Genetically determined reproductive aging and coronary heart disease: a bidirectional two-sample Mendelian Randomization

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Conflicts of interest/Competing interests

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Abstract

Background: Accelerated reproductive aging, in women indicated by early natural menopause, is associated with increased coronary heart disease (CHD) risk in observational studies. Conversely, an adverse CHD risk profile has been suggested to accelerate menopause.

Objectives: To study the direction and evidence for causality of the relationship between reproductive aging and (non-)fatal CHD and CHD risk factors in a bidirectional Mendelian Randomization (MR) approach, using age at natural menopause (ANM) genetic variants as a measure for genetically determined reproductive aging in women. We also studied the association of these variants with CHD risk (factors) in men.

Design: Two-sample MR, using both cohort data as well as summary statistics, with four methods: simple and weighted median-based, standard inverse-variance weighted (IVW) regression, and MR-Egger regression.

Participants: Data from EPIC-CVD and summary statistics from UK Biobank and publicly available GWAS were pooled for the different analyses.

Main Outcome Measures: CHD, CHD risk factors and ANM.

Results: Across different methods of MR no association was found between genetically determined reproductive aging and CHD risk in women (Relative Risk Estimate $(RRE)_{IVW}$=0.99, 95% confidence interval (CI):0.97;1.01), or any of the CHD risk factors. Similarly, no associations were found in men. Neither did the reversed analyses show evidence for an association between CHD (risk factors) and reproductive aging.

Conclusion: Genetically determined reproductive aging is not causally associated with CHD risk (factors) in women, nor were the genetic variants associated in men. We found no evidence for a reverse association in a combined sample of women and men.

Keywords: Reproductive aging, Mendelian Randomization, coronary heart disease, risk factors

Ethics approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The studies were approved by local medical ethical committees as described in the Supplemental methods(1).

Introduction

Coronary heart disease (CHD) is the leading cause of death in both men and women(2). Accelerated reproductive aging, as indicated by early menopause in women, has been associated with increased risk of CHD(3–7). The mechanisms underlying these associations are not fully understood yet; deterioration of traditional coronary heart disease risk factors, in particular cholesterol, has been suggested to play a role(8,9). For example, women with an early menopause might be exposed to higher levels of these CHD risk factors longer, which might result in the association with CHD in observational studies.

In observational studies, it is difficult to disentangle the potential independent effect of accelerated reproductive aging on CHD risk from the effect of general aging, as residual confounding can still be present. Furthermore, reversed causality can also play a role here, as women with an unfavorable CHD risk profile have been reported to experience accelerated reproductive aging(10). Mendelian Randomization (MR) designs, exploiting the principle of random independent segregation of alleles at meiosis, are a means to establish causality in situations where randomized clinical trials are impossible(11,12). In MR studies, single nucleotide polymorphisms (SNPs) associated with the exposure as found in genome-wide association studies (GWAS) are used as instrumental variables.

A GWAS has been conducted by Day et al. (2015) for the reproductive aging trait age at natural menopause (ANM) in 69,360 women of European decent with ANM between 40 and 60 years(13). This GWAS reported 56 SNPs, mainly implicated in genome stability (DNA repair), immune function and mitochondrial biogenesis(13) which are biological processes not specific for women and known to be impaired upon aging. Furthermore, a recent CHD GWAS showed that only 10 of the 241 CHD variants were sex-specific(14), which did not include any of the 56 ANM variants. Therefore, we hypothesize that the same 56 ANM variants, even though they may not be associated with reproductive aging in men,

could still be associated with CHD in both women and men, due to the biological processes they are involved in and their link to biological aging.

A recent study in three cohorts(15) showed that an earlier ANM, genetically determined by the 56 SNPs, was associated with an increased CHD risk in women (meta-analysed HR=1.12, 95%CI: 1.02-1.24), but not in men (meta-analysed HR=1.05, 95%CI: 0.94-1.16). However, although studying relationships between genetic risk scores and disease risk provides higher statistical power than formal MR analysis, it is also associated with substantial risk of false positive findings due to horizontal pleiotropy(16). A formal MR analysis by the authors in publicly available data from the Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) consortium from 2011 did not confirm a causal role of ANM in CHD risk(15). In addition, the authors did not investigate cardiovascular risk factors as an outcome nor studied the reversed association.

The aims of the present study are to disentangle putative causal links between reproductive aging and fatal or non-fatal CHD and CHD risk factors in women. In addition, we also studied the reversed association, e.g. between fatal or non-fatal CHD and CHD risk factors and reproductive aging, since hypotheses exist that an adverse CHD risk profile may cause advanced reproductive aging(10,17). Finally, we aim to assess whether the genetic variants are associated with CHD and traditional CHD risk factors in men as well.

Methods

In order to study the different hypotheses as described in the introduction, we conducted four different MR analyses:

1. MR to study the association between ANM variants and CHD risk
2. MR to study the association between ANM variants and CHD risk factors
3. MR to study the association between CHD variants and ANM
4. MR to study the association between CHD risk factor variants and ANM

Below we described the methods for each MR. In addition, Figure 1 shows which data sources were used for each MR, further explained below.

