

ORIGINAL RESEARCH

Body Mass Index and Hypertension as Mediators of the Association Between Age at Menarche and Subclinical Atherosclerosis: A Sex-Specific Mendelian Randomization Analysis

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BACKGROUND: Early age at menarche (AAM) has been associated with a higher risk of carotid artery intima-media thickness (cIMT), an indicator of subclinical vascular disease, albeit the mechanisms underlying this association remain elusive. A better understanding of the relationship between AAM, modifiable cardiometabolic risk factors, and subclinical atherosclerosis may contribute to improved primary prevention and cardiovascular disease treatment. We aimed to investigate the putative causal role of AAM on cIMT, and to identify and quantify the potentially mediatory effects of cardiometabolic risk factors underlying this relationship.

METHODS AND RESULTS: We conducted linkage disequilibrium score regression analyses between our exposure of interest, AAM, our outcome of interest, cIMT and potential mediators of the AAM-cIMT association to gauge cross-trait genetic overlap. We considered as mediators the modifiable anthropometric risk factors body mass index (BMI), systolic blood pressure (SBP), lipid traits (total cholesterol, triglycerides, high-density lipoprotein cholesterol, and low-density lipoprotein cholesterol), and glycemic traits (fasting glucose). We then leveraged the paradigm of Mendelian randomization to infer causality between AAM and cIMT, and to identify whether cardiometabolic risk factors served as potential mediators of this effect. Our analyses showed that genetically predicted AAM was inversely associated with cIMT, BMI, SBP, and triglycerides, and positively associated with high-density lipoprotein, low-density lipoprotein, and total cholesterol. We showed that the effect of genetically predicted AAM on cIMT may be partially mediated through BMI (20.1% [95% CI, 1.4% to 38.9%]) and SBP (13.5% [95% CI, 0.5%–26.6%]). Our cluster-specific Mendelian randomization revealed heterogeneous causal effect estimates of age at menarche on BMI and SBP.

CONCLUSIONS: We highlight supporting evidence for a potential causal association between earlier AAM and cIMT, and almost one third of the effect of AAM on cIMT may be mediated by BMI and SBP. Early intervention aimed at lowering BMI and hypertension may be beneficial in reducing the risk of developing subclinical atherosclerosis due to earlier age at menarche.

Key Words: age at menarche ■ BMI ■ cIMT ■ Mendelian randomization ■ SBP

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CLINICAL PERSPECTIVE

What Is New?

- This Mendelian randomization study provides evidence that genetically predicted earlier age at menarche is potentially causally associated with carotid intima-media thickness, a marker of subclinical atherosclerosis.
- Our results suggest that the association between age at menarche and carotid intima-media thickness may be partially mediated by adult body mass index and systolic blood pressure.

What Are the Clinical Implications?

- These findings further support the adverse health effects of earlier age at menarche.
- Early intervention aimed at lowering body mass index and hypertension may be beneficial in reducing the risk of developing subclinical atherosclerosis due to earlier age at menarche.

Nonstandard Abbreviations and Acronyms

AAM	age at menarche
cIMT	carotid intima-media thickness
FG	fasting glucose
IVW	inverse-variance weighted
LDSC	linkage disequilibrium score
MR	Mendelian randomization
TC	total cholesterol

Cardiovascular disease (CVD) is one of the leading causes of morbidity and death in women worldwide.¹ Emerging evidence suggests that sex-specific risk factors such as age at menarche (AAM), age at menopause, and timing and number of births also contribute to a woman's unique CVD risk, in addition to the traditionally established CVD risk factors including hyperlipidemia, hypertension, diabetes, and obesity. AAM in particular is considered to be an independent risk factor for lifetime risk of CVD events, CVD death, and overall death in women.^{2,3} Epidemiological studies have highlighted an association between earlier menarche (<10 years of age) in women and a significantly higher relative risk of coronary heart disease than women who experienced menarche at a later onset (12–13 years of age).^{2,4} These findings suggest that sex-specific factors play an important role in lifetime CVD risk that is potentially independent of traditional

risk factors in women; however, mechanisms underlying this association remain unknown.

Earlier AAM has also been associated with other traditional cardiometabolic risk factors, including higher BMI,^{5,6} higher blood pressure, and abnormal glycemia in adolescents.⁷ It has been reported that the relationship between AAM and body mass index (BMI) may mediate the association between AAM and cardiovascular health outcomes, albeit findings from prospective studies have reported contradictory results when adjusting for BMI in relation to menarche onset and cardiometabolic outcomes in adults.^{6,7} Similarly, it remains unclear whether systolic blood pressure (SBP) and glycemic and lipid traits serve as a potential confounder in the AAM and CVD relationship.^{8–10} Previous studies consistently demonstrated the potential causal association of AAM with CVD outcomes, coronary artery disease,^{11,12} and ischemic heart disease.¹³ Several studies have additionally proposed that AAM is associated with various cardiometabolic risk factors, including SBP, BMI, blood lipid levels, and markers of subclinical atherosclerosis (ie, cIMT). All these cardiometabolic traits and diseases are considered risk factors for future CVD events. However, most of them have not examined the potential mediatory effect of these risk factors. Whether the previously reported associations between AAM and CVD reflect a causal effect and whether they are mediated by BMI and other cardiometabolic risk factors remains to be determined.

cIMT, a marker of subclinical vascular disease, predicts future cardiovascular events including myocardial infarction and stroke and its timely recognition can slow or prevent the progression to overt CVD. The association between AAM and cIMT has been investigated in only a few observational studies, with contradictory findings.¹⁴ Similarly, there is some evidence to suggest an association between AAM, lipid traits, glycemic traits and blood pressure, although the evidence is also conflicting.^{15,16} A better understanding of the relationship between pubertal timing, modifiable cardiometabolic risk factors, and subclinical atherosclerosis may contribute to early intervention and improved disease management.

The use of genomic analyses such as linkage disequilibrium score (LDSC) regression and MR may enable us to unravel the complex associations between cardiometabolic risk factors, AAM, and cIMT. LDSC is a method that provides unbiased estimates for the genetic correlation between 2 traits.^{17,18} MR is an instrumental variable method that exploits the random assignment of exposure-associated risk alleles, enabling us to detect the potentially causal relationships between risk factors and disease outcomes.¹⁹ MR framework can overcome limitations of observational studies by using genetic variants as instrumental variables that are randomly distributed at conception,

thereby mimicking randomized controlled trials,²⁰ and are less prone to reverse causation, measurement error, and reverse causality.²¹ Numerous studies have investigated the potential causal associations of AAM with CVDs, but a vast number of those have mainly focused on the clinical CVD outcomes including coronary artery disease, myocardial infarction, and stroke.^{12,13,22,23} No MR study has investigated the causal associations between AAM and preclinical vascular disease as measured by cIMT.

In this study, we aimed to (1) estimate cross-trait genetic correlations and identify genome-wide genetic overlap between AAM, cIMT, and modifiable cardiometabolic risk factors; (2) perform univariable MR analyses to test if genetic liability to AAM causally increases the risk of subclinical atherosclerosis using cIMT as a marker; and (3) perform 2-step MR to investigate whether clinically relevant cardiometabolic risk factors mediate effects of genetic liability to AAM on cIMT.

METHODS

An overview of the study design is shown in Figure 1.

Genetic Association Data Sources

Age at Menarche

Genetic associations of AAM were obtained from a large-scale sex-specific genome-wide association study

(GWAS) meta-analysis among 329 345 women of European ancestry (ReproGen consortium [N=179 117], 23andMe [N=76 831], and UK Biobank [N=73 397],²⁴ Table S1).

Carotid Intima-Media Thickness

We performed a sex-stratified GWAS of cIMT (mean of the maximum, unit of measurement [μm]) to identify genetic instruments associated with cIMT in the UK Biobank (UKBB) sample (Figure 2, Table S2). A description of UKBB resources and cIMT phenotyping have been described in detail previously^{25,26} (UKBB Resource 511, <https://biobank.ctsu.ox.ac.uk/crystal/refer.cgi?id=511>). In brief, a GWAS of cIMT was performed on 22 000 participants of European-only ancestry (mean age, 56.5 [range, 40–69] years). For the GWAS of cIMT in the UKBB set, we use natural log-transformed values of the mean of the maximum cIMT for normality. We restricted the samples to European-only individuals on the basis of the UKBB data field 21 000 (<https://biobank.ctsu.ox.ac.uk/crystal/field.cgi?id=21000>). The GWAS of cIMT was performed using a linear mixed noninfinitesimal model as implemented in BOLT-LMM software.²⁷

Modifiable Cardiometabolic Risk Factors

Anthropometric Traits

Genetic associations of adult BMI (kg/m^2) were obtained from a UKBB GWAS among 193 570 female

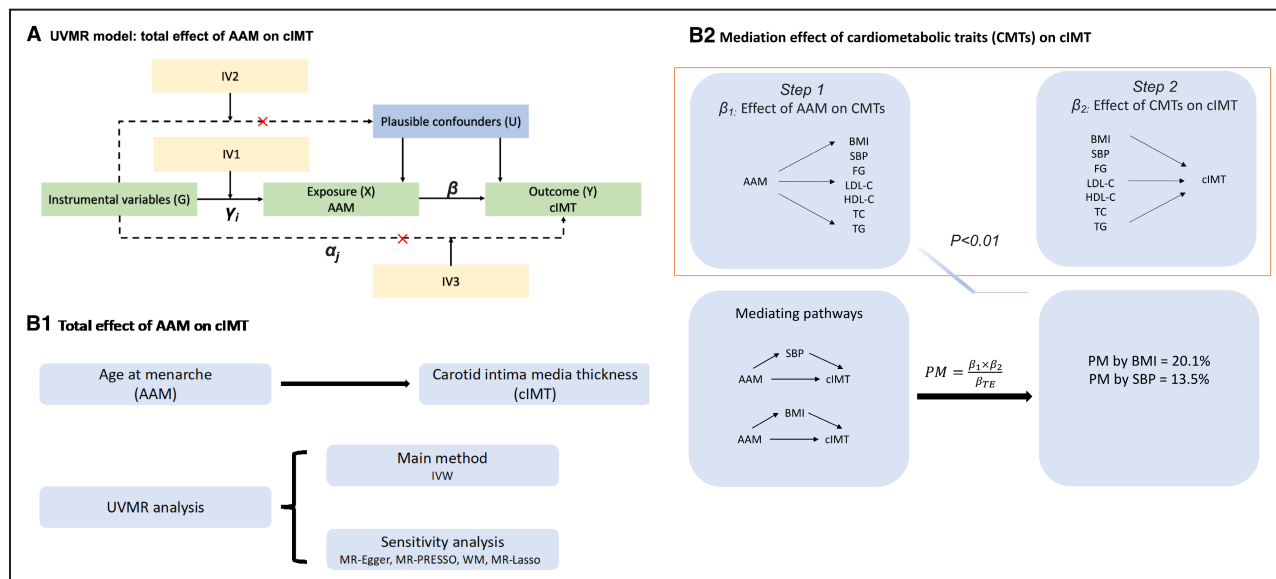


Figure 1. Schematic design showing (A) the main principles of Mendelian randomization and (B) workflow of the proposed causal relationships between AAM, cardiometabolic risk factors, and cIMT.

