association Study; MVP, Million Veteran Program

Supplementary Figure III - Manhattan plot for the AAA GWAS



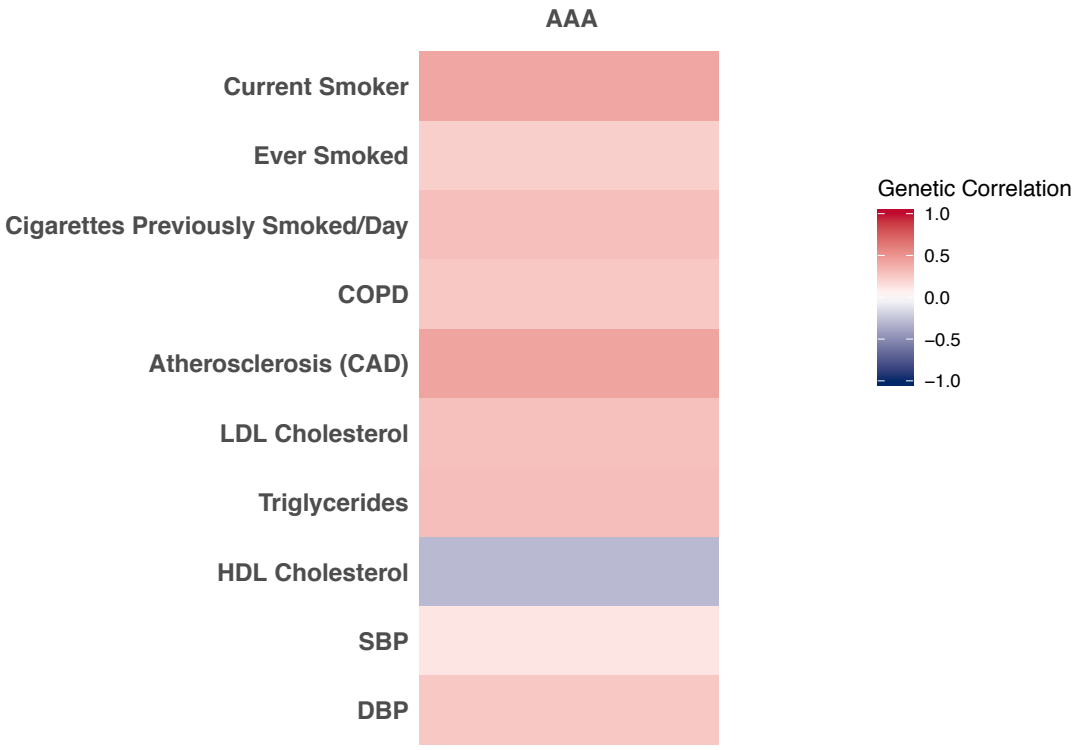
Supplementary Figure III - Plot of -log10(*P*) for association of genotyped and imputed variants by chromosomal position for all autosomal polymorphisms analyzed in the AAA GWAS. Logistic regression two-sided P values are displayed.
Abbreviations: AAA, Abdominal Aortic Aneurysm; GWAS, genome-wide association study

Supplementary Figure IV - Forest plot for association of the *CHRNA3* locus and AAA risk stratified by smoking status