1. Methods for MR to study the association between ANM variants and CHD risk

Outcome

The main outcome was the first event of fatal or non-fatal CHD, defined by codes 410–414 of the International classification of diseases Ninth Edition (ICD-9) and codes I20–I25 of the Tenth Edition (ICD-10).

Data sources

A genome-wide meta-analysis by Day et al. (2015) identified 56 SNPs associated with ANM among 70,000 women of European descent, 54 common HapMap SNPs and two Exome chip SNPs(13), a list of SNPs can be found in Supplemental Table 1(1). As described by Day et al. (2015) all SNPs passed the threshold of p<5e-8 in the joint model. Pleiotropic effects were investigated by searching the GRASP database and NHGRI catalogue for the SNPs or their proxies (R^2>0.5)(13). We used the 56 ANM variants as instrument for genetically determined

reproductive aging in women, and also investigated whether these variants were associated with CHD in men.

We studied these variants in relation to CHD outcome data from 417,579 participants of European descent (including 49,150 CHD cases) from three studies: the EPIC-CVD case-cohort study(18), the UK Biobank(19), and a modified version of the CARDIoGRAMplusC4D consortium (m-CARDIoGRAMplusC4D) since we could only include those studies that provided us with sex-specific summary data (Cardiogenics, Thiseas, AMC-PAS, Duke 2, CCGB 2, ITH 2, OHGS A2, OHGS B2, OHGS C2, Germifs I, Germifs II, Germifs III, Germifs IV, LIFE-Heart and LURIC(20)). Details of the three studies (EPIC-CVD, UK Biobank, and m-CARDIoGRAMplusC4D), including ethical approval and definitions of fatal or non-fatal CHD in each study, can be found in the Supplemental methods(1).

Genotyping

EPIC-CVD participants were genotyped with the Human Core Exome array, Illumina 660 Quad array, and Omni Exome Express array(21). Genotyping in the UK Biobank was performed using the Affymetrix UK BiLEVE Axiom array and the Affymetrix UK Biobank Axiom Array(19,22). The m-CARDIoGRAMplusC4D studies have used various genotyping methods as described previously(20).

Statistical analysis

We verified whether the ANM variants were a valid instrument for the MR analysis in women by calculating the F-statistic according to the method described previously(23), using the SD (5.8 years) for ANM from the imputed data in the EPIC-CVD subcohort and the beta's for the ANM variants from the GWAS(13).

Regarding the outcome CHD, summary statistics (odds ratios and standard errors for the SNP-CHD associations) were derived through the contact persons of UK Biobank and the included CARDIoGRAMplusC4D studies. For the EPIC-CVD case-cohort study, Prentice-weighted Cox proportional hazards regression adjusted for age, country, the first two principal components and array was used to calculate hazard ratios and standard errors for the associations between reproductive aging SNPs and CHD outcome.

We performed a 2-sample MR using four separate methods to estimate causal effects for binary (CHD) outcomes: the simple median-based method, the weighted median-based method, the standard inverse-variance weighted (IVW) regression and the MR-Egger regression using the 'Mendelian Randomization' package in R(24). The IVW provides a consistent estimate and assumes that all assumptions of the instrumental variable are met, the median based and MR-Egger methods provide estimates under weaker assumptions, with the MR-Egger additionally providing an intercept that represents the average pleiotropic effect(25,26). When unbalanced horizontal pleiotropy is absent, results of all methods are expected to be consistent(27). We first conducted sex-specific MR analyses for ANM on CHD in all three studies (UK Biobank, m-CARDIoGRAMplusC4D, EPIC-CVD) separately. Subsequently, we pooled the estimates with a fixed effect model as is standard in MR studies. All analyses were conducted with R version 3.2.0(28).

2. Methods for MR to study the association between ANM variants and CHD risk factors

Outcomes

The outcomes used for this MR are traditional CHD risk factors: total cholesterol, high density lipoprotein (HDL) cholesterol, triglycerides, HbA1c and glucose. More risk factors

are associated with CHD, but we could only use risk factors of which publicly available summary statistics were accessible.

Data sources

We again used the 56 variants from the study by Day et al. (2015)(13) as instrument for the exposure ANM. We applied these variants in MR analyses using data from EPIC-CVD and UK Biobank combined with publicly available GWAS summary statistics from the Global Lipids Genetics Consortium (n=188,577) (29) (total cholesterol, HDL cholesterol, triglycerides) and MAGIC (n=122,744) (30,31) (HbA1c, fasting glucose) for the CHD risk factor outcomes. Details on these consortia can be found in the Supplemental methods(1).

Genotyping

As described above EPIC-CVD participants were genotyped with the Human Core Exome array, Illumina 660 Quad array, and Omni Exome Express array(21). Genotyping in the UK Biobank was performed using the Affymetrix UK BiLEVE Axiom array and the Affymetrix UK Biobank Axiom Array(19,22). The Global Lipids Genetics Consortium and MAGIC used different assays as described previously(29–31). Finally, the 56 SNPs were selected according to the study by Day et al (2015) as described above and in (13).