Based on the MR assumptions, the genetic variants are assumed to affect cIMT through AAM only, not through confounding factors. AAM indicates age at menarche; BMI, body mass index; cIMT, carotid artery intima-media thickness; FG, fasting glucose; HDL, high-density lipoprotein cholesterol; IVW-RE, random-effects inverse variance-weighted; LDL, low-density lipoprotein cholesterol; MR, Mendelian randomization; MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier; PM, proportion mediated; SBP, systolic blood pressure; SNPs, single nucleotide polymorphism; TC, total cholesterol; TG, triglycerides; UVMR, univariate Mendelian randomization; and WM, weighted median.

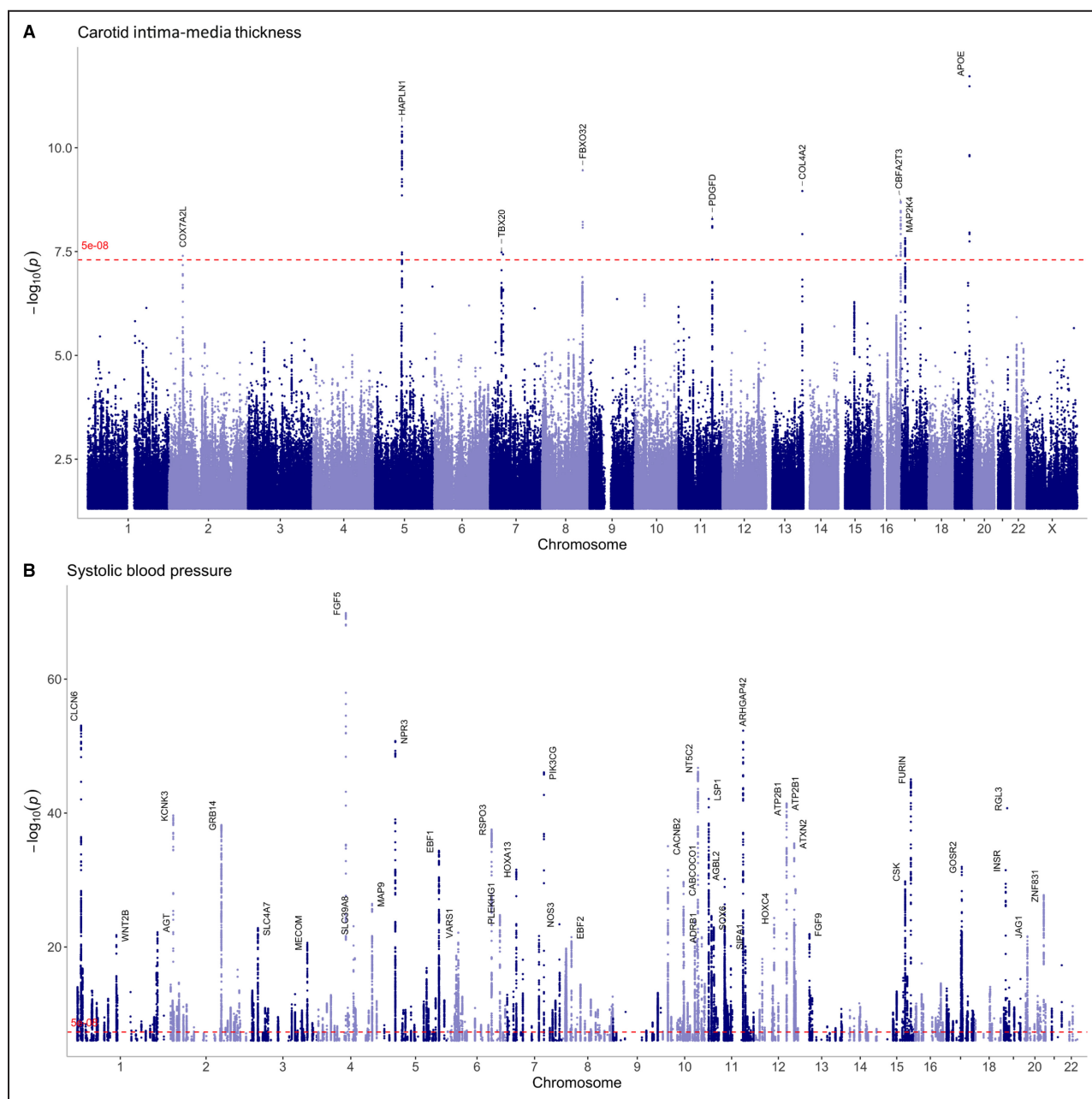


Figure 2. Genome-wide association study (A) cIMT and (B) SBP.

SNPs with $P < 0.05$ and $1e-5$ were plotted for cIMT and SBP, respectively. For SBP, SNPs with $P < 5e-20$ were annotated to nearest gene to avoid overlap (genome build 37). Red dotted line indicates genome-wide threshold of $P < 5e-8$. cIMT indicates carotid artery intima-media thickness; SBP, systolic blood pressure; and SNPs, single-nucleotide polymorphisms.

participants (<http://www.nealelab.is/uk-biobank/>).²⁸ Inverse rank-normal transformation was applied to the raw BMI value constructed using height and weight measured during the initial center visit (Table S1).

Systolic Blood Pressure

We performed a sex-specific GWAS of systolic blood pressure (SBP; mmHg) using data on up to 230 000 European-only women participants (mean age, 56.60

[SD, 7.87] years; mean SBP, 138.0064 [SD, 21.178] mmHg) using the UKBB resources (Figure 2, Table S3). We adjusted for medication use by adding 15 mmHg to SBP measurements for individuals reported to be taking blood pressure-lowering medication (N=94 289, UKBB data field 6153 [<https://biobank.ndph.ox.ac.uk/ukb/field.cgi?id=6153>]). The sex-specific GWAS was performed using BOLT-LMM software,²⁷ and the analysis was adjusted for age and the first 4 principal components on the basis of the UKBB data.

Glycemic Traits

Instrumental variables associated with fasting glucose (FG) were extracted from a large-scale sex-specific GWAS from the meta-analyses of glucose and insulin-related traits consortium (MAGIC) ($n=73\,089$ women).²⁹ Briefly, FG was measured in whole blood and corrected to plasma level using the correction factor of 1.13, and individuals were excluded from the analysis if they had a physician diagnosis of diabetes, were on diabetes treatment (oral or insulin), or had a fasting plasma glucose ≥ 7 mmol/L²⁹ (Table S1).

Lipids

Instrument variables associated with high-density lipoprotein cholesterol (HDL-C; mmol/L), low-density lipoprotein cholesterol (LDL-C; mmol/L), triglycerides (mmol/L), and total cholesterol (TC; mmol/L) were retrieved from publicly available GWAS summary data from the Neale laboratory [UKBB—Neale laboratory; <http://www.nealelab.is/uk-biobank/>].²⁸ The analyses included European-only participants and were adjusted for age, age², sex, and the first 20 principal components.

The studies used in our analysis were approved by their respective institutional review boards, and informed consent was provided by all participants. Further details on study cohorts used for the analysis are provided in Table S1.

Cross-Trait LDSC

We applied LDSC to quantify the genetic overlap between AAM, BMI, SBP, HDL-C, LDL-C, triglycerides, TC, FG, and cIMT using the LDSC software with GWAS summary statistics data on up to 1.2 million HapMap single-nucleotide polymorphisms (SNPs).^{17,18} Briefly, LDSC estimates the genetic covariance by regressing the cross-products of summary test statistics (Z1Z2 statistics) for 2 traits against its LDSC.^{17,18} The slope (coefficient) represents genetic correlation. We used the precomputed LDSCs of European samples estimated using the 1000 Genomes reference panel provided on the website of this software (<http://github.com/bulik/ldsc>). The analysis was restricted to HapMap3 SNPs, as these are well-imputed in most studies.³⁰ The “munge_sumstats.py” and “ldsc.py” from the LDSC command line tool were used to convert the GWAS summary statistics for LDSC regression and to conduct LDSC, respectively. To account for multiple testing, we applied a 5% false discovery rate.

Two-Sample Univariable MR

We performed a 2-sample univariable MR to investigate the effect of AAM on cIMT. Figure 1 shows the overall study design that we have adopted for this

analysis. We selected SNPs associated with AAM at P value $< 5 \times 10^{-8}$. SNPs with a minor allele frequency $\leq 5\%$ and F -statistics < 10 were excluded. Correlated SNPs ($r^2 \geq 0.001$) were excluded by keeping the one with the strongest association with AAM (ie, with the smallest P value). Genetic associations of cIMT for these SNPs were then obtained from the GWAS of cIMT.

To further investigate the mediating effects of our selected modifiable risk factors on the AAM–cIMT association, we applied a 2-step MR strategy.³¹ Specifically, we performed 2-sample univariable MR analyses for investigating the potential causal effect of AAM on BMI, SBP, HDL-C, LDL-C, triglycerides, TC, FG, and the potential causal effect of on BMI, SBP, HDL-C, LDL-C, triglycerides, TC, and FG on cIMT to assess the mediating effect. To estimate the proportion mediated, we used the formula $PM = \frac{\beta_1 \times \beta_2}{\beta_{TE}}$, where β_1 represents the causal effect of the exposure on the mediator (step 1), β_2 represents the causal effect of the mediator on the outcome (step 2), and β_{TE} represents the total causal effect of the exposure on the outcome.³¹ We estimated the 95% CIs for proportion mediated using the delta method.³¹ We used the same criteria as described previously to select genetic instruments in each of these MR analyses. Genetic associations of the outcome of interest in each MR analysis for the selected genetic instrument were then obtained from the corresponding GWAS.