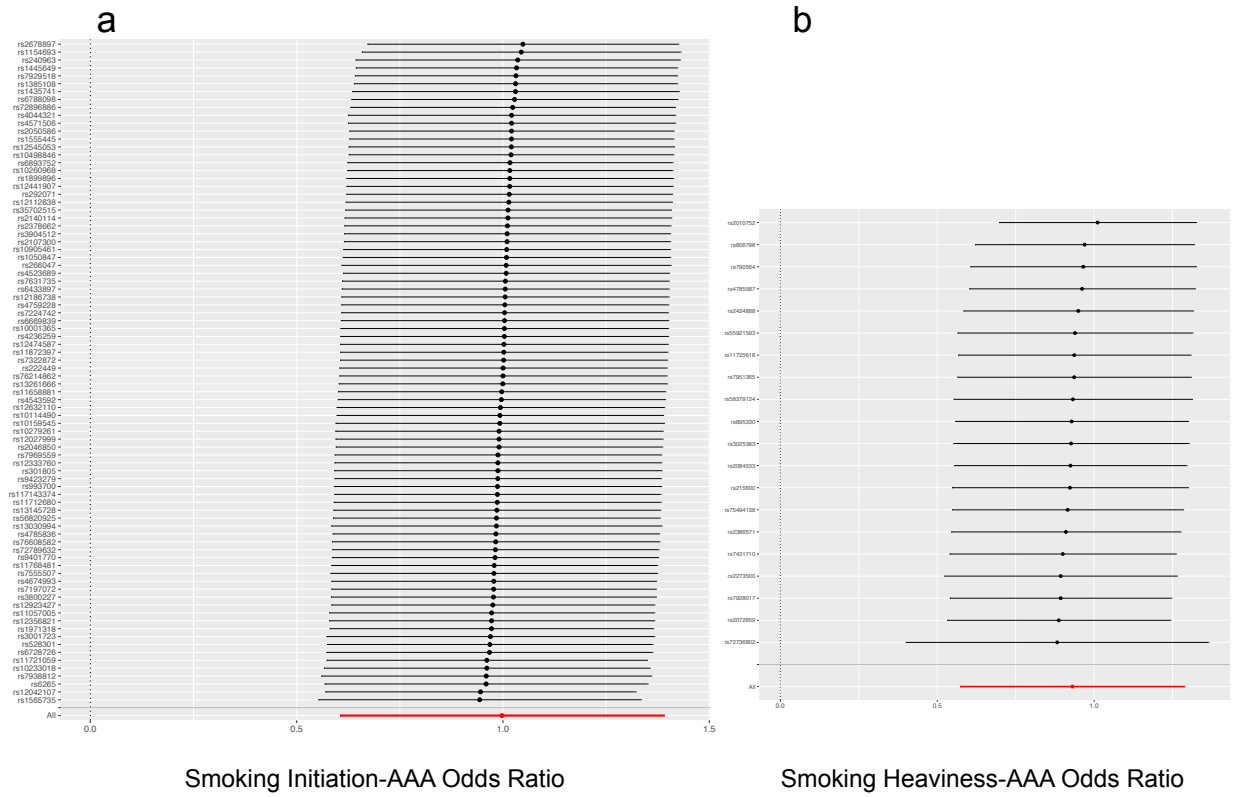
Supplementary Figure IV - When stratifying European MVP participants by smoking status (ever smokers vs. never smokers), nearly all the DNA sequence variant-AAA association signal resides within the ever smoker group. Previous reports of variation at the *CHRNA3* locus demonstrated that carriers of the AAA risk allele have a reduced likelihood of cigarette smoking cessation[55], and suggests that the AAA-*CHRNA3* association is driven by a greater burden of tobacco exposure in those who carry the nicotine dependence/AAA risk allele. Individuals without a smoking designation in the VHA electronic health record were excluded from this analysis. Logistic regression two-sided values of P are displayed.
Abbreviations: MVP, Million Veteran Program; AAA, Abdominal Aortic Aneurysm; VA, Veterans Affairs; CI, Confidence Interval

Supplementary Figure V - Genetic correlation of AAA with risk factors for disease and rupture