Statistical analysis

For the CHD risk factor outcomes, we derived effect estimates and standard errors for the reproductive aging SNPs with the cardiovascular risk factors total cholesterol, HDL cholesterol and triglycerides in the Global Lipids Genetics Consortium(29), and in MAGIC(30,31) for diabetes risk factors HbA1c and fasting glucose using Phenoscanner(32) or the original publication. In the random subcohort of EPIC-CVD, we first imputed the missing observational data of EPIC-CVD (non-genetic data only) using multiple imputation

with the MICE package in R(33) with 10 imputations and 50 iterations, including the CVD risk factors, SNPs and other baseline characteristics as predictors. Subsequently, we derived regression coefficients with linear regression in the subcohort only, separately in each imputation, using the same adjustments as for CHD. Thereafter we pooled the results with Rubin's Rule(34), a method designed to pool parameter estimates of multiple imputed datasets, taking into account that the imputed datasets are drawn from the same source dataset. The estimates for the UK Biobank data were downloaded from the Nealelab(35).

We performed a 2-sample MR using four separate methods to estimate causal effects for continuous (total cholesterol, HDL cholesterol, triglycerides, apolipoprotein A (apoA1), apolipoprotein B (apoB), C-reactive protein (CRP), glucose, HbA1c) outcomes: the simple median-based method, the weighted median-based method, the standard inverse-variance weighted (IVW) regression and the MR-Egger regression using the 'Mendelian Randomization' package in R(24). MR analyses were performed for each cardiovascular risk factor in each study separately (EPIC-CVD, UK Biobank, Global Lipids Genetics Consortium, MAGIC) and then pooled using a fixed effects model. Sex-specific analyses were possible in EPIC-CVD and UK Biobank, and pooled results both sexes conducted with Global Lipids Genetics Consortium and MAGIC. All analyses were conducted with R version 3.2.0(28).

3. Methods for MR to study the association between CHD variants and ANM (reversed association)

Outcomes

For this MR ANM was the outcome defined as the age at last naturally occurring menstrual period followed by at least 12 consecutive months of amenorrhea(13).

Data sources

In order to study causality of the reversed association we used the genome-wide significant variants for CHD in the CARDIoGRAMplusC4D(20) (n=185,000) data as instrument for the exposure CHD. The ReproGen (n=70,000)(13) data were used for ANM as the outcome. We used the sex-combined GWAS summary statistics for the exposure, as sex-specific summary statistics were not available. The outcome ANM is only available in women, so the outcome variants are in women only.

Genotyping

The CARDIoGRAMplusC4D consortium consists of 40 different studies with all slightly different methods for genotyping. Details can be found in the paper by Nikpay et al (2015)(20). The 56 SNPs selected according to the study by Day et al (2015) from the ReproGen study were used as outcome variants. Details described above and in (13).

Statistical analysis

We performed a 2-sample MR using four separate methods to estimate causal effects for the continuous ANM outcome: the simple median-based method, the weighted median-based method, the standard inverse-variance weighted (IVW) regression and the MR-Egger regression using the 'Mendelian Randomization' package in R(24). The analysis were conducted for both sexes combined. All analyses were conducted with R version 3.2.0(28).

4. Methods for MR to study the association between CHD risk factor variants and ANM (reversed association)

Outcomes

For this MR ANM was the outcome defined as the age at last naturally occurring menstrual period followed by at least 12 consecutive months of amenorrhea(13).

Data sources

In order to study causality of the reversed association we used genome-wide significant variants from publicly available GWAS summary statistics of the Global Lipids Genetics Consortium (n=188,577) (29) (total cholesterol, low density lipid (LDL) cholesterol), the International Consortium for Blood Pressure GWAS (n=200,000) (36) (systolic blood pressure, diastolic blood pressure), and the GIANT consortium (n=339,224) (37) (body mass index) as instruments for the exposures, and ReproGen (n=70,000) (13) for ANM as the outcome. We used the sex-combined GWAS summary statistics for the exposure, as sex-specific summary statistics were not available. The outcome ANM is only available in women, so the outcome variants are in women only.

Genotyping

We used the genetic variants as defined in the Global Lipids Genetics Consortium, the International Consortium for Blood Pressure GWAS and the GIANT consortium. Details on genotyping and SNP selection can be found in (29,36,37) respectively. The 56 SNPs selected according to the study by Day et al (2015) from the ReproGen study were used as outcome variants. Details described above and in (13).

Statistical analyses

We performed a 2-sample MR using four separate methods to estimate causal effects for continuous (total cholesterol, LDL cholesterol, systolic blood pressure, diastolic blood pressure and body mass index) outcomes: the simple median-based method, the weighted

median-based method, the standard inverse-variance weighted (IVW) regression and the MR-Egger regression using the 'Mendelian Randomization' package in R(24). These analyses with ANM as an outcome were conducted for both sexes combined. All analyses were conducted with R version 3.2.0(28).