SNP-specific causal effects for the selected genetic instruments were estimated using the Wald ratio, that is, SNP–outcome association divided by SNP–exposure association.³² To obtain the MR effect estimates, we pooled the SNP-specific estimates using inverse-variance weighted (IVW) random effects model as the main method,³³ and we used weighted median and MR-Egger regression as sensitivity methods to assess the robustness of IVW estimates and horizontal pleiotropic effects.^{34,35} We also performed MR-Lasso, which is an extension of IVW method by excluding the invalid SNPs that had a nonzero direct effect on the outcome.³⁶ Potential outlier SNPs identified using Mendelian Randomization Pleiotropy Residual Sum and Outlier (MR-PRESSO) were excluded from the analysis.³⁷ As AAM and cIMT had overlapping samples with the selected modifiable risk factors, we applied a recent statistical method, MRlap, which calculates corrected causal estimates, accounting for sample overlap, winner's curse, and weak instrument bias in MR.³⁸ To orient the direction of causality, we applied Steiger filtering, which excludes the SNPs with a larger variance explained in the outcome than in the exposure.³⁹ We accounted for multiple comparisons of 5 outcome categories using Bonferroni correction with a P value threshold of 0.01 (0.05/5).

To investigate the heterogeneity, we applied MR-Clust to identify genetic variants that may have reflected distinct causal mechanisms.⁴⁰ In brief, MR-Clust uses

an expectation-maximization algorithm to assign genetic variants contributing to the causal effect estimate in a similar direction, magnitude, and precision to the same cluster.⁴⁰ In this study, we assigned a genetic variant to a cluster if the conditional probability was ≥ 0.8 . Here, we focused only on the substantive clusters, not the null cluster of genetic variants, which do not suggest a causal effect or the junk clusters of genetic variants that cannot be assigned to any substantive clusters.

Next, for each cluster, we performed gene-based pathway enrichment analysis using ingenuity pathway analysis (www.qiagen.com/ingenuity) to provide biological insights into the observed cluster-specific estimates. SNPs in each cluster that had the conditional probability ≥ 0.8 were mapped to genes using the University of California Santa Cruz Genome Browser queried using mysql.⁴¹ We used a 20-kb positional mapping window to assign SNPs to gene for pathway analysis.

Statistical Analysis

All analyses were performed in R 3.6.1 (R Foundation for Statistical Computing, Vienna, Austria). GWAS of cIMT was performed using BOLT-LMM²⁶ using an in-house pipeline. Genetic correlation across the traits were computed using the software package LDSC (<https://github.com/bulik/ldsc>). MR analyses were performed using the *TwoSampleMR* (<https://mrcieu.github.io/TwoSampleMR/>), *MR-PRESSO* (<https://github.com/rondolab/MR-PRESSO>), *MendelianRandomization* (<https://cran.r-project.org/web/packages/MendelianRandomization>), *mrclust* packages (<https://github.com/cnfoley/mrclust>), and *MRlap* (<https://github.com/n-mounier/MRlap>).

RESULTS

Linkage Disequilibrium Score Regression

Cross-trait LDSC regression revealed strong inverse genetic correlation between AAM and BMI ($r_g = -0.32$, $SE = 0.02$, $P = 2.2 \times 10^{-58}$), SBP ($r_g = -0.09$, $SE = 0.01$, $P < 0.001$), and triglycerides ($r_g = -0.10$, $SE = 0.01$, $P = 5.9 \times 10^{-8}$) and positive genetic correlation between AAM and HDL ($r_g = 0.14$, $SE = 0.02$, $P = 2.2 \times 10^{-12}$) after a 5% false discovery rate (Figure 3, Table S4). In addition, we examined phenotypic correlation and cross-trait LDSC regression between BMI and SBP. The analysis indicated a phenotypic correlation of 0.26 ($P < 0.001$) and a genetic correlation (r_g) of 0.21 ($P < 0.001$), suggesting that BMI is correlated with SBP both phenotypically and genetically.

Association of AAM With cIMT

Our analysis showed that genetically predicted earlier AAM was associated with higher cIMT using

genetic variants passing Steiger filtering ($\beta_{IVW} = -0.008$, $P_{IVW} = 0.002$ [95% CI, -0.013 to -0.003]; Figure 4, Table, Tables S5 through S7). Sensitivity methods showed consistent direction of effect, and MR-Egger did not suggest potential pleiotropy. Neither MR-Lasso nor MR-PRESSO identified invalid or outlier SNPs. The MRlap estimates were consistent with the IVW estimates ($\beta_{MRlap} = -0.109$, $P_{MRlap} = 0.009$; Table S6, Figure S1). While not applying Steiger filtering, high heterogeneity was observed ($P_{Heterogeneity} = 0.022$) and SNPs with potential direct effect on the outcome were identified by MR-Lasso. However, MR-Lasso estimate (excluding invalid SNPs) was consistent with IVW estimates ($\beta_{MR-Lasso} = -0.010$, $P_{MR-Lasso} = 0.001$ [95% CI, -0.015 to -0.004]).

Mediating Effects of BMI and SBP

Genetically predicted earlier AAM was associated with higher adult BMI ($\beta_{IVW} = -0.109$, $P_{IVW} = 3.1 \times 10^{-20}$ [95% CI, -0.132 to -0.085]; Figure 4, Table, Tables S8 and S9). MR-Egger suggested potential pleiotropy ($P_{Pleiotropy} = 0.031$); however, MR-Lasso estimate ($\beta_{MR-Lasso} = -0.100$, $P_{MR-Lasso} = 1.9 \times 10^{-29}$ [95% CI, -0.117 to -0.082]; Table) was consistent with the IVW estimate. MRlap estimates ($\beta_{MRlap} = -0.21$, $P_{MRlap} = 3 \times 10^{-18}$; Table S9; Figure S1) showed significant and consistent direction of effect with the IVW estimate. To further address the heterogeneity and pleiotropy that we observed between AAM and BMI, we excluded SNPs that were also associated with BMI at $P < 0.05$. After this exclusion, AAM and BMI MR estimates remained significant (Table S10; $\beta_{IVW} = -0.029$, $P_{IVW} = 6.4 \times 10^{-4}$ [95% CI, -0.04 to -0.012]), and we did not observe any heterogeneity ($P_{Heterogeneity} = 0.55$; Table S10) or pleiotropy ($P_{Pleiotropy} = 0.79$; Table S10). Additionally, all MR methods showed consistent direction of effect (Table S10). Genetically predicted adult BMI was positively associated with cIMT ($\beta_{IVW} = 0.016$, $P_{IVW} = 0.002$ [95% CI, 0.006 – 0.025]; Table, Table S11, Figure S1). All sensitivity methods as well as MRlap that accounted for sample overlap showed consistent direction of effect, and MR-Egger did not suggest potential pleiotropy (Figure S1). Proportion mediated by adult BMI for the AAM–cIMT association was estimated to be 20.1% (95% CI, 1.4%–38.9%).

Genetically predicted earlier AAM was also associated with higher SBP ($\beta_{IVW} = -0.471$, $P_{IVW} = 0.004$ [95% CI, -0.795 to -0.147]; Figure 4, Table, Table S5). MR-Egger estimate ($\beta_{MR-Egger} = 0.342$, $P_{MR-Egger} = 0.529$; Table) showed the opposite direction to the IVW estimate, but MR-Lasso estimate ($\beta_{MR-Lasso} = -0.362$, $P_{MR-Lasso} = 0.004$ [95% CI, -0.610 to -0.114]; Figure 4, Table) was consistent with the IVW estimate. Genetically predicted adult SBP was positively associated with cIMT ($\beta_{IVW} = 0.002$, $P_{IVW} = 1.3 \times 10^{-22}$ [95% CI, 0.002 – 0.003]; Figure 4, Table,

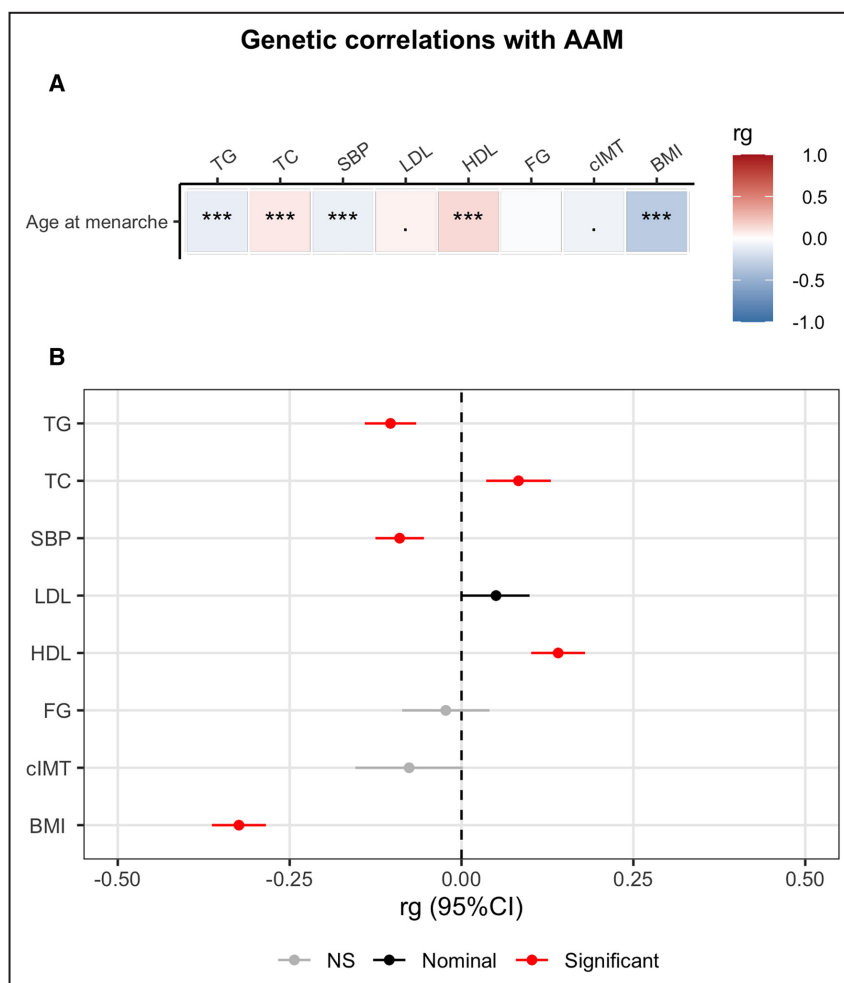


Figure 3. Genetic correlation estimates between AAM, cardiometabolic risk factors, and cIMT (A) heatmap of genetic correlation (rg) results and (B) Forest plot.

A. Significant genetic correlations at an false discovery rate of 5% are marked with an asterisk while the dot shows nominally significant genetic correlation with raw P value ≤ 0.05 and false discovery rate > 0.05 . BMI indicates body mass index; cIMT, carotid artery intima-media thickness; FG, fasting glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; NS, not significant; SBP, systolic blood pressure; TC, total cholesterol; and TG, triglycerides.