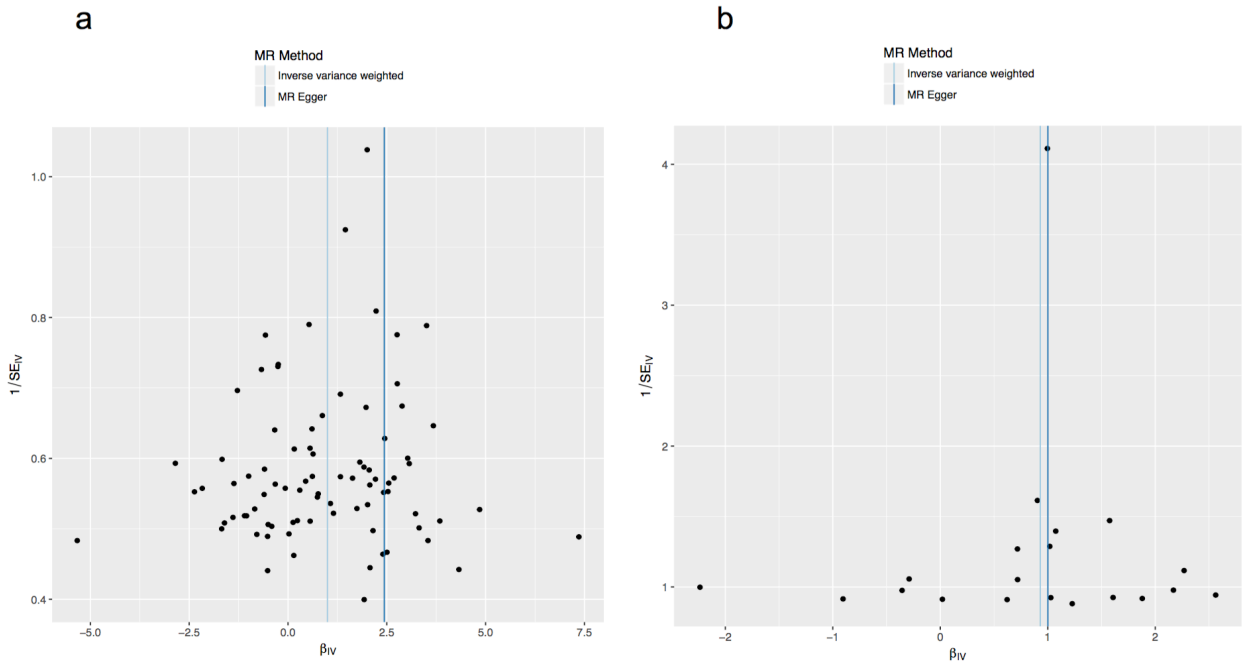
Supplementary Figure V - Using linkage disequilibrium score regression[16], summary statistics for AAA from the current study (N = 7,642 AAA cases and 172,172 controls) and 10 AAA risk factors were used to calculate a genetic correlation between traits (r_g). Nine of ten risk factors demonstrated a significant genetic correlation with AAA after Bonferroni correlation (r_g P < 0.005 for all except systolic blood pressure). The genetic correlation values, associated standard errors, (two-sided) values of P, and sample sizes for each trait are depicted in Table V in the supplement.
Abbreviations: AAA, Abdominal Aortic Aneurysm; COPD, Chronic Obstructive Pulmonary Disease; CAD, Coronary Artery Disease; LDL, Low-Density Lipoprotein; HDL, High-Density Lipoprotein; SBP, Systolic Blood Pressure; DBP, Diastolic Blood Pressure

Supplementary Figure VI - Diagnostic leave-one-out plot for smoking exposure-AAA Mendelian randomization analysis