Results

1. Results MR to study the association between ANM variants and CHD risk

The F-statistic for genetically determined reproductive aging in women in EPIC-CVD was 93.7, indicating that our instrument was strong in women. Table 1 shows the results for the analysis of the association between genetically determined reproductive aging and CHD risk, stratified by sex and by study (UKBiobank, m-CARDIoGRAMplusC4D, and EPIC-CVD). In women (Figure 2 and Table 1), the IVW analyses in each study separately showed no causal association between genetically determined reproductive aging and CHD, nor when studies were pooled together (Relative Risk Estimate[RRE]$_{IVW}$=0.99; 95% confidence interval [CI]=0.97;1.01). The MR-Egger method indicated no pleiotropic effects (intercept=0.004, p=0.318) and resulted in an RRE$_{MR\text{-}Egger}$ of 0.97 (95%CI=0.94;1.02) in the pooled data. Similar results were found for men (Figure 3 and Table 2) with a pooled RRE$_{IVW}$ of 1.00 (95%CI=0.97;1.02), also indicating no pleiotropic effects (RRE$_{MR\text{-}Egger}$=1.00 (95%CI=0.95;1.05), intercept=0.000, p=0.948).

2. Results MR to study the association between ANM variants and CHD risk factors

Figure 4 (women) and Figure 5 (men) show the IVW results for the association between genetically determined reproductive aging and cardiovascular risk factors. Supplemental table 2(1) shows the results for all four MR methods. For each one-year decrease in genetically determined reproductive aging, total cholesterol levels decreased with 0.009 mmol/L in

women in the IVW-analysis, however this was not statistically significant (95%CI= -0.019;0.001). Similarly, genetically determined reproductive aging was not causally associated with total cholesterol in men ($beta_{IVW}$=0.005 mmol/L, 95%CI= -0.005;0.015). Furthermore, no causal association was found for HDL cholesterol, triglycerides, ApoA1, ApoB, CRP, glucose, and HbA1c in both women as well as men (Figure 4, Figure 5, Supplemental table 2(1)).

3. Results MR to study the association between CHD variants and ANM

Figure 6 and Table 2 show the results for the association between CHD genetically determined reproductive aging for all four MR methods, investigating causality of the reversed association. The IVW analysis shows that CHD was not causally associated with earlier genetically determined reproductive aging ($beta_{IVW}$=0.063, 95%CI=-0.050;0.176).

4. Results MR to study the association between CHD risk factor variants and ANM

Figure 6 and Table 2 show the results for the association between CHD risk factors and genetically determined reproductive aging for all four MR methods, investigating causality of the reversed association. The IVW analysis shows that total cholesterol ($beta_{IVW}$=-0.002, 95%CI=-0.006;0.002), LDL cholesterol ($beta_{IVW}$=0.003, 95%CI=-0.001;0.006), systolic blood pressure ($beta_{IVW}$=0.015, 95%CI=-0.018;0.049), diastolic blood pressure ($beta_{IVW}$=0.023, 95%CI=-0.031;0.077) and body mass index ($beta_{IVW}$=-0.069, 95%CI=-0.0294;0.0156) were not causally associated with earlier genetically determined reproductive aging.

Discussion

This study did not find a causal association between reproductive aging and CHD risk or CHD risk factors, including cholesterol levels, in women. This study does not provide evidence for an association between genetic variants for female reproductive aging and CHD risk or CHD risk factors in men. Furthermore, this study does not provide evidence for causality of the reversed association, since we do not find a causal association between CHD and CHD risk factors and genetically determined reproductive aging.

Our findings regarding CHD are partly in contrast with one previous study investigating the association between ANM SNPs and CHD death, which reported a significantly increased risk of CHD death with a weighted genetic risk score (wGRS) in women, but not in men(15). However, our findings are in line with those of the MR analysis in women, presented in the same paper, using CARDIoGRAMplusC4D data only, which was also null. The discrepancy between the wGRS and MR findings is potentially due to the fact that the wGRS analysis was adjusted for several known CVD risk factors (current smoking, body mass index, hypertension, type 2 diabetes, total cholesterol, and lipid treatment). This might induce a biased association between the genetic variant and the outcome through confounder(s), also known as collider bias(38,39). In addition, the number of cases used for the wGRS analyses was small (only 541 CHD deaths in women), so a chance finding cannot be ruled out either. However, the discrepancy between studies might also be caused by the heterogeneity of outcome definitions. These definitions slightly differ between this study (CHD) and the previously published study (CVD), the composite CVD also includes stroke and congestive heart failure. This should be taken into account when interpreting the results.

Our MR-study suggests that the association between genetically determined reproductive aging and CHD is not causal. However, most observational studies do find an

association between early age at menopause and CHD in women. A possible explanation is that observational studies are susceptible to residual confounding. Postmenopausal women are by definition older than premenopausal women, making it challenging to separate the effects of biological aging from the various phases of the reproductive aging process. Hence, residual confounding due to age may still be present in observational studies. Another possible explanation might be survivor bias in the GWAS we used. It is possible that women, who died of a CHD event before they went through menopause, although this is very rare(17), were not included in the GWAS. Therefore, variants associated with both ANM and CHD might not have been found. Furthermore, reverse causation could be a potential problem in observational studies. Although most studies assume that an early ANM increases CHD risk, it might be possible that an unfavorable cardiovascular risk profile, or accelerated vascular aging, causes an early ANM. One previous observational study showed indeed that higher cholesterol levels prior to menopause were associated with earlier menopause(10). One other observational study found no association between premenopausal CVD and subsequent age at menopause(40), if anything, women who developed CVD before menopause had a lower risk of becoming postmenopausal than women without premenopausal CVD (HR=0.98 for CVD and HR=0.90 for MI), indicating that menopause occurred later in these women(40), but none of these results were statistically significant due to the small number of premenopausal cases. However, another study did find an accelerated menopause for women with CVD prior to the age of 35(17). Our reversed MR analysis does not support evidence for a reversed association where CHD increases the risk of early menopause.