Table S11). All sensitivity methods including MRlap showed consistent direction of effect, but MR-Egger suggested potential pleiotropy ($P_{\text{Pleiotropy}} = 1.0 \times 10^{-4}$). We next excluded all AAM SNPs that were also associated with SBP at $P < 0.05$. After applying this exclusion, IVW and MR-Lasso were significant (Table S10; $P < 0.05$) and there was no indication of potential heterogeneity or pleiotropy (Table S10; $P_{\text{heterogeneity}} > 0.1$ and $P_{\text{Pleiotropy}} > 0.1$). The proportion mediated by adult SBP for the AAM–cIMT association was estimated to be 13.5% (95% CI, 0.5%–26.6%).

Among the biomarkers under investigation, genetically predicted AAM was positively associated with TC and HDL (cholesterol: $\beta_{\text{IVW}} = 0.021$, $P_{\text{IVW}} = 0.001$ [95% CI, 0.005–0.037]; HDL: $\beta_{\text{IVW}} = 0.038$, $P_{\text{IVW}} = 2.2 \times 10^{-4}$ [95%

CI, 0.018–0.059]; Table and Tables S5 and S8) and with higher triglycerides ($\beta_{\text{IVW}} = -0.028$, $P_{\text{IVW}} = 0.002$ [95% CI, -0.047 to -0.010]; Figure 4, Table and Tables S5 and S8). Although potential pleiotropy was not suggested, MR-Egger showed consistent direction with the IVW method for the above associations except for triglycerides. However, among these biomarkers, we found that genetically predicted cholesterol was positively associated with cIMT ($\beta_{\text{IVW}} = 0.013$, $P_{\text{IVW}} = 7.6 \times 10^{-4}$ [95% CI, 0.005–0.021]; Figure 4, Table and Table S11). In addition, genetically predicted LDL was also positively associated with cIMT ($\beta_{\text{IVW}} = 0.017$, $P_{\text{IVW}} = 1.1 \times 10^{-5}$ [95% CI, 0.010–0.026]; Figure 4, Table and Table S11). In cases where MR-Lasso identified outlier SNPs for each AAM and cardiometabolic risk factor MR, we

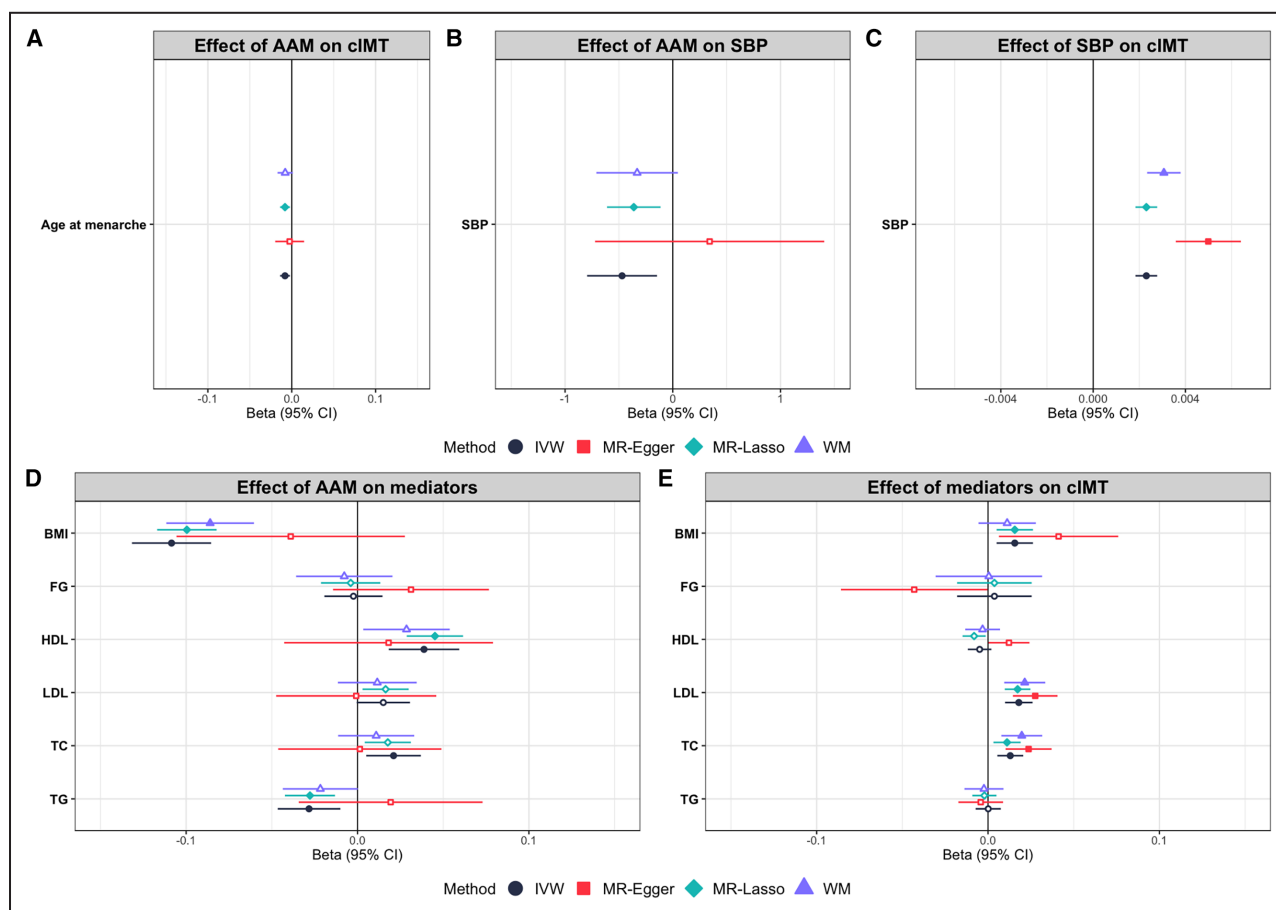


Figure 4. Forest plot of effect estimates from Mendelian randomization analysis. Line segments represent 95% CIs.

Solid symbols indicate associations with a P -value smaller than or equal to 0.01. MR-Lasso is not performed for the effect of AAM/BMI/SBP on cIMT because no invalid SNPs were identified by MR-Lasso. BMI indicates body mass index; cIMT, carotid artery intima-media thickness; FG, fasting glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC, total cholesterol; and TG, triglycerides.

performed a subsequent IVW analysis after excluding these outliers. The resulting estimates consistently displayed significant effects in a consistent direction with the main analysis (Table S8). The results from MRlap were consistent with the primary IVW method for all modifiable risk factors tested (Table S9).

Cluster-Specific MR Estimates

We found substantial heterogeneity in the AAM–BMI and AAM–SBP analyses even after excluding invalid SNPs identified using Steiger filtering and MR-PRESSO (Table and Table S5). Next, we performed cluster-specific MR using MR-Clust to test whether the clusters had different causal effects on BMI and SBP than the overall causal effect using all SNPs. Cluster-specific SNPs were used as IVs to run the analysis. Using MR-Clust,⁴⁰ we identified 3 clusters for AAM–BMI analysis and 3 clusters for AAM–SBP analysis (Figures 5 through 7 and Table S12). All 3 cluster-specific MR estimates for AAM–BMI varied in terms of

number of SNPs and showed inverse causal association with BMI. Cluster_1 ($N_{\text{SNP}}=7$, $\beta=-0.15$, $P=2 \times 10^{-64}$; Figures 5 through 7 and Table S12) showed less strong causal association as compared with clusters 2 and 3 (Cluster_2 [$N_{\text{SNP}}=22$, $\beta=-0.41$, $P=2 \times 10^{-239}$], Cluster_3 [$N_{\text{SNP}}=29$, $\beta=-0.42$, $P<0.001$; Figures 5 through 7 and Table S12). We found substantial heterogeneity in cluster-specific estimates for SBP (Figures 5 and 7 and Table S12). Cluster_1 ($N_{\text{SNP}}=5$, $\beta=3.93$, $P=3.6 \times 10^{-08}$), Cluster_3 ($N_{\text{SNP}}=6$, $\beta=2.27$, $P=8.4 \times 10^{-08}$) showed positive causal estimates between AAM–SBP while Cluster_2 ($N_{\text{SNP}}=24$, $\beta=-4.34$, $P=3.9 \times 10^{-79}$) showed an inverse causal AAM–SBP association (Figures 5 and 7 and Table S12).

To provide further insights into the cluster-specific results, we next performed ingenuity pathway analysis (www.qiagen.com/ingenuity) to identify canonical pathways associated to SNPs in each of the clusters. We performed ingenuity pathway analyses by grouping SNPs that had an inclusion probability $>80\%$ for each cluster originating from MR-Clust (Figures 5

Table 1. Relationships Between AAM, cIMT, BMI, and SBP From MR With Steiger Filtering

Exposure	Outcome	Number of SNPs	Method*	Beta	SE	P value	95% CI (lower)	95% CI (upper)	Heterogeneity P value	Pleiotropy P value
Main analysis (AAM–cIMT)										
AAM	cIMT	183	IVW-RE	−0.008	0.003	0.002	−0.013	−0.003	0.984	NA
AAM	cIMT	183	WM	−0.008	0.005	0.094	−0.017	0.001	NA	NA
AAM	cIMT	183	MR-Egger	−0.002	0.009	0.779	−0.020	0.015	0.983	0.502
AAM	cIMT	183	MR-Lasso	No invalid SNPs were identified						
2-step MR: (1) AAM–BMI; (2) BMI–cIMT										
AAM	BMI	176	IVW-RE	−0.109	0.012	3.1E-20	−0.132	−0.085	3.9E-30	NA
AAM	BMI	176	WM	−0.086	0.013	4.2E-11	−0.112	−0.060	NA	NA
AAM	BMI	176	MR-Egger	−0.039	0.034	0.253	−0.106	0.028	1.4E-28	0.031
AAM	BMI	115	MR-Lasso	−0.100	0.009	1.9E-29	−0.117	−0.082	NA	NA
BMI	cIMT	132	IVW-RE	0.016	0.005	0.002	0.006	0.025	0.858	NA
BMI	cIMT	132	WM	0.011	0.009	0.191	−0.006	0.028	NA	NA
BMI	cIMT	132	MR-Egger	0.041	0.018	0.022	0.006	0.076	0.878	0.133
BMI	cIMT	132	MR-Lasso	No invalid SNPs were identified						
2-step MR: (1) AAM–SBP; (2) SBP–cIMT										
AAM	SBP	187	IVW-RE	−0.471	0.166	0.004	−0.795	−0.147	1.8E-20	NA
AAM	SBP	187	WM	−0.331	0.193	0.086	−0.709	0.047	NA	NA
AAM	SBP	187	MR-Egger	0.342	0.543	0.529	−0.722	1.406	5.8E-20	0.118
AAM	SBP	133	MR-Lasso	−0.362	0.127	0.004	−0.610	−0.114	NA	NA
SBP	cIMT	217	IVW-RE	0.002	2.4E-04	1.3E-22	0.002	0.003	0.637	NA
SBP	cIMT	217	WM	0.003	3.7E-04	1.1E-16	0.002	0.004	NA	NA
SBP	cIMT	217	MR-Egger	0.005	0.001	4.6E-11	0.004	0.006	0.863	1.0E-04
SBP	cIMT	217	MR-Lasso	No invalid SNPs were identified						

AAM indicates age at menarche; BMI, body mass index; cIMT, carotid intima-media thickness; IVW-RE, inverse variance-weighted random effects model; MR, Mendelian randomization; MR-PRESSO, Mendelian Randomization Pleiotropy Residual Sum and Outlier; NA, not applicable; SBP, systolic blood pressure; SNPs, single-nucleotide polymorphisms; and WM, weighted median.