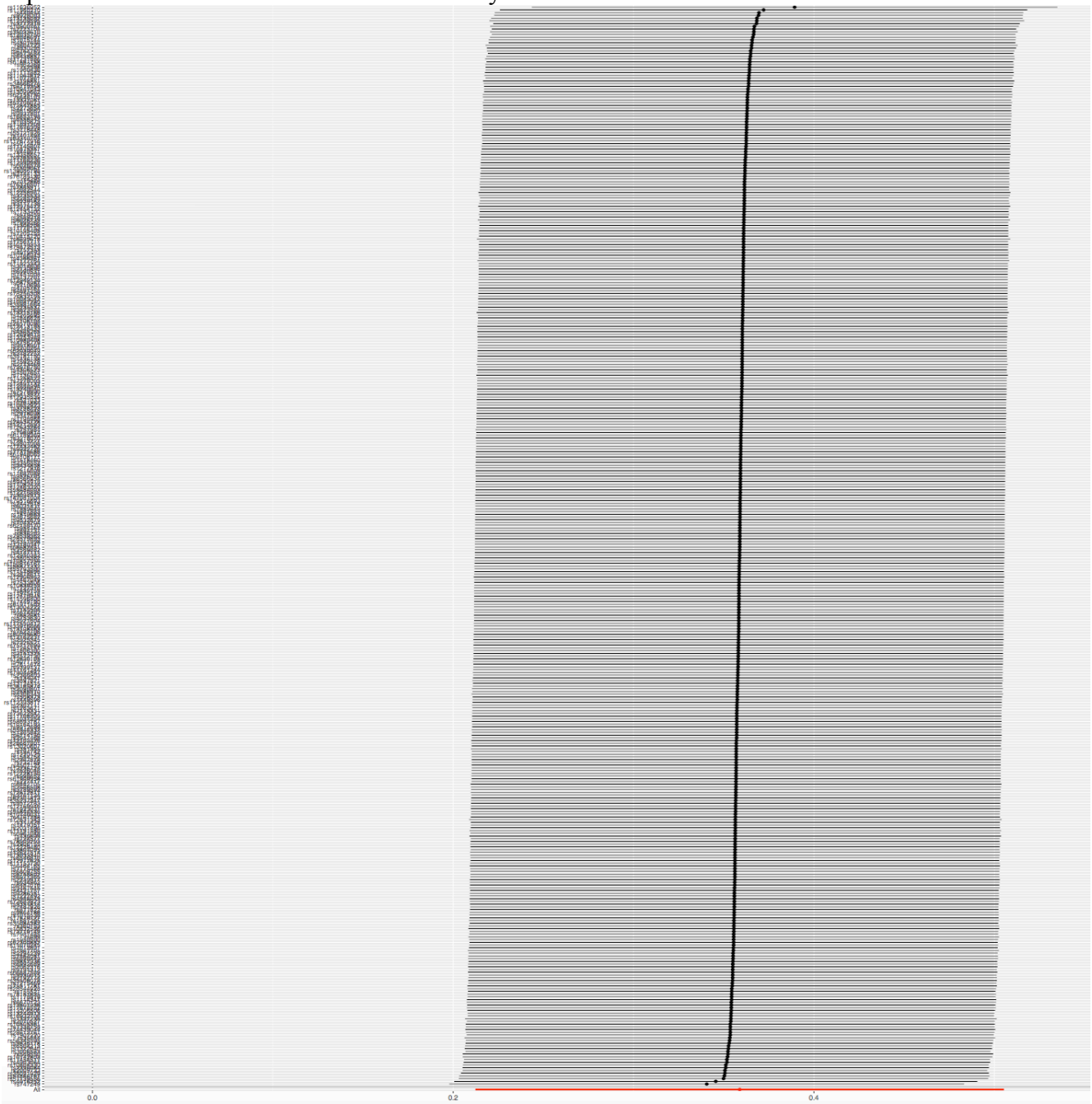
Supplementary Figure VI - Diagnostic leave-one-out plots were generated for each of the smoking exposures with a positive Mendelian randomization result - smoking initiation (a) and smoking heaviness (b) - with the AAA outcome. In each case, minimal evidence of a single association driving the inverse-variance weighted result was observed.

Supplementary Figure VII - Diagnostic funnel plot for smoking exposure-AAA Mendelian randomization analysis