MR uses SNPs, that are randomly assigned by birth, as instrumental variables, and as such provides a method to assess causality(41). However, an MR study makes several assumptions, that have to be taken into account(42). The first assumption is that the genetic

marker is associated with the exposure. The SNPs used in our study were all associated with ANM at a p-value <5e-6 in the latest and largest GWAS(13). As discussed above, this may not be true in men. The second and third assumptions are that the association between the genetic marker and the outcome is explained exclusively through the exposure of interest and is unconfounded. This is often referred to as the absence of pleiotropy, which means that the genetic variant is not associated with other phenotypes. Although our Phenoscanner search showed that a few of the SNPs are associated with age at menarche or sex hormone levels, and thus that some pleiotropy may be present, our MR-Egger analysis showed no indication of pleiotropy, since all intercepts were zero or very close to zero and non-significant(26). We therefore assume that our results are not biased by pleiotropy.

Strengths and limitations

Strengths of this study are that, to the best of our knowledge, this is the largest MR study of associations between reproductive aging and CHD to date with 20,169 CHD events in women and 27,397 in men. We used several methods for MR analyses all yielding consistent results for the tested hypotheses, and in women the instrument we used was strong (F-statistic 93.7). Some limitations need to be acknowledged. First, we cannot establish whether the ANM risk score is a valid instrument for reproductive aging in men. The F-statistic is calculated using observed menopausal age in women, but men do not have a similar trait with an abrupt stop in reproductive potential. Since the SNPs we used are mainly implicated in mechanisms that are not specific for women, and the SNPs were not sex-specific, we hypothesized that there are common mechanisms of reproductive aging for women and men, and that, therefore, the same variants can be used as marker for genetically determined reproductive aging in men. However, it needs to be acknowledged that corresponding phenotypic traits in men need to be further investigated. Second, the GWAS on ANM included women with an ANM between 40 and 60 years only and therefore did not

include women with an extremely early menopause (<40) or premature ovary insufficiency (POI). Most of the observational studies did include women with an extremely early menopause or POI, and two recent systematic reviews and meta-analyses of observational studies showed that POI is associated with both fatal and non-fatal CHD and CVD(43,44). Although we could not study an effect of extremely early menopause in our MR study, a recent GWAS on early menopause revealed no new genetic variants for early menopause and showed that the genetic etiology of early menopause overlaps with that of ANM. Thus early menopause is at least partly explained by the same polygenic variants as ANM(45). The GWAS also excluded women using HRT, while long-term HRT use might be associated with lower CHD risk. However, this only induces confounding in observational studies and not in MR studies. Third, we cannot fully rule out reversed causation since the analyses are conducted in a sample of men and women combined, a consequence of using publicly accessible data, while the outcome (ANM) is estimated in women only. In order to rule out reversed causation these analyses should be reran in women and men separately. Fourth, our analyses with glucose were based on both fasting (MAGIC) and non-fasting estimates (EPIC-CVD). Although both are associated with an increased CVD risk(46,47) it might not be appropriate to combine them, since different SNPs might drive the association and underlying mechanisms could be different. Fifth, some prevalent cases might have been present at the start of the study, which can be problematic in observational studies. However, in an MR one can argue that study entry is not 'time zero', but the allocation of genetic variants at conception is, therefore all events are incident events. Furthermore, there were no women that had an event before they became postmenopausal.

Conclusion

In summary, we found no convincing evidence that reproductive aging is causally associated with CHD and CHD risk factors in women, nor are the SNPs related to CHD and CHD risk

factors in men. We neither found evidence for causality of the reverse association in a combined sample of women and men. However, there is a discrepancy between the definition of CHD in the studies used and we could only analyze men and women together in the reversed association. Still, our results suggest that the association between early menopause and CHD risk in observational studies might be the result of residual confounding, reversed causation, or reflect a shared etiology that results in both earlier menopause and higher CHD risk.

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Data availability

Restrictions apply to the availability of some or all data generated or analyzed during this study to preserve patient confidentiality or because they were used under license. The corresponding author will on request detail the restrictions and any conditions under which access to some data may be provided.

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Figure 1. Visual of the data sources used for each Mendelian Randomization

Figure 2. Results for the MR of ANM variants and coronary heart disease in women of the four different Mendelian Randomization methods used.

Figure 3. Results for the MR of ANM variants and coronary heart disease in men of the four different Mendelian Randomization methods used.

Figure 4. Results for the MR of ANM variants and coronary heart disease risk factors in women for the Inversed Variance Weighted (IVW) method.

Figure 5. Results for the MR of ANM variants and coronary heart disease risk factors in men for the Inversed Variance Weighted (IVW) method.

Figure 6. Results for the MR of CHD and CHD risk factors and ANM for the Inversed Variance Weighted (IVW) method. We used the sex-combined GWAS summary statistics for the exposure, as sex-specific summary statistics were not available. The outcome ANM is only available in women, so the outcome variants are in women only.