*MR-Lasso identified invalid SNPs for AAM–BMI and AAM–SBP. For the rest of the analysis, MR-Lasso is equivalent to IVW method, thus the results are not presented to avoid duplication. Potential outliers (MR-PRESSO) were only identified for AAM–BMI and AAM–SBP analyses. For these analyses, we presented the results excluding potential outliers identified from MR-PRESSO.

through 7 and Table S13). Genes from 3 clusters associated with BMI were mapped to distinct canonical pathways with BAG2 Signaling Pathway (Cluster_1), Uracil Degradation II (Reductive) (Cluster_2), and Methylglyoxal Degradation III (Cluster_3) showing the highest enrichment (Table S14). Genes from 3 clusters associated with SBP were mapped to Chronic Myeloid Leukemia Signaling, Senescence Pathway, and Endocannabinoid Cancer Inhibition Pathway (Cluster_3), Thrombopoietin Signaling (Cluster_2) (Table S15).

DISCUSSION

Here, we investigated the SNP-based genetic correlation and potential causal relationship between AAM and cIMT using LDSC and the MR paradigm, respectively. To the best of our knowledge, this is the first study to report genetic correlation and causal associations between AAM and cIMT, and to investigate

whether the effect of AAM on cIMT is mediated by modifiable risk factors including anthropometric, glycaemic, lipid traits, and blood pressure. First, our cross-trait LDSC identified an inverse genetic correlation between AAM, BMI, and cIMT, suggesting that shared genetic variants and interconnected biological pathways predispose to these traits. Second, our univariable MR revealed that genetic liability to earlier AAM is potentially causally deleterious to cIMT. Finally, using a 2-step MR framework, we found that BMI and SBP account for as much as 20.1% and 13.5% of the causal effect of AAM on cIMT, respectively. Previous MR studies consistently demonstrated the potential causal association of AAM with CVD outcomes, including coronary artery disease^{11,12} and ischemic heart disease.¹³ Several studies have additionally proposed that AAM is associated with various cardiometabolic risk factors, including SBP, BMI, blood lipid levels, and markers of subclinical atherosclerosis (ie, cIMT). All these cardiometabolic traits/diseases are considered risk factors for future CVD events. Therefore, it is reasonable to

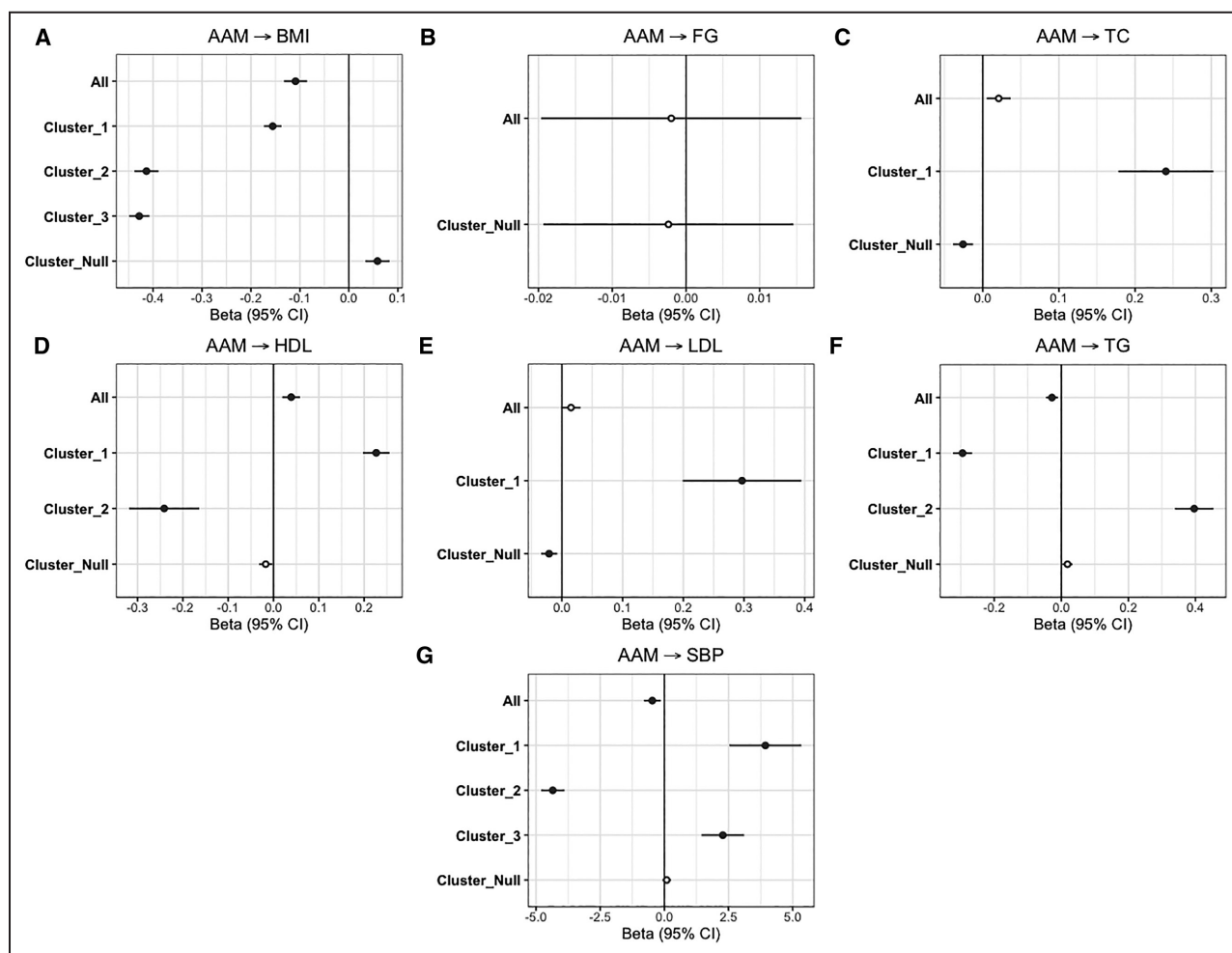


Figure 5. Forest plot of effect estimates from cluster specific Mendelian randomization performed using MR-Clust.

Line segments represent 95% CIs. BMI indicates body mass index; cIMT, carotid artery intima-media thickness; FG, fasting glucose; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; SBP, systolic blood pressure; TC (mmol/L), total cholesterol; and TG (mmol/L), triglycerides.

claim that implementing early preventive interventions to address these potential risk factors could be effective in preventing CVD events. Our study contributes valuable findings in this regard by demonstrating that one third of the effect of AAM on cIMT is mediated by modifiable CVD risk factors, namely, BMI and SBP.

Genetic Correlations Among the Traits Under Investigation

First, we investigated the genetic overlap between AAM, cIMT, and modifiable cardiometabolic risk factors and found that AAM showed stronger and significant inverse genetic correlation with BMI, and triglycerides than cIMT and other cardiometabolic risk factors investigated. Our findings are consistent with previous studies,^{24,42} and suggest that AAM and BMI may share genetic variants that may implicate shared biological pathways between both traits. The genetic

correlation between AAM, cIMT, and SBP were smaller as compared with BMI and nonsignificant, suggesting that these traits play a smaller role in the underlying biology of AAM than BMI. To test whether the observed correlations could reflect causal associations, we used MR to identify potential causal links between these traits. Our findings from cross-trait LDSC were mostly concordant with our MR findings.

Despite the genetic association and MR effect that we identified between AAM and cIMT, the absence of evidence for genetic correlation between the traits is noteworthy. MR employs a selection of genetic variants, as opposed to LDSC analysis, which may reflect an association that was otherwise canceled out by opposing effects in a genome-wide correlation. The genome-wide genetic correlation between 2 traits can be influenced by multiple common causes and may be negligible in cases where some genetic loci exhibit a positive genetic correlation between

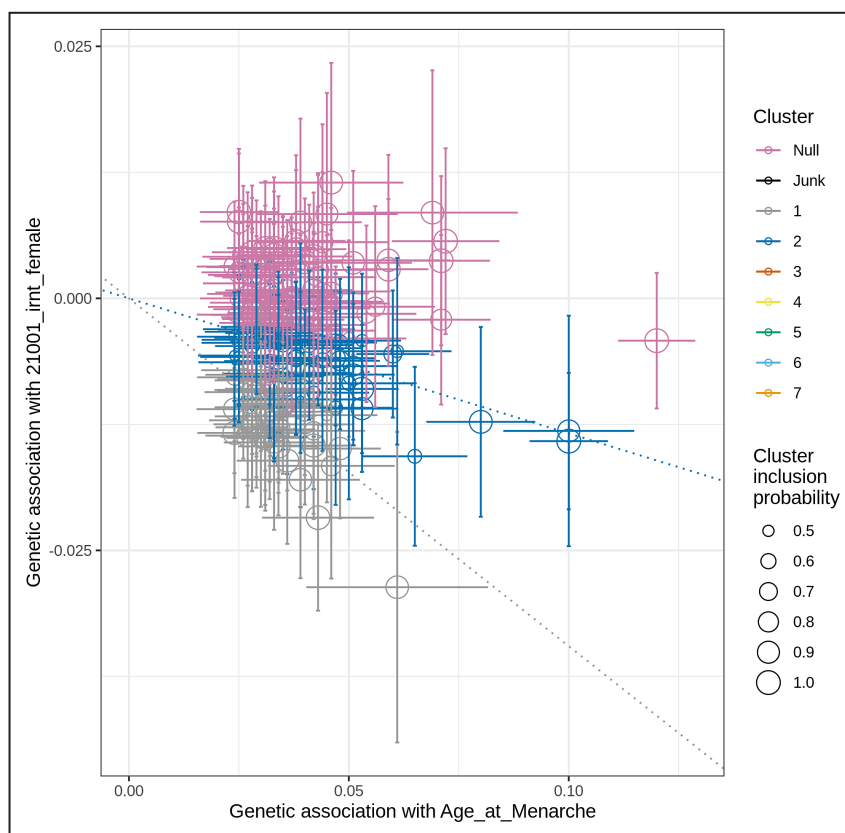


Figure 6. Clustering of genetic variants for the Mendelian randomization analysis of age at menarche with adult body mass index.

the 2 traits, while others show a negative genetic correlation.

