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Genetic variants associated with earlier age at menopause increase the risk of cardiovascular events in women

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Abstract

Objective—To better understand the relationship between cardiovascular disease risk and age-at-natural menopause using genetic data.

Methods—Early menopause is associated with cardiovascular disease risk. We constructed a genetic risk score comprising 56 age-at-natural menopause decreasing alleles in men and women from the Framingham Heart Study, the Atherosclerosis Risk in Communities Study and the Rotterdam Study. If the genetic predisposition to earlier age-at-natural menopause is associated with increased cardiovascular disease risk, it is reasonable to ask whether the risk is shared by men carrying the alleles despite not experiencing menopause. We estimated the hazard ratio for the score for time to first cardiovascular event. To investigate the possible genetic pleiotropy between age-at-natural menopause and cardiovascular disease, we performed cross-trait linkage disequilibrium score regressions between age-at-natural menopause and cardiovascular disease and risk factors using genome-wide association studies.

Results—Approximately 22,500 cardiovascular disease free participants at baseline were analyzed (9,808 men, 12,760 women). Each additional unit of the genetic propensity to earlier age-at-natural menopause increased the hazard of both cardiovascular disease and cardiac death in women (cardiovascular disease: hazard ratio=1.10 [1.04-1.16], $P=9.7\times10^{-4}$, cardiac death: 1.12 [1.02-1.24], P=0.03) while no effect was observed for either outcome in men (hazard ratio=0.99 [0.95-1.04], P=0.71; 1.05 [0.94-1.16], P=0.34). We found significant negative genetic correlations

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in women but not men between age-at-natural menopause and cardiovascular disease and risk factors.

Conclusion—Genetic variants associated with earlier age-at-natural menopause are associated with increased cardiovascular disease risk in women but not men, suggesting sex-specific genetic effects on cardiovascular disease risk.

Keywords

Age-at-natural menopause; cardiovascular disease; genetic risk score; genetic correlation; pleiotropy; DNA damage response pathway

Introduction

Early menopause is associated with an increased risk of osteoporosis, type 2 diabetes, cardiovascular disease and all-cause mortality.[1-4] While the correlation between age-at-natural-menopause (ANM) and cardiovascular disease risk is well known, the basis for that correlation is incompletely understood.

Genome-wide association studies (GWAS) have identified 18 common genetic loci associated with ANM. A recent study of nearly 70,000 women, with both common and low-frequency coding variants from Hapmap2 reference sample and exome chip, was able to triple the number of independent signals associated with ANM (N=56).[5] Moreover, this study found an overwhelming enrichment of DNA damage response (DDR) genes among the ANM-associated GWAS loci, possibly explaining up to approximately two-thirds of the associations. However, few studies have investigated the genetic relationship between cardiovascular disease risk and ANM and it is unknown whether the genetic predisposition to earlier menopause also increases the risk of cardiovascular disease, or whether the increased risk in women with earlier menopause is due to non-genetic factors. Moreover, if the genetic predisposition to earlier ANM is also associated with increased cardiovascular disease risk, then it is reasonable to ask whether the increased risk is shared by men carrying ANM-lowering alleles as well. Thus, we sought to better understand the relationship between cardiovascular risk and ANM in men and women using genetic data. We investigated whether the association was due to genetic factors, and explored whether the genetic factors affected males and females equally. For this purpose, we constructed for the first time a genetic risk score (GRS) comprising the ANM decreasing alleles in women and men who also carried the alleles despite not experiencing menopause. We first determined the time to the first cardiovascular event in relation to the GRS in men and women from the Framingham Heart Study (FHS), the Atherosclerosis Risk in Communities (ARIC) Study and the Rotterdam Study (RS). We then investigated the possible genetic pleiotropy and causality between ANM and cardiovascular disease and risk factors using data from large-scale sex-stratified GWAS, Linkage Disequilibrium (LD) score regressions and Mendelian randomization (MR) analyses. Finally, we examined the role of the DDR pathway by dividing the GRS in two sub-scores, according to the belonging or not of ANM SNPs to this pathway.