Table 1. Relative risk estimates of the association between genetically determined reproductive aging and coronary heart disease for individual studies and the pooled cohorts

	UK Biobank OR (95%CI)	**m-CARDIoGRAMplusC4D** OR (95%CI)	**EPIC-CVD** HR (95%CI)	**Pooled relative risk estimates**
		Women		
Simple median	0.99 (0.96;1.02)	0.97 (0.89;1.06)	0.97 (0.78;1.21)	0.99 (0.96;1.02)
Weighted median	0.99 (0.96;1.02)	0.97 (0.89;1.06)	1.08 (0.88;1.33)	0.99 (0.96;1.02)
IVW*	0.99 (0.97;1.02)	0.98 (0.91;1.05)	1.02 (0.89;1.17)	0.99 (0.97;1.01)
MR-Egger	0.98 (0.94;1.02)	0.88 (0.76;1.02)	1.29 (0.91;1.83)	0.97 (0.94;1.02)
		Men		
Simple median	0.99 (0.95;1.02)	1.05 (0.99;1.12)	0.98 (0.81;1.18)	1.01 (0.98;1.04)
Weighted median	0.99 (0.96;1.03)	1.05 (0.99;1.12)	0.84 (0.71;1.01)	1.01 (0.98;1.04)
IVW*	0.99 (0.96;1.01)	1.03 (0.99;1.08)	0.93 (0.82;1.05)	1.00 (0.97;1.02)
MR-Egger	0.98 (0.93;1.03)	1.02 (0.92;1.12)	0.85 (0.62;1.16)	1.00 (0.95;1.05)

*Inverse-variance weighted

Table 2. Estimates of the association between coronary heart disease and CHD risk factors and genetically determined reproductive aging.

	CHD beta (95%CI)	Total cholesterol beta (95%CI)	LDL cholesterol beta (95%CI)	Systolic blood pressure beta (95%CI)	Diastolic blood pressure beta (95%CI)	Body mass index beta (95%CI)
Simple median	0.064 (-0.104;0.232)	0.000 (-0.005;0.005)	0.004 (-0.002;0.009)	-0.006 (-0.030;0.019)	0.018 (-0.022;0.058)	-0.385 (-0.658;-0.112)
Weighted median	0.057 (-0.100;0.231)	-0.005 (-0.009-0.001)	0.000 (-0.004;0.004)	-0.013 (-0.036;0.009)	-0.008 (-0.043;0.028)	-0.114 (-0.379;0.151)
IVW*	0.063 (-0.050;0.176)	-0.002 (-0.006;0.002)	0.003 (-0.001;0.006)	0.015 (-0.018;0.049)	0.023 (-0.031;0.077)	-0.069 (-0.294;0.156)
MR-Egger	-0.005 (-0.260;0.251)	-0.007 (-0.013;0.000)	0.001 (-0.005;0.008)	-0.004 (-0.112;0.104)	-0.060 (-0.237;0.117)	0.418 (-0.116;0.953)

We used the sex-combined GWAS summary statistics for the exposure, as sex-specific summary statistics were not available. The outcome ANM is only available in women, so the outcome variants are in women only.
*Inverse-variance weighted