Increased intima-media thickness is a marker of subclinical atherosclerosis and medial hypertrophy, which has been shown to predict future cardiovascular events including myocardial infarction and stroke. Several conventional observational studies have investigated the inverse association of AAM with cardiovascular risk factors, including BMI, blood pressure, and cIMT.^{43,44} Although observational and MR studies have consistently shown an inverse association between AAM and BMI and SBP, the association between AAM and cIMT is still controversial. For example, a cross-sectional study failed to identify any association between AAM <12 years and cIMT,⁴⁵ while a community-based study reported an inverse and independent association between early AAM and internal cIMT in Black women.⁴⁶ Additionally, those studies were primarily focused on examining the association between AAM and cIMT and did not examine the mediating effects of BMI and SBP. Our 2-step MR analysis indicated that BMI and SBP could serve as potential mediators on the effect of AAM on cIMT.

Effect of AAM on cIMT via BMI

Our finding of the mediating role of BMI on the effect of an earlier AAM on higher cIMT was consistent with existing knowledge. The relationship between BMI and cIMT is established, with a number of studies reporting that higher childhood BMI is associated with thicker cIMT.^{47,48} Previous studies have also shown that children with obesity are more likely to have an early AAM, with fat distribution affecting this relationship.^{44,49} Few studies have explored the relationship between increased adiposity and cIMT in adults. A previous MR analysis indicated that earlier AAM causes higher adult BMI.⁵⁰ Although the exact biological mechanisms underlying the observed mediation are yet to be fully discovered, there could be several plausible biological mechanisms for explaining the relationships. As previously hypothesized,⁵⁰ it is possible that early AAM leads to an altered hormonal state and subsequent changes in psychological state and physiological or metabolic rates of girls at adolescence, leading to weight gain and associated complications of higher adiposity later in life. This remains to be investigated in future studies.

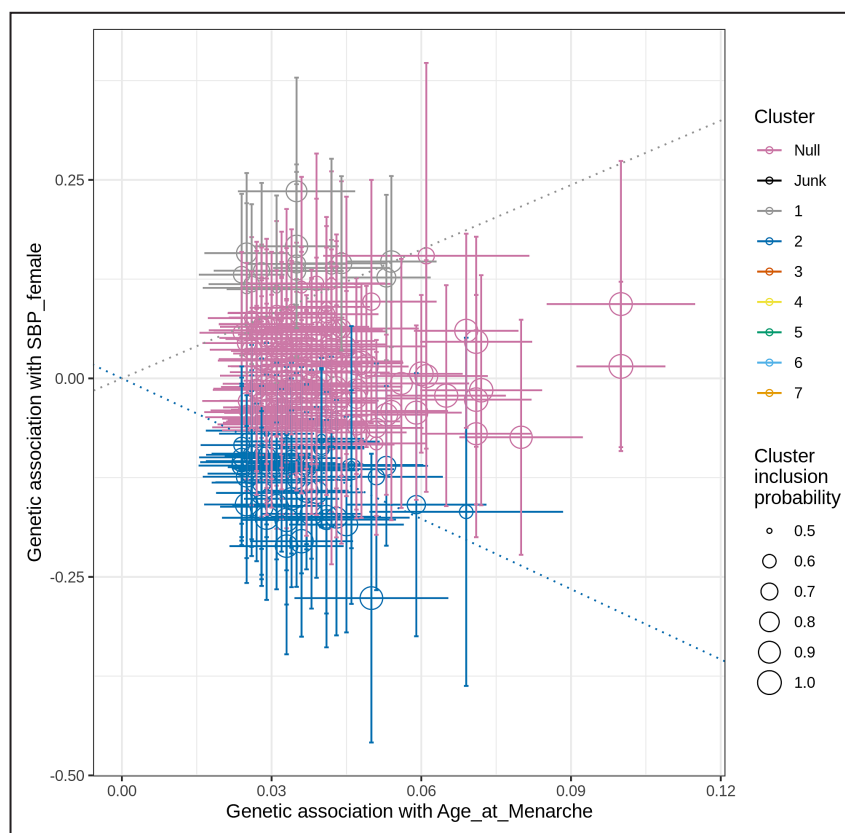


Figure 7. Clustering of genetic variants for the Mendelian randomization analysis of age at menarche with adult systolic blood pressure.

Effect of AAM on SBP and cIMT

There have been inconsistent findings regarding the relationship between AAM and SBP in prior epidemiological studies, with a variety of studies showing that AAM is inversely associated with hypertension,^{51,52} while others show that AAM is positively associated with hypertension.^{51,53,54} Several cross-sectional studies have suggested that SBP is linearly associated with cIMT in individuals with and without hypertension.⁵⁵ On the other hand, and in line with our findings, Fan et al identified an inverse causal relationship between AAM and SBP, with SBP mediating the effect of AAM on CAD using the MR framework on an Asian population nested in the Taiwan Biobank.¹² Other observational studies in Asian population groups have identified a linear relationship between increased SBP and cIMT.⁵⁶ Overall, these findings provide supporting evidence for the mediatory role of SBP in the effect of earlier AAM on cIMT and promote interventions aimed at lowering SBP to reduce risk of cIMT thickening attributable to earlier AAM and its clinical sequelae. As suggested by a recent article, early AAM is a feature of early vascular aging in adults with primary hypertension, and primary hypertension is known to cause vascular damage that may increase cIMT and CVD risk.⁵⁷

Effect of AAM on Modifiable Cardiometabolic Risk Factors

Our MR analyses identified an inverse association between AAM and triglycerides and positive association between AAM and HDL levels. These associations have been previously reported in a MR study investigating the association of genetically predicted AAM and myocardial infarction.²³ Associations between dyslipidemia and earlier AAM have also been highlighted in traditional epidemiological studies.⁴³ Additionally, previous studies have highlighted significant effects of genetically predicted AAM on fasting blood glucose levels using GWAS summary data from sex-combined data sets for blood glucose.^{58,59} Our study did not replicate this association using sex-specific GWAS estimates for exposure and outcome data sets. This discrepancy in findings highlights the need to increase the generation and use of sex-specific GWAS in downstream MR analyses to identify possible sex-specific causal pathways in relevant disease phenotypes. Overall, our findings suggest genetically predicted earlier AAM have a potentially causal effect on increased cIMT through BMI and adiposity-related mechanisms that are also reflected through adverse lipid profiles.

Strengths and Limitations

Our study has a number of strengths:

1. We leverage state-of-the-art MR methodology to examine the potential causal cardiometabolic effects of earlier AAM, an experiment that is not practically and ethically suitable in a randomized controlled trial setting.
2. The GWAS data and genetic instruments used in the cross-trait LDSC regression and MR analyses were derived from the largest female-specific GWAS of AAM, cIMT, BMI, SBP, lipids traits, and FG.
3. We used multiple sensitivity methods to improve robustness of model assumptions and our analysis showed consistent direction of effect estimates supporting the validity of our findings.

Our study has several limitations:

1. The sex-specific GWAS of AAM, BMI, cIMT, SBP, and lipid traits were derived from UKBB resources that partly overlapped. While overlapping samples are not known to produce any systematic bias for the genetic correlation analysis, it could potentially attenuate the MR findings. Previous studies have shown that sample overlap in MR can increase type 1 error and could potentially lead to bias results for classic MR methods.⁶⁰ We performed sensitivity analysis using the recently proposed MRlap method to address the sample overlap.³⁸ Our findings remain consistent; however, we observed that, for some traits, the effect size was larger, with wider CIs. Further replication of our results in independent data sets is warranted.
2. Our 2-step MR was based on the assumption that there was no exposure $\times$ mediator interaction.³¹ The presence of exposure $\times$ mediator interaction is known to produce biased estimates from both 2-step and multivariable MR. However, the size of the bias is thought to be lower in the MR analyses as compared with the non-instrumental variable based methods.³¹
3. The GWAS resources drawn in the current study were drawn from the European-only women participants of UKBB. Therefore, our findings may not be applicable to women of other populations and ethnicities, as allelic differences between ethnicities may produce different effect estimates.
4. We did not include childhood BMI in the mediation analysis as the GWAS summary level data available from the Genetic Investigation of Anthropometric Traits consortium had a relatively small sample size.
5. Our choice to employ 2-step MR instead of multivariable MR to investigate the mediating effects of BMI and SBP was influenced by several factors. First, we were primarily interested in estimating the mediating effect of BMI and SBP individually and the potential

impact of intervening on these mediators. Second, the power of MR analyses is reported to decrease as the number of mediators increases in the multivariable MR model.³¹ Although MR mediation analysis has advantages compared with non-instrumental variable regression-based methods and counterfactual approaches, which are still susceptible to issues such as unmeasured confounding and reverse causality in conventional observational studies, MR mediation analysis has limitations as well. For example, MR mediation analysis assumes a linear association and does not account for exposure–mediator interaction.³¹ Finally, MR relies on core assumptions that impact the reliability of causal inference. We aimed to ensure the robustness of our findings through the implementation of multiple sensitivity analyses and filters on genetic instrument selection to overcome potential pleiotropic effects. Causal inferences made through our MR analyses should be interpreted carefully, as MR estimates are only suggestive of potential effects of genetic predisposition of an exposure on an outcome of interest but do not provide evidence that therapeutic intervention targeting the exposure will change the outcome.

Clinical and Public Health Implications

Our findings suggest that early interventions targeting SBP and BMI may result in significant health benefits by reducing cIMT and its associated adverse health outcomes in women. Despite the growing availability of sex-specific data focused on cardiovascular health care, the implementation and translation of findings stemming from such data in practical settings has been slow. Given the lack of progress in improving the incidence and mortality rates of CVD among younger women, our results motivate further research aimed at enhancing our understanding of female cardiovascular health and risk factors. This knowledge will be crucial to advance the global prevention, diagnosis, and treatment of CVD in women.