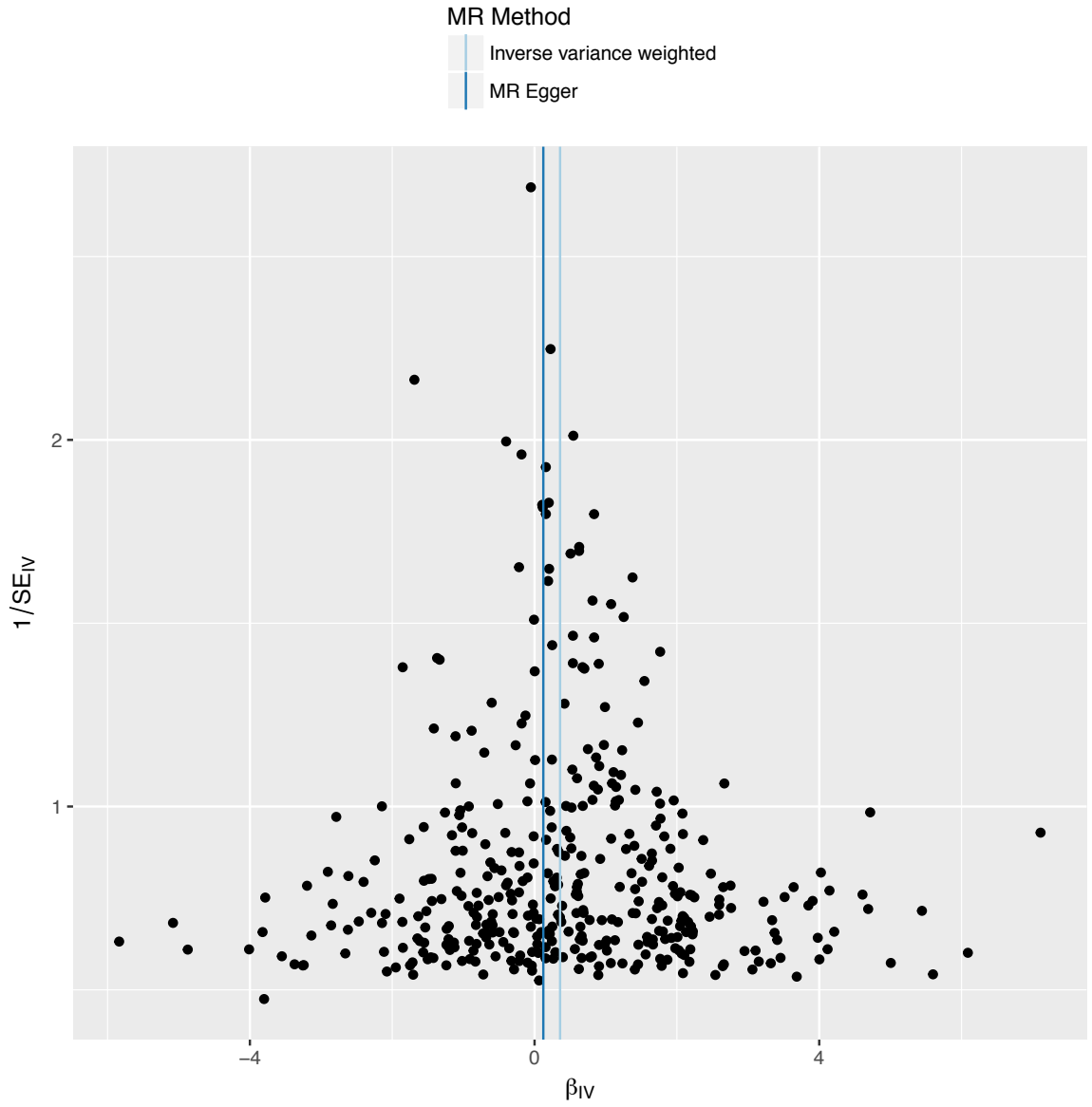
Supplementary Figure VII - A funnel plot depicting the reciprocal of the standard error (Y-axis) of the instrumental variable estimate against the instrumental variable estimates (X-axis) for smoking initiation (a) and smoking heaviness (b). Ideally, these plots should demonstrate a symmetric funnel, in which more precise estimates are less variable. Minimal asymmetry was observed
Abbreviations: MR, Mendelian Randomization; IV, Instrumental Variable

Supplementary Figure VIII - Diagnostic leave-one-out plot for diastolic blood pressure exposure-AAA Mendelian randomization analysis



Supplementary Figure VIII - A diagnostic leave-one-out plot was generated for the diastolic blood pressure exposure Mendelian randomization analysis with the AAA outcome. Minimal evidence of a single association driving the inverse-variance weighted result was observed.