Figure 1

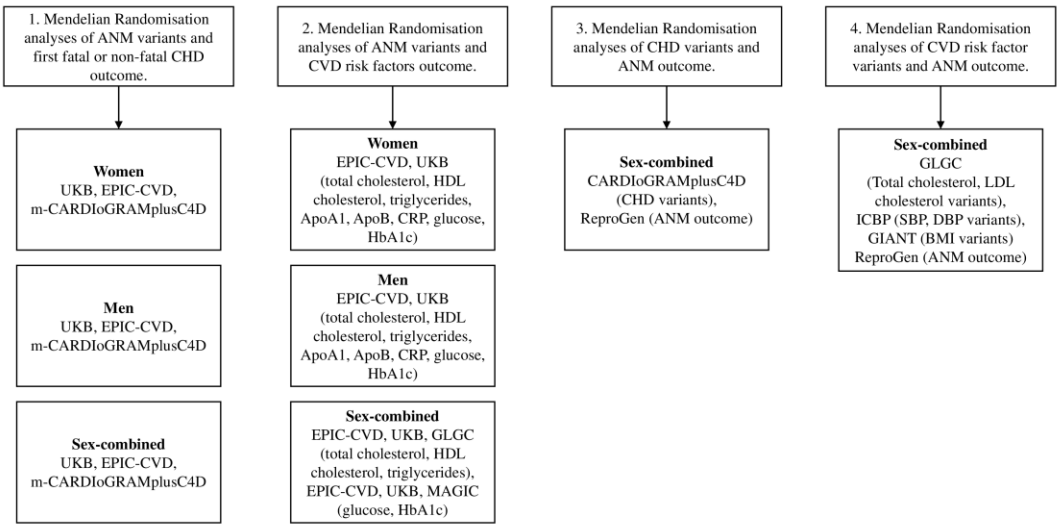

Figure 2

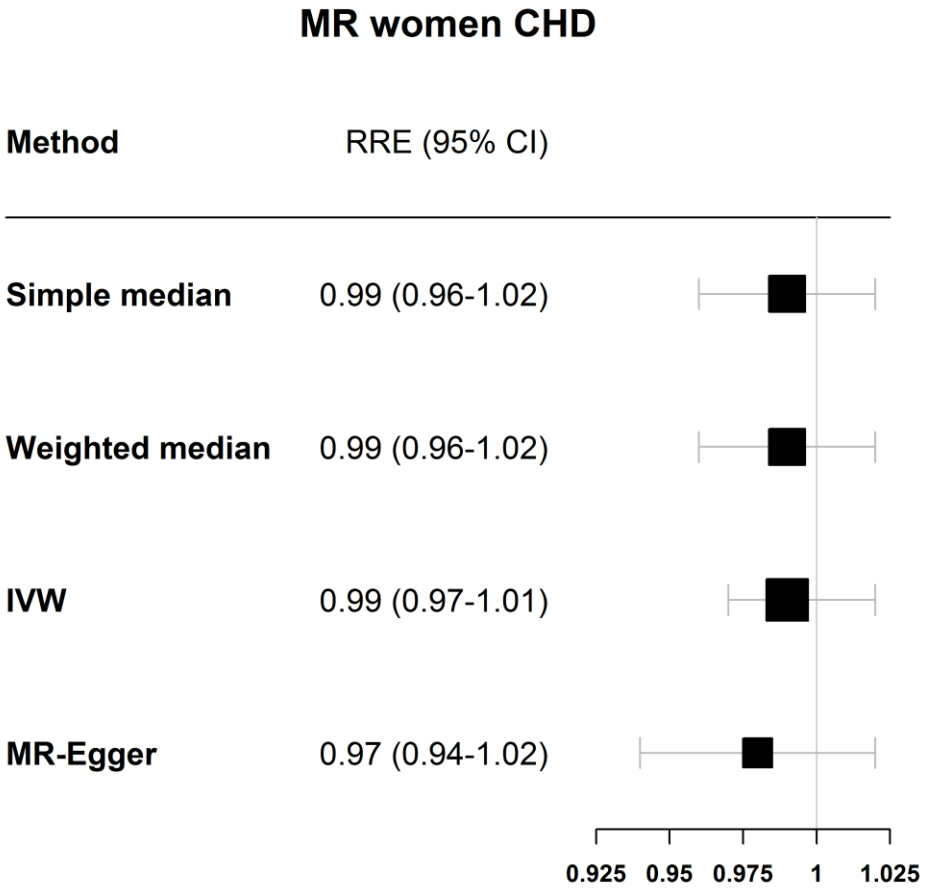

Figure 3