CONCLUSIONS

Our findings suggest that an earlier AAM is potentially causally associated with higher cIMT and that one third of the effect of AAM on cIMT may be mediated by the modifiable risk factors BMI and SBP. Those findings may indicate that early intervention aimed at lowering BMI and hypertension may be beneficial in reducing the risk of developing subclinical atherosclerosis due to earlier age at menarche. A better understanding of the relationship between reproductive factors and CVD may contribute to better prevention and management of this disease.

ARTICLE INFORMATION

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Disclosures

None.

Supplemental Material

Tables S1–S15

Figure S1

REFERENCES

- Mehta LS, Beckie TM, DeVon HA, Grines CL, Krumholz HM, Johnson MN, Lindley KJ, Vaccarino V, Wang TY, Watson KE, et al. Acute myocardial infarction in women: a scientific statement from the American Heart Association. *Circulation*. 2016;133:916–947. doi: [10.1161/CIR.0000000000000351](https://doi.org/10.1161/CIR.0000000000000351)
- Lee JJ, Cook-Wiens G, Johnson BD, Braunstein GD, Berga SL, Stanczyk FZ, Pepine CJ, Bairey Merz CN, Shufelt CL. Age at menarche and risk of cardiovascular disease outcomes: findings from the National Heart Lung and blood institute-sponsored Women's ischemia syndrome evaluation. *J Am Heart Assoc*. 2019;8:e012406. doi: [10.1161/JAHA.119.012406](https://doi.org/10.1161/JAHA.119.012406)
- Lakshman R, Frouhi NG, Sharp SJ, Luben R, Bingham SA, Khaw KT, Wareham NJ, Ong KK. Early age at menarche associated with cardiovascular disease and mortality. *J Clin Endocrinol Metab*. 2009;94:4953–4960. doi: [10.1210/jc.2009-1789](https://doi.org/10.1210/jc.2009-1789)
- Canoy D, Beral V, Balkwill A, Wright FL, Kroll ME, Reeves GK, Green J, Cairns BJ, Million Women Study C. Age at menarche and risks of coronary heart and other vascular diseases in a large UK cohort. *Circulation*. 2015;131:237–244. doi: [10.1161/CIRCULATIONAHA.114.010070](https://doi.org/10.1161/CIRCULATIONAHA.114.010070)
- Pierce MB, Leon DA. Age at menarche and adult BMI in the Aberdeen children of the 1950s cohort study. *Am J Clin Nutr*. 2005;82:733–739. doi: [10.1093/ajcn/82.4.733](https://doi.org/10.1093/ajcn/82.4.733)
- Dreyfus J, Jacobs DR Jr, Mueller N, Schreiner PJ, Moran A, Carnethon MR, Demerath EW. Age at menarche and Cardiometabolic risk in adulthood: the coronary artery risk development in young adults study. *J Pediatr*. 2015;167:344–352. doi: [10.1016/j.jpeds.2015.04.032](https://doi.org/10.1016/j.jpeds.2015.04.032)
- Prentice P, Viner RM. Pubertal timing and adult obesity and cardiometabolic risk in women and men: a systematic review and meta-analysis. *Int J Obes*. 2013;37:1036–1043. doi: [10.1038/ijo.2012.177](https://doi.org/10.1038/ijo.2012.177)
- Zhang L, Yang L, Wang C, Yuan T, Zhang D, Wei H, Li J, Lei Y, Sun L, Li X, et al. Mediator or moderator? The role of obesity in the association between age at menarche and blood pressure in middle-aged and elderly Chinese: a population-based cross-sectional study. *BMJ Open*. 2022;12:e051486. doi: [10.1136/bmjopen-2021-051486](https://doi.org/10.1136/bmjopen-2021-051486)
- Farahmand M, Ramezani Tehrani F, Azizi F. Whether age of menarche is influenced by body mass index and lipoproteins profile? A retrospective study. *Iran J Reprod Med*. 2012;10:337–342.
- Jung H, Sung YA, Hong YS, Song DK, Hong SH, Lee H. Relationship between age at menarche and metabolic diseases in Korean postmenopausal women: the Korea National Health and nutrition examination survey 2016–2018. *PLoS One*. 2023;18:e0280929. doi: [10.1371/journal.pone.0280929](https://doi.org/10.1371/journal.pone.0280929)
- Cao M, Cui B. Negative effects of age at menarche on risk of cardiometabolic diseases in adulthood: a Mendelian randomization study. *J Clin Endocrinol Metab*. 2020;105:522. doi: [10.1210/clinem/dgz071](https://doi.org/10.1210/clinem/dgz071)
- Fan HY, Huang YT, Chen YY, Hsu JB, Li HY, Su TC, Lin HJ, Chien KL, Chen YC. Systolic blood pressure as the mediator of the effect of early menarche on the risk of coronary artery disease: a Mendelian randomization study. *Front Cardiovasc Med*. 2022;9:1023355. doi: [10.3389/fcvm.2022.1023355](https://doi.org/10.3389/fcvm.2022.1023355)
- Chen J, Chen H, Zhu Q, Liu Q, Zhou Y, Li L, Wang Y. Age at menarche and ischemic heart disease: an update mendelian randomization study. *Front Genet*. 2022;13:942861. doi: [10.3389/fgene.2022.942861](https://doi.org/10.3389/fgene.2022.942861)
- Hardy R, Maddock J, Ghosh AK, Hughes AD, Kuh D. The relationship between pubertal timing and markers of vascular and cardiac structure and function in men and women aged 60–64 years. *Sci Rep*. 2019;9:11037. doi: [10.1038/s41598-019-47164-x](https://doi.org/10.1038/s41598-019-47164-x)
- Stockl D, Meisinger C, Peters A, Thorand B, Huth C, Heier M, Rathmann W, Kowall B, Stockl H, Doring A. Age at menarche and its association with the metabolic syndrome and its components: results from the KORA F4 study. *PLoS One*. 2011;6:e26076. doi: [10.1371/journal.pone.0026076](https://doi.org/10.1371/journal.pone.0026076)
- Pierce MB, Kuh D, Hardy R. Role of lifetime body mass index in the association between age at puberty and adult lipids: findings from men and women in a British birth cohort. *Ann Epidemiol*. 2010;20:676–682. doi: [10.1016/j.annepidem.2010.05.015](https://doi.org/10.1016/j.annepidem.2010.05.015)
- Bulik-Sullivan BK, Loh PR, Finucane HK, Ripke S, Yang J, Schizophrenia Working Group of the Psychiatric Genomics C, Patterson N, Daly MJ, Price AL, Neale BM. LD score regression distinguishes confounding from polygenicity in genome-wide association studies. *Nat Genet*. 2015;47:291–295. doi: [10.1038/ng.3211](https://doi.org/10.1038/ng.3211)
- Bulik-Sullivan B, Finucane HK, Anttila V, Gusev A, Day FR, Loh PR; ReproGen C, Psychiatric Genomics C, Genetic consortium for anorexia nervosa of the Wellcome Trust Case Control C, Duncan L, Perry JR. An atlas of genetic correlations across human diseases and traits. *Nat Genet*. 2015;47:1236–1241. doi: [10.1038/ng.3406](https://doi.org/10.1038/ng.3406)
- Davey Smith G, Hemani G. Mendelian randomization: genetic anchors for causal inference in epidemiological studies. *Hum Mol Genet*. 2014;23:R89–R98. doi: [10.1093/hmg/ddu328](https://doi.org/10.1093/hmg/ddu328)
- Hingorani A, Humphries S. Nature's randomised trials. *Lancet*. 2005;366:1906–1908. doi: [10.1016/S0140-6736\(05\)67767-7](https://doi.org/10.1016/S0140-6736(05)67767-7)
- Smith GD, Ebrahim S. Mendelian randomization: can genetic epidemiology contribute to understanding environmental determinants of disease? *Int J Epidemiol*. 2003;32:1–22. doi: [10.1093/ije/dyg070](https://doi.org/10.1093/ije/dyg070)
- Au Yeung SL, Jiang C, Cheng KK, Xu L, Zhang W, Lam TH, Leung GM, Schooling CM. Age at menarche and cardiovascular risk factors using Mendelian randomization in the Guangzhou biobank cohort study. *Prev Med*. 2017;101:142–148. doi: [10.1016/j.ypmed.2017.06.006](https://doi.org/10.1016/j.ypmed.2017.06.006)
- Zheng J, Chen K, Huang T, Shao C, Li P, Wang J, Wang W, Zhang K, Meng X, Gao J, et al. Genetically determined lifestyle and Cardiometabolic risk factors mediate the Association of Genetically Predicted age at menarche with genetic predisposition to myocardial infarction: a two-step,