Supplementary Figure IX - Diagnostic funnel plot for diastolic blood pressure exposure-AAA Mendelian randomization analysis



Supplementary Figure IX - A funnel plot depicting the reciprocal of the standard error (Y-axis) of the instrumental variable estimate against the instrumental variable estimates (X-axis) for diastolic blood pressure. Ideally, these plots should demonstrate a symmetric funnel, in which more precise estimates are less variable. Minimal asymmetry was observed.
Abbreviations: MR, Mendelian Randomization; IV, Instrumental Variable

Legends for Supplementary Excel File Tables

Supplementary Table I - Odds Ratios and P values for 10 previously identified genome-wide significant AAA loci in MVP discovery GWAS analysis (N = 7,642 AAA cases and 172,172 controls)

Supplementary Table II - Logistic regression odds ratios and two-sided P values for 14 novel genome-wide significant AAA loci in MVP discovery and replication analyses

Supplementary Table III - Logistic regression odds ratios and two-sided P values for suggestive loci in MVP discovery that did not meet prespecified criteria for replication (N = up to 12,614 cases and 272,030 controls - MVP+Stage2a; N = up to 10,477 cases and 279,641 controls - MVP+Stage2b)

Supplementary Table IV - Logistic regression phenome-wide association results (PheWAS) for AAA risk DNA sequence variants surviving Bonferroni Correction (two-sided P < 1.7E-6). All associations are aligned to the AAA risk allele

Supplementary Table V - LD Score regression genetic correlation (r_g) results for AAA and its risk factors (and risk factors for rupture). AAA summary statistics from MVP (N = 7,642 AAA cases and 172,172 controls) and the data source provided below were used for this analysis. Two-sided values of P are displayed

Supplementary Table VI - Logistic (smoking initiation, smoking cessation) and linear (smoking heaviness) regression effect estimates, standard errors, and two-sided P values of variants used for Mendelian randomization analysis. Effect estimates/P values are taken from 2019 GSCAN GWAS analysis summary-level data from up to 1,232,091 participants

Supplementary Table VII - Logistic regression association effect estimates, standard errors, and two-sided P values for the smoking phenotype-AAA Mendelian randomization analyses

Supplementary Table VIII - Linear regression SBP and DBP effect estimates, standard errors, and two-sided P values of variants used for Mendelian randomization analysis. Effect estimates/P values are taken from 2019 ICBP+UK Biobank discovery GWAS analysis summary statistics in up to 757,601 participants

Supplementary Table IX - Logistic regression association effect estimates, standard errors, and two-sided P values for the blood pressure-AAA Mendelian randomization analyses. Effect estimates are scaled to reflect odds of outcome per 10mmHg increase in each blood pressure trait.

Supplementary Table X - Logistic regression association results for AAA risk DNA sequence variants with cerebral, lower extremity, and iliac artery aneurysms. All

associations are aligned to the AAA risk allele and two-sided values of P are displayed. Nominally significant associations (P< 0.05) are highlighted in blue

Supplementary Table XI - Logistic regression association results for AAA risk DNA sequence variants with cerebral, lower extremity, and iliac artery aneurysms after excluding AAA cases. All associations are aligned to the AAA risk allele and two-sided values of P are displayed. Associations significant (P< 0.05) after excluding AAA cases from the analysis are highlighted in blue. A greater attenuation of association signal for the AAA risk variants with iliac artery and lower extremity aneurysms than for cerebral aneurysms was observed.

Supplementary Table XII - Overlap of diagnoses between AAA and lower extremity aneurysms/iliac artery aneurysms/cerebral aneurysms in MVP 2.1 data

Supplementary Table XIII - Variants used for 3 different weighted AAA polygenic risk scores (PRS_{AAA}), generated from MVP AAA summary statistics (N = 7,642 AAA cases and 172,172 controls)

Supplementary Table XIV - Demographic and clinical characteristics of Mayo Clinic Vascular Disease Biorepository AAA ascertained case-control cohort. All participants are of European ancestry

Supplementary Table XV - Baseline characteristics and AAA prevalence across MVP release 3.0 European and African ancestry individuals, BioMe, and the Penn Medicine Biobank

Supplementary Table XVI - Prevalence of AAA among the individuals in the top 5% PRS_{AAA} in MVP (African and European ancestries), as well as Penn Medicine Biobank and BioMe (European ancestry) stratified by study, age, and gender

Supplementary Table XVII - Prevalence of AAA MVP release 3.0 (African and European ancestries), as well as Penn Medicine Biobank and BioMe (European ancestry) stratified by PRS quintile

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