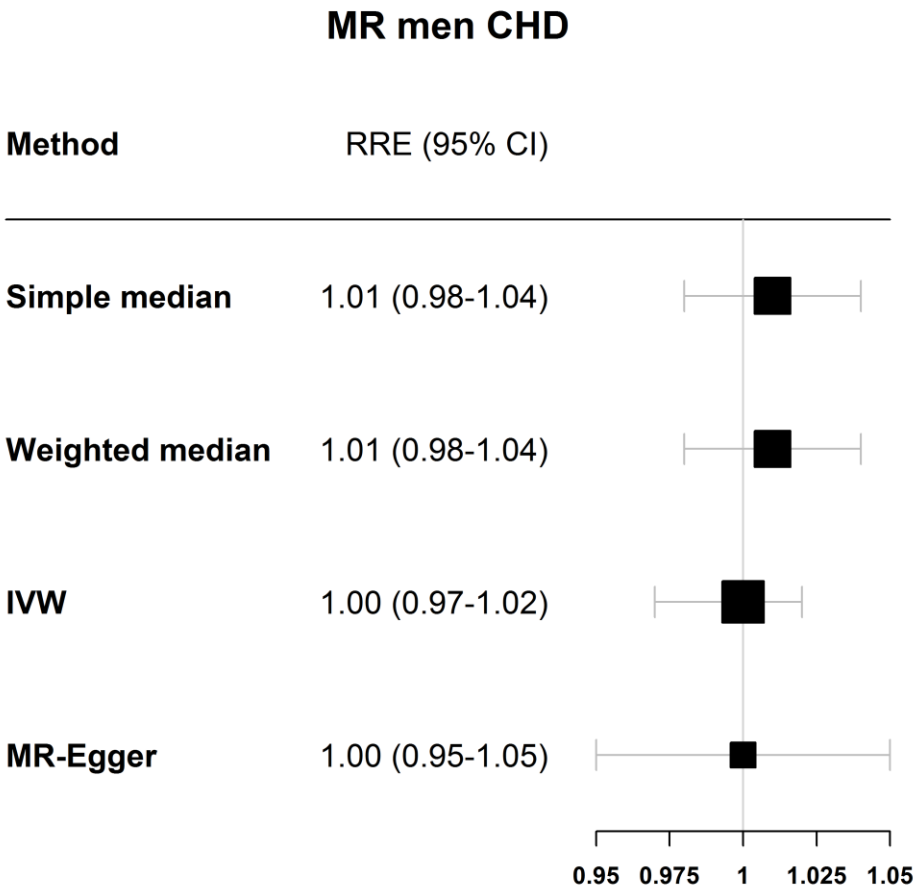

Figure 4

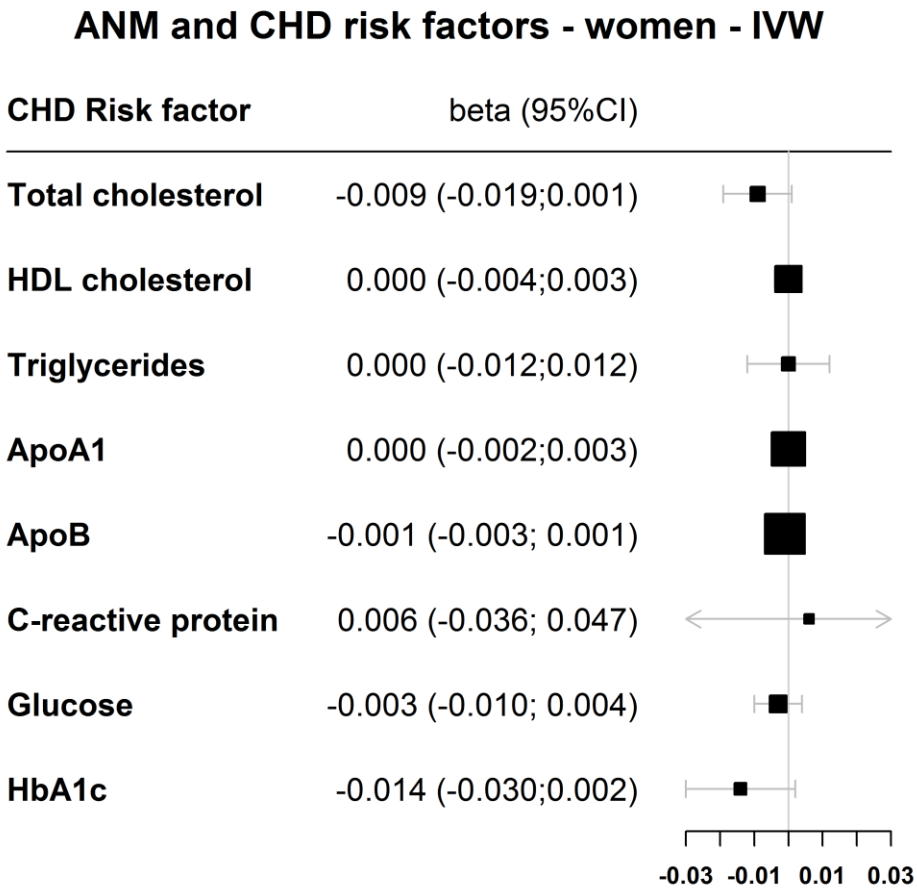

Figure 5

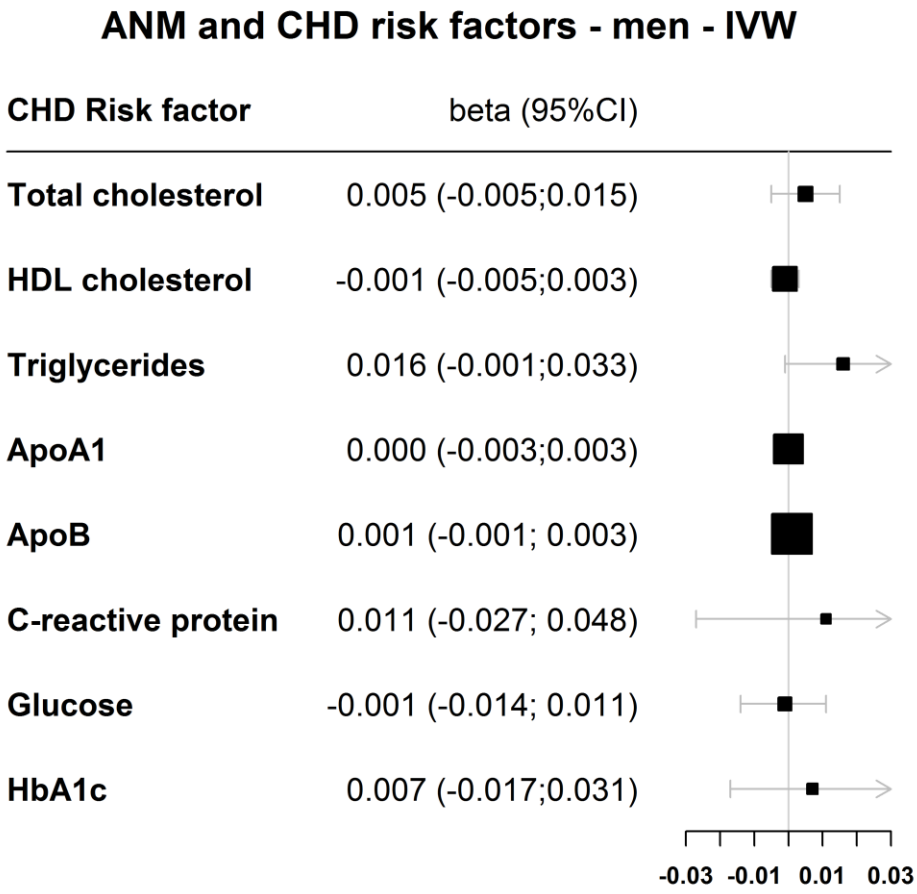

Figure 6