Methods

Populations/Participants

We included participants with phenotype and genotype data. We excluded participants with prevalent CVD at baseline and missing covariates. A total of 22,568 CVD free participants at baseline were included in the meta-analysis of time to the first cardiovascular event. Participants were drawn from three population-based cohort studies including the FHS (N=7,560 total, 4,182 women), ARIC (N=7,656 total, 4,125 women) and the RS (N=5,438 total, 3,370 women for Rotterdam Study I (RS-I) and N=1,914 total, 1,083 women for Rotterdam Study II (RS-II)). These three population-based cohort studies are part of The Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium.[6] They have a long follow-up, validated CVD events, information on ANM and post-menopausal women. These studies also all have genome-wide genotype data, allowing us to assess the genetic score for ANM. Detailed descriptions of all studies are provided in Supplementary Material and Table S1.

Statistical Analysis

Computation of the Genetic Risk Score—The largest meta-analysis on ANM to date identified 56 independent SNPs (54 common Hapmap2 variants and two low-frequency exome-chip coding variants).[5] We extracted these 56 independent SNPs from Haplotype Reference Consortium, 1000 Genomes imputed data or exome-chip genotyped data (Supplementary Material). We used imputed data for the 54 Hapmap SNPs and exome-chip genotypes when available for the two exome-chip SNPs. Imputed data were also used to fill in a somewhat small number of participants who were not genotyped on the exome chip. The genotypes or imputed genotype dosages of the 56 SNPs were used to construct a genetic risk score (GRS) using an effect-weighted score method such that for the i^{th} individual:

$\mathrm{GRS_i}=\sum_{j=1}^{k}\beta_j G_{ij}$ where k=56 is the number of independent genome-wide associated SNPs, β_j is the estimated effect size from the published ANM meta-analysis[5] for the ANM-reducing allele of the j^{th} SNP and G_{ij} is the number of copies of the ANM-reducing allele at the j^{th} SNP carried by the i^{th} individual (G_{ij} is between 0 and 2).

Association analyses—Association between the GRS and time to the first cardiovascular event was evaluated using a Cox proportional-hazards model. Two cardiovascular endpoints were defined as 1) coronary heart disease death and 2) a composite endpoint including myocardial infarction, stroke, congestive heart failure or death from coronary heart disease. Participants with prevalent cardiovascular disease (CVD) at baseline including myocardial infarction, angina, stroke, heart failure, and intermittent claudication (peripheral artery disease) were excluded. The study entry point was age at baseline (DNA blood draw). Outcome definitions per study are available in Supplementary Material. All analyses were adjusted for sex, age at entry point, the first 10 genotype principal components, familial relatedness, study center and known cardiovascular factors (current smoking, body mass index, hypertension, type 2 diabetes, total cholesterol and lipid treatment). Covariate definitions per study are available in Supplementary Material. The log hazard ratio was scaled according to the effect size of a 1-unit increase in polygenic risk

score on ANM to obtain an estimated log hazard ratio for a 1-year decrease in genetically predicted ANM. To evaluate the influence of sex, we added a GRS*sex interaction term to the models. For ease of interpretation, analyses were also performed in men and women separately. An additional adjustment on ANM was also performed in women.

Genetic correlation—Cross-trait LD score regression[7] was used to estimate the overall genetic correlation between ANM and eight individual traits (CVD or CVD risk factors, see Supplementary Table S2) from published European studies using datasets available in the GRASP database (available on 01/01/2017).[8,9] For these studies, sex-specific results were available. We estimated genetic correlations using the --rg flag in the ldsc software package, with LD Scores from 1000 Genomes Project Europeans and default settings.[7] Briefly, this method regresses the product of effect size estimates for trait 1 and trait 2 for each SNP against LD Score. The product of the slope and a constant estimates the genetic correlation, and the intercept estimates the product of the number of overlapping samples and the correlation between phenotypes among the overlapping samples. We reported significant genetic correlations with a False Discovery Rare (FDR) lower or equal to 5%.

Mendelian randomization—A Mendelian randomization (MR) analysis on ANM with coronary artery disease (CAD) was carried out in women using the single-SNP results of the Hapmap ANM meta-analysis[5] and the CARDIoGRAMplusC4D CAD meta-analysis[10] for the 54 ANM Hapmap SNPs included in the GRS using a method described in \https://cran.rproject.org/web/packages/gtx/vignettes/ashg2012.pdf.

GRS sub-scores according to belonging of ANM SNPs to DNA repair pathway —The ANM SNPs were found to be strongly enriched in DNA damage response (DDR) pathways in the ANM meta-analysis.[5] To investigate the role of this pathway, we repeated the analyses with a GRS divided in two sub-scores, according to the belonging (GRS1) or not (GRS2) of the ANM SNPs to this pathway (see Supplementary Table S1).

Results

Description of the studies

A total of 22,568 CVD free participants at baseline (4,970 composite endpoint events, 1,056 coronary heart disease deaths) were analyzed, including 9,808 men (2,495 composite endpoint events, 515 coronary heart disease deaths) and 12,760 women (2,475 composite endpoint events, 541 coronary heart disease deaths), among which there were 9,003 post-menopausal women (2,086 composite endpoint events, 484 coronary heart disease deaths).

The characteristics of the studies are shown in Table 1. Participants from the Rotterdam Study were older than the participants in FHS or ARIC (68.7 +/- 9.0 in RSI and 64.2 +/- 7.7 in RSII *versus* 51.3 +/- 15.5 in FHS and 54.1 +/- 5.7 in ARIC) and all women were menopausal. The follow-up time for ARIC was twice as long as the follow-up in FHS or Rotterdam (20.05 +/- 7.14 *versus* 10.9 +/- 3.9 in FHS; 12.07 +/- 5.92 in RSI and 8.31 +/- 2.11 in RSII). The longer follow-up in ARIC and the higher mean age at baseline in Rotterdam were likely contributing to the higher number of cardiovascular events in these

studies compared to FHS (2,286 in ARIC and 1,923 in RSI *versus* 533 in FHS for the composite endpoint).

Participants from the studies also have differences in cardiovascular risk factors. The Rotterdam Study participants had a higher prevalence of diabetes (9.4% in RSI and 11.7% in RSII *versus* 5.6% in FHS and 7.5% in ARIC) and hypertension (53.6% in RSI and 59.6% in RSII *versus* 31.2% in FHS and 24.1% in ARIC) compared to other studies. Participants of both the Rotterdam Study and ARIC were more likely to be smokers (prevalence 22.7% in RSI; 22.2% in RSII and 24.35% in ARIC *versus* 14.8% in FHS).

Association analyses between GRS and time to the first cardiovascular event

As expected, a higher GRS of ANM decreasing alleles was strongly associated with earlier ANM (β_{GRS} = −1.06 [−1.17-0.94]). The GRS explained between 1% and 7% of the ANM variance. The ANM GRS and GRS*sex interaction terms were not significant for the coronary heart disease death outcome (HR_{GRS}=1.02 [0.89-1.17], P_{GRS}=0.70 and $HR_{GRS*sex}$=1.16 [0.99-1.35], $P_{GRS*sex}$=0.14). For the composite endpoint, GRS*sex interaction term was significant (HR_{GRS}= 0.96 [0.90-1.03], P_{GRS}=0.32 and $HR_{GRS*sex}$=1.10 [1.01-1.19], $P_{GRS*sex}$=0.02). Sex-stratified analyses (Table 2) showed that GRS increased cardiovascular event risk in women (HR=1.12 [1.02-1.24], P=0.03 for coronary heart disease deaths and HR=1.10 [1.04-1.16], $P=9.7\times10^{-4}$ for the composite endpoint) while no effect was observed in men (HR=1.05 [0.94-1.16], P=0.34 for coronary heart disease deaths and HR=0.99 [0.95-1.04], P=0.71 for the composite endpoint). See detailed results by study in Table 2. Thus, a 1-unit decrease in genetically predicted ANM increases the hazard of coronary heart disease death by 12% and of the composite endpoint by 10% in women.

We found that the GRS remained associated with time to the first coronary heart disease death but not cardiovascular diseases in women when analyses were adjusted for ANM (HR=1.13 [1.07-1.25], P=0.02), suggesting a genetic pleiotropy in women between ANM and coronary deaths (Table S3).

GRS sub-scores according to belonging of ANM SNPs to DNA repair pathway

The two GRS sub-scores were strongly associated with earlier ANM (β_{GRS1} = −1.05 [−1.18-0.91] and β_{GRS2} = −0.82 [−1.06-0.59]). We did not observe significant associations of either GRS sub-score with cardiovascular disease risk (composite endpoint) in males or females. However, we observed an association of GRS2 (the sub-score comprising the ANM SNPs that do not belong to the DDR pathway) with coronary deaths in females (HR=1.30 [1.07-1.59], P=0.008).

Genetic correlation

To test the genetic correlations between ANM and CVD outcomes or CVD risk factors, we performed cross-trait LD Score regression using summary statistics from published GWAS meta-analyses of eight traits for which sex specific analyses were available (Supplementary Table S2). We observed significant negative correlations between ANM and four of the eight traits in women but not in men (hip circumference: rg=−0.12; P = 0.001; body mass index

(BMI): rg=−0.13; P=0.002; weight: rg=−0.12; P= 0.003; coronary artery disease (CAD): rg= −0.22; P = 0.01). See detailed results in Table S2.

Mendelian randomization

We carried out a MR analysis on ANM with CAD in women using the single-SNP results of the Hapmap ANM[5] and CAD[10] meta-analyses. We did not detect a putative causal effect of ANM on CAD in women (P=0.52).

Discussion

We investigated for the first time the genetic relationship between ANM and CVD risk in the FHS, the ARIC and the Rotterdam studies. We used a GRS comprising ANM-reducing alleles that were genome-wide significant in a recent large meta-analysis[5] to determine the time to the first incident CVD event and observed that a genetic predisposition to earlier ANM increased CVD risk in women whereas in men ANM-reducing alleles did not have an effect on CVD risk. We observed a genetic pleiotropy between ANM and coronary deaths, suggesting that some variants affect both ANM and coronary deaths. As the 54 Hapmap SNPs explained only 6% of the variance in ANM,[5] we investigated the overall genetic correlation of ANM with CAD and CVD risk factors with cross-trait LD score regressions. We described for the first time a significant negative genetic correlation between ANM and CAD in women but not in men. We also confirmed the negative genetic correlation between ANM and BMI[5] in women and we reported a new negative genetic correlation between ANM and hip circumference in women but not in men. These significant genetic correlations between ANM and CAD and CVD risk factors in women are consistent with genetic pleiotropy. Some variants affect both ANM and CAD and CVD risk factors in women and these variants that decrease ANM also increase CAD and CVD risk factors such as BMI or hip circumference in women.

We observed that a 1-unit decrease in genetically predicted ANM increases the hazard of coronary heart disease death by 12% and of the composite endpoint by 10% in women. This is higher than what is known in the epidemiology literature about the association between ANM and either mortality or CVD.[11,12] A study on a cohort of 12,115 postmenopausal women living in Utrecht, Netherlands, aged 50-65 years showed that for each year's delay in the menopause the cardiovascular mortality risk decreased by 2%.[11] Data from a prospective cohort study of US adults examined the relation between age at natural menopause and all-cause and cause-specific mortality among women who never used hormone therapy, who never smoked, and who experienced natural menopause between the ages of 40 and 54 years and showed that all-cause mortality rates were higher among women who reported that menopause occurred at age 40-44 years compared with women who reported that menopause occurred at age 50-54 years (rate ratio (RR) = 1.04, 95% confidence interval (CI): 1.00, 1.08).

Similar strategies using a GRS have been previously conducted to examine relationship between genetic variants associated with ANM and diseases such as polycystic ovary syndrome, gonadotrophins and ovarian volume[13] and breast cancer.[5,13-15] A recent study evaluated the link between genetic variants associated with coronary heart disease and type 2

diabetes and ANM in over 50,000 women from three large consortia (ITMAT/Broad/CARe (IBC), ReproGen and EPIC-InterAct). In this study, the association between cardio-metabolic disease and earlier timing of menopause was not causal but this finding does not exclude the possibility that the reverse association can be causal.[16] It should be noted that few studies consider that sex can modify the effect of genetic variants on cardiovascular diseases.[17]

Cardiovascular disease remains the leading cause of death in women in the United States. Women are often older at time of first presentation, as we observe in all cohorts participating in this study (Table 1) making it difficult to distinguish the effects of menopause from that of age. Women with an early menopause are at an increased risk of CVD.[1-3] Cardiovascular risk factor changes occurring with menopause have been considered one of the biological mechanisms, as menopause is often accompanied by unfavorable levels of cardiovascular risk factors such as blood pressure, serum cholesterol, and body mass index.[18] Interestingly, our cross-trait LD score regression analysis showed a significant negative genetic correlation between ANM and BMI in women, suggesting genetic pleiotropy where some of the variants that decrease ANM also increase BMI. Increased cardiovascular risk has been proposed as a consequence of menopause but the alternative hypothesis, that increased premenopausal cardiovascular risk promotes early menopause, has also been examined.[19] Atherosclerosis risk could determine the ANM, possibly by inducing ischemic damage in the ovaries or through direct effects on the endocrine system.[19] The complex mechanisms by which estrogen influences coronary heart disease risk are incompletely understood. The depletion of estrogen at menopause could increase endothelial dysfunction, lipid deposition in the vasculature, and other changes that can precipitate the development of atherosclerosis over time.[20] Interestingly, ANM SNPs were enriched for the DDR pathway as DNA damage and repair also plays a role in the vasculature and is recognized as a factor in the progression of atherosclerosis.[21-24] We identified an association of ANM SNPs that do not belong to the DDR pathway with coronary deaths. A prolonged exposure to lack of estrogen may primarily influence coronary heart disease risk through menopause timing. Further investigation is needed to understand the role of these SNPs on coronary deaths.

A major strength of the studies included in this project is the use of prospectively collected longitudinal risk-factor data and ANM, and modeling that allowed estimation of risk factors at a specific age, thus permitting an analysis not confounded by age. As ANM was determined prospectively in many women, it minimized errors in reported menopausal ages. A measurement error in the reporting of ANM or a residual negative confounding in the ANM GWAS studies from which the GRS weights were derived would be likely to attenuate effects. Another strength of this project is that cardiovascular events were validated with medical records and the cardiovascular risk factors were directly measured (except current smoking which was self-reported in FHS and ARIC).

This study has some limitations. The significance of the GRS effect in the meta-analysis of time to the first cardiovascular event in women was mainly driven by the signal in the FHS although the other studies had consistent direction of effects. This could be due to differences in the study characteristics or to heterogeneity of the outcome definitions. We performed a sensitivity analysis by removing the FHS and we still observed that the GRS

increased cardiovascular event risk in women (HR=1.07 [1.01-1.14], P=0.03 for the composite endpoint). Further, removing younger Gen 3 with few events did not have any meaningful effect on estimates in the FHS. We also observed a difference in effect by age in the FHS sample that requires a larger sample to investigate further.

The regression estimates and standard errors available for most studies in the GRASP database are truncated to two decimal places. Thus, our genetic correlation estimates between ANM and other traits may be attenuated. We conducted a MR analysis in women to investigate the putative causal effect of ANM on CAD. We assumed that the possible genetic pleiotropy between ANM and CAD was not due to the ANM genetic variants included in the GRS. Indeed, few of the 54 ANM Hapmap SNPs were nominally associated with CAD in the CARDIoGRAMplusC4D GWAS performed in women. If pleiotropy exists, this method may not be appropriate as pleiotropy invalidates assumptions of MR.[25]

Conclusion

In summary, we show that the genetic predisposition to earlier ANM increased CVD risk in women. Our findings of negative genetic correlations between ANM and CAD and CVD risk factors in women suggest a genetic pleiotropy: some genetic variants associated with earlier ANM are associated with increased CAD risk and CVD risk factors (hip circumference, BMI, weight). There was no evidence that the ANM decreasing variants affect the risk factors and CAD in males. Thus, we demonstrate the usefulness of genetics and sex-specific analyses in further understanding the relationship between cardiovascular risk and ANM.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Table 1

Descriptive table of the populations included in the meta-analysis

	FHS				ARIC				Rotterdam Study I visit 1			Rotterdam Study II visit 1		
	All	**Men**	**Women**	**Post-ANM* women**	**All**	**Men**	**Women**	**Post-ANM* women**	**All**	**Men**	**Post-ANM* women**	**All**	**Men**	**Post-ANM* women**
N (%)	7,560 (100.0)	3,378 (44.7)	4,182 (55.3)	1,844 (24.4)	7,656 (100.0)	3,531 (46.1)	4,125 (53.9)	2,706 (35.3)	5,438 (100.0)	2.068 (38.0)	3,370 (62.0)	1,914 (100.0)	831 (43.4)	1,083 (56.6)
Age[a], mean (SD)	51.3 (15.5)	50.2 (14.7)	52.2 (16.1)	60.9 (12.7)	54.1 (5.7)	54.3 (5.7)	53.8 (5.7)	55.1 (5.4)	68.7 (9.0)	67.6 (8.1)	69.5 (9.4)	64.2 (7.7)	63.7 (7.1)	64.6 (8.1)
Follow-up time[b], mean (SD)	10.8 (3.9)	10.7 (3.9)	11.0 (3.9)	11.8 (4.3)	20.1 (7.1)	18.6 (7.7)	21.3 (6.4)	21.3 (6.3)	12.1 (5.9)	11.2 (6.0)	12.6 (5.8)	8.3 (2.1)	8.1 (2.3)	8.5 (1.9)
ANM*, mean (SD)	-	-	-	49.9 (3.9)	-	-	-	47.6 (5.1)	-	-	48.8 (5.0)	-	-	48.9 (5.8)
Cardiac deaths, N (%)	209[e] (2.8)	96[e] (2.8)	113[e] (2.7)	76[e] (4.1)	235 (3.1)	152 (4.3)	83 (2.0)	63 (2.3)	573 (10.5)	245 (11.9)	328 (9.7)	39 (2.0)	22 (2.7)	17 (1.6)
Cardiac death age, mean (SD)	81.1 (12.4)	76.9 (13.7)	84.6 (9.9)	86.2 (8.4)	73.7 (7.9)	73.4 (8.2)	74.2 (7.3)	75.3 (7.1)	82.8 (7.8)	80.6 (7.4)	84.4 (7.7)	77.5 (11.7)	73.5 (12.0)	82.7 (9.1)
Composite CVD[c], N (%)	533[e] (7.1)	246[e] (7.3)	287[e] (6.9)	189[e] (10.3)	2,286 (29.9)	1,316 (37.3)	970 (23.5)	679 (25.1)	1,923 (35.4)	813 (39.3)	1,110 (32.9)	228 (11.9)	120 (14.4)	108 (10.0)
Composite CVD[c] age, mean (SD)	77.1 (12.9)	73.2 (13.1)	80.6 (11.8)	82.0 (10.5)	74.1 (8.0)	72.9 (8.4)	75.1 (7.5)	76.4 (7.3)	79.7 (8.4)	77.1 (7.7)	81.6 (8.4)	73.4 (9.8)	70.5 (8.7)	76.6 (9.9)
Body Mass Index[a], mean (SD)	27.3 (5.4)	28.1 (4.5)	26.6 (5.9)	27.0 (5.8)	26.8 (4.7)	27.3 (3.9)	26.3 (5.3)	26.4 (5.2)	26.3 (3.6)	25.6 (2.9)	26.7 (3.9)	27.2 (4.0)	26.8 (3.3)	27.5 (4.4)
Smoking[a], N (%)	1,122 (14.8)	510 (15.1)	612 (14.6)	263 (14.3)	1,864 (24.4)	843 (23.9)	1,021 (24.8)	638 (23.6)	1,234 (22.7)	624 (30.2)	610 (18.1)	425 (22.2)	209 (25.2)	216 (19.9)
Lipid treatment[a], N (%)	789 (10.4)	428 (12.7)	361 (8.6)	209 (11.3)	226 (3.0)	96 (2.7)	130 (3.2)	95 (3.5)	105 (1.9)	35 (1.7)	70 (2.1)	191 (10.0)	81 (9.7)	110 (10.2)
Hypertension[a], N (%)	2,358 (31.2)	1,131 (33.5)	1,227 (29.3)	737 (40.0)	1,843 (24.1)	881 (25.0)	962 (23.3)	672 (24.8)	2,916 (53.6)	1,037 (50.1)	1,879 (55.8)	1,141 (59.6)	497 (59.8)	644 (59.5)
T2 diabetes[a], N (%)	422 (5.6)	227 (6.7)	195 (4.7)	115 (6.2)	571 (7.5)	299 (8.5)	272 (6.6)	190 (7.0)	511 (9.4)	184 (8.9)	327 (9.7)	223 (11.7)	123 (14.8)	100 (9.2)
Total cholesterol[a], mean (SD)	196.6 (38.3)	195.1 (38.2)	197.8 (38.4)	207.7 (39.1)	212.7 (40.6)	208.8 (37.9)	216.6 (42.2)	220.4 (42.5)	256.0[d](46.0)	243.6[d] (44.5)	264.1 (45.2)[d]	225.5 (37.5)	217.3 (37.1)	232.0 (36.0)

Participants with a prevalent cardiovascular disease (CVD) at baseline including myocardial infarction, angina, stroke, heart failure, and intermittent claudication (peripheral artery disease) were excluded.

[a] measured at baseline

[b] Follow-up time for the composite cardiovascular disease outcome

[c] a composite end point of CVD events defined as myocardial infarction, stroke, congestive heart failure and death from coronary heart disease

[d] Measurements were non-fasting at baseline Rotterdam Study-I (RS-I-1)

[e] Number of events in the FHS cohorts (original cohort/offspring/gen3): cardiac deaths (ALL:100/104/5, men: 37/56/3, women: 63/48/2, post-menopausal women: 47/28/1), composite endpoint (ALL: 208/281/44, men: 69/148/29, women: 139/133/15, post-menopausal women: 93/93/3)

[f] Age at natural menopause

Table 2

Sex-stratified association analyses between the Genetic Risk Score (GRS) and time to the first cardiovascular event using a Cox proportional-hazards model from age at baseline[a] for a total of 22,568 cardiovascular diseases (CVD) free individuals at baseline

Coronary Heart Disease Death, N events=1,056						
	Women			Men		
Study	**HR[c]**	**95% CI**	**p**	**HR[c]**	**95%CI**	**p**
FHS	1.29	1.08-1.54	**0.005**	0.90	0.73-1.12	0.36
ARIC	1.14	0.89-1.45	0.29	1.23	1.01-1.50	0.04
RSI	1.04	0.91-1.18	0.58	1.00	0.86-1.16	0.99
RSII	1.13	0.55-2.32	0.73	1.57	0.77-3.21	0.21
Meta-Analysis	1.12	1.02-1.24	**0.03**	1.05	0.94-1.16	0.34
Composite Endpoint[b], N events=4,970						
Study	**HR[c]**	**95% CI**	**p**	**HR[c]**	**95%CI**	**p**
FHS	1.22	1.08-1.37	**0.001**	0.91	0.80-1.05	0.19
ARIC	1.06	0.93-1.22	0.36	1.00	0.94-1.07	0.91
RSI	1.06	0.99-1.14	0.11	0.99	0.91-1.07	0.76
RSII	1.27	0.95-1.69	0.11	1.08	0.81-1.45	0.60
Meta-Analysis	1.10	1.04-1.16	**9.7×10^{-4}**	0.99	0.95-1.04	0.71

[a] Analyses were adjusted for sex, age at entry point, principal components, familial relatedness, study center and known cardiovascular factors (current smoking, body mass index, hypertension, type 2 diabetes, total cholesterol and lipid treatment). The Hazard Ratios (HRs) are presented per 1 unit increase in GRS. A 1-unit increase in GRS corresponds to a