- two-sample Mendelian randomization study. *Front Cardiovasc Med*. 2022;9:821068. doi: [10.3389/fcvm.2022.821068](https://doi.org/10.3389/fcvm.2022.821068)
24. Day FR, Thompson DJ, Helgason H, Chasman DI, Finucane H, Sulem P, Ruth KS, Whalen S, Sarkar AK, Albrecht E, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. *Nat Genet*. 2017;49:834–841. doi: [10.1038/ng.3841](https://doi.org/10.1038/ng.3841)
 25. Bycroft C, Freeman C, Petkova D, Band G, Elliott LT, Sharp K, Motyer A, Vukcevic D, Delaneau O, O'Connell J, et al. The UK biobank resource with deep phenotyping and genomic data. *Nature*. 2018;562:203–209. doi: [10.1038/s41586-018-0579-z](https://doi.org/10.1038/s41586-018-0579-z)
 26. Coffey S, Lewandowski AJ, Garratt S, Meijer R, Lynum S, Bedi R, Paterson J, Yaqub M, Noble JA, Neubauer S, et al. Protocol and quality assurance for carotid imaging in 100,000 participants of UK biobank: development and assessment. *Eur J Prev Cardiol*. 2017;24:1799–1806. doi: [10.1177/2047487317732273](https://doi.org/10.1177/2047487317732273)
 27. Loh PR, Tucker G, Bulik-Sullivan BK, Vilhjalmsdottir BJ, Finucane HK, Salem RM, Chasman DI, Ridker PM, Neale BM, Berger B, et al. Efficient Bayesian mixed-model analysis increases association power in large cohorts. *Nat Genet*. 2015;47:284–290. doi: [10.1038/ng.3190](https://doi.org/10.1038/ng.3190)
 28. The Neale Lab. GWAS Analysis of the UK Biobank. 2018. Accessed July 15, 2022. <http://www.nealelab.is/uk-biobank/>.
 29. Lagou V, Magi R, Hottenga JJ, Grallert H, Perry JRB, Bouatia-Naji N, Marullo L, Rybin D, Jansen R, Min JL, et al. Sex-dimorphic genetic effects and novel loci for fasting glucose and insulin variability. *Nat Commun*. 2021;12:24. doi: [10.1038/s41467-020-19366-9](https://doi.org/10.1038/s41467-020-19366-9)
 30. International HapMap C, Altshuler DM, Gibbs RA, Peltonen L, Altshuler DM, Gibbs RA, Peltonen L, Dermitzakis E, Schaffner SF, Yu F, et al. Integrating common and rare genetic variation in diverse human populations. *Nature*. 2010;467:52–58. doi: [10.1038/nature09298](https://doi.org/10.1038/nature09298)
 31. Carter AR, Sanderson E, Hammerton G, Richmond RC, Davey Smith G, Heron J, Taylor AE, Davies NM, Howe LD. Mendelian randomisation for mediation analysis: current methods and challenges for implementation. *Eur J Epidemiol*. 2021;36:465–478. doi: [10.1007/s10654-021-00757-1](https://doi.org/10.1007/s10654-021-00757-1)
 32. Burgess S, Small DS, Thompson SG. A review of instrumental variable estimators for Mendelian randomization. *Stat Methods Med Res*. 2017;26:2333–2355. doi: [10.1177/0962280215597579](https://doi.org/10.1177/0962280215597579)
 33. Lawlor DA, Harbord RM, Sterne JA, Timpson N, Davey Smith G. Mendelian randomization: using genes as instruments for making causal inferences in epidemiology. *Stat Med*. 2008;27:1133–1163. doi: [10.1002/sim.3034](https://doi.org/10.1002/sim.3034)
 34. Burgess S, Thompson SG. Interpreting findings from Mendelian randomization using the MR-egger method. *Eur J Epidemiol*. 2017;32:377–389. doi: [10.1007/s10654-017-0255-x](https://doi.org/10.1007/s10654-017-0255-x)
 35. Bowden J, Davey Smith G, Haycock PC, Burgess S. Consistent estimation in Mendelian randomization with some invalid instruments using a weighted median estimator. *Genet Epidemiol*. 2016;40:304–314. doi: [10.1002/gepi.21965](https://doi.org/10.1002/gepi.21965)
 36. Slob EAW, Burgess S. A comparison of robust Mendelian randomization methods using summary data. *Genet Epidemiol*. 2020;44:313–329. doi: [10.1002/gepi.22295](https://doi.org/10.1002/gepi.22295)
 37. Verbanck M, Chen CY, Neale B, Do R. Detection of widespread horizontal pleiotropy in causal relationships inferred from Mendelian randomization between complex traits and diseases. *Nat Genet*. 2018;50:693–698. doi: [10.1038/s41588-018-0099-7](https://doi.org/10.1038/s41588-018-0099-7)
 38. Mounier N, Kutalik Z. Bias correction for inverse variance weighting Mendelian randomization. *Genet Epidemiol*. 2023;47:314–331. doi: [10.1002/gepi.22522](https://doi.org/10.1002/gepi.22522)
 39. Hemani G, Tilling K, Davey SG. Orienting the causal relationship between imprecisely measured traits using GWAS summary data. *PLoS Genet*. 2017;13:e1007081. doi: [10.1371/journal.pgen.1007081](https://doi.org/10.1371/journal.pgen.1007081)
 40. Foley CN, Mason AM, Kirk PDW, Burgess S. MR-Clust: clustering of genetic variants in Mendelian randomization with similar causal estimates. *Bioinformatics*. 2021;37:531–541. doi: [10.1093/bioinformatics/btaa778](https://doi.org/10.1093/bioinformatics/btaa778)
 41. Navarro Gonzalez J, Zweig AS, Speir ML, Schmelter D, Rosenbloom KR, Raney BJ, Powell CC, Nassar LR, Maulding ND, Lee CM, et al. The UCSC genome browser database: 2021 update. *Nucleic Acids Res*. 2021;49:D1046–D1057. doi: [10.1093/nar/gkaa1070](https://doi.org/10.1093/nar/gkaa1070)
 42. Day FR, Bulik-Sullivan B, Hinds DA, Finucane HK, Murabito JM, Tung JY, Ong KK, Perry JRB. Shared genetic aetiology of puberty timing between sexes and with health-related outcomes. *Nat Commun*. 2015;6:8842. doi: [10.1038/ncomms9842](https://doi.org/10.1038/ncomms9842)
 43. Remsberg KE, Demerath EW, Schubert CM, Chumlea WC, Sun SS, Siervogel RM. Early menarche and the development of cardiovascular disease risk factors in adolescent girls: the Fels longitudinal study. *J Clin Endocrinol Metab*. 2005;90:2718–2724. doi: [10.1210/jc.2004-1991](https://doi.org/10.1210/jc.2004-1991)
 44. Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS, Bogalusa Heart S. The relation of menarcheal age to obesity in childhood and adulthood: the Bogalusa heart study. *BMC Pediatr*. 2003;3:3. doi: [10.1186/1471-2431-3-3](https://doi.org/10.1186/1471-2431-3-3)
 45. Stockl D, Peters A, Thorand B, Heier M, Koenig W, Seissler J, Thiery J, Rathmann W, Meisinger C. Reproductive factors, intima media thickness and carotid plaques in a cross-sectional study of postmenopausal women enrolled in the population-based KORA F4 study. *BMC Womens Health*. 2014;14:17. doi: [10.1186/1472-6874-14-17](https://doi.org/10.1186/1472-6874-14-17)
 46. Bhuiyan AR, Srinivasan SR, Chen W, Fernandez C, Xu JH, Berenson GS. Timing of menarche related to carotid artery intima-media thickness in black and white young adult women: the Bogalusa heart study. *Ann Epidemiol*. 2015;25:414–419. doi: [10.1016/j.annepidem.2015.02.001](https://doi.org/10.1016/j.annepidem.2015.02.001)
 47. Freedman DS, Patel DA, Srinivasan SR, Chen W, Tang R, Bond MG, Berenson GS. The contribution of childhood obesity to adult carotid intima-media thickness: the Bogalusa heart study. *Int J Obes*. 2008;32:749–756. doi: [10.1038/sj.ijo.0803798](https://doi.org/10.1038/sj.ijo.0803798)
 48. Kotsis VT, Staboulis SV, Papamichael CM, Zakopoulos NA. Impact of obesity in intima media thickness of carotid arteries. *Obesity (Silver Spring)*. 2006;14:1708–1715. doi: [10.1038/oby.2006.196](https://doi.org/10.1038/oby.2006.196)
 49. Lassek WD, Gaulin SJ. Brief communication: menarche is related to fat distribution. *Am J Phys Anthropol*. 2007;133:1147–1151. doi: [10.1002/ajpa.20644](https://doi.org/10.1002/ajpa.20644)
 50. Gill D, Brewer CF, Del Greco MF, Sivakumaran P, Bowden J, Sheehan NA, Minelli C. Age at menarche and adult body mass index: a Mendelian randomization study. *Int J Obes*. 2018;42:1574–1581. doi: [10.1038/s41366-018-0048-7](https://doi.org/10.1038/s41366-018-0048-7)
 51. Werneck AO, Oyeyemi AL, Cyrino ES, Ronque ERV, Szwarcwald CL, Coelho ESMJ, Silva DR. Association between age at menarche and blood pressure in adulthood: is obesity an important mediator? *Hypertens Res*. 2018;41:856–864. doi: [10.1038/s41440-018-0079-4](https://doi.org/10.1038/s41440-018-0079-4)
 52. Shen L, Wang L, Hu Y, Liu T, Guo J, Shen Y, Zhang R, Miles T, Li C. Associations of the ages at menarche and menopause with blood pressure and hypertension among middle-aged and older Chinese women: a cross-sectional analysis of the baseline data of the China health and retirement longitudinal study. *Hypertens Res*. 2019;42:730–738. doi: [10.1038/s41440-019-0235-5](https://doi.org/10.1038/s41440-019-0235-5)
 53. Liu G, Yang Y, Huang W, Zhang N, Zhang F, Li G, Lei H. Association of age at menarche with obesity and hypertension among southwestern Chinese women: a new finding. *Menopause*. 2018;25:546–553. doi: [10.1097/GME.0000000000001027](https://doi.org/10.1097/GME.0000000000001027)
 54. Chen L, Zhang L, Chen Z, Wang X, Zheng C, Kang Y, Zhou H, Wang Z, Gao R. Age at menarche and risk of hypertension in Chinese adult women: results from a large representative nationwide population. *J Clin Hypertens (Greenwich)*. 2021;23:1615–1621. doi: [10.1111/jch.14321](https://doi.org/10.1111/jch.14321)
 55. Ferreira JP, Girerd N, Bozec E, Machu JL, Boivin JM, London GM, Zannad F, Rossignol P. Intima-media thickness is linearly and continuously associated with systolic blood pressure in a population-based cohort (STANISLAS cohort study). *J Am Heart Assoc*. 2016;5:5. doi: [10.1161/JAHA.116.003529](https://doi.org/10.1161/JAHA.116.003529)
 56. Ren L, Shi M, Wu Y, Ni J, Bai L, Lu H, Tu J, Wang J, Ning X. Correlation between hypertension and common carotid artery intima-media thickness in rural China: a population-based study. *J Hum Hypertens*. 2018;32:548–554. doi: [10.1038/s41371-018-0074-x](https://doi.org/10.1038/s41371-018-0074-x)
 57. Litwin M, Feber J. Origins of primary hypertension in children: early vascular or biological aging? *Hypertension*. 2020;76:1400–1409. doi: [10.1161/HYPERTENSIONAHA.120.14586](https://doi.org/10.1161/HYPERTENSIONAHA.120.14586)
 58. Xing W, Lv Q, Li Y, Wang C, Mao Z, Li Y, Li J, Yang T, Li L. Genetic prediction of age at menarche, age at natural menopause and type 2 diabetes: a Mendelian randomization study. *Nutr Metab Cardiovasc Dis*. 2023;33:873–882. doi: [10.1016/j.numecd.2023.01.011](https://doi.org/10.1016/j.numecd.2023.01.011)
 59. Bubach S, Horta BL, Goncalves H, Assuncao MCF. Early age at menarche and metabolic cardiovascular risk factors: mediation by body composition in adulthood. *Sci Rep*. 2021;11:148. doi: [10.1038/s41598-020-80496-7](https://doi.org/10.1038/s41598-020-80496-7)
 60. Burgess S, Davies NM, Thompson SG. Bias due to participant overlap in two-sample Mendelian randomization. *Genet Epidemiol*. 2016;40:597–608. doi: [10.1002/gepi.21998](https://doi.org/10.1002/gepi.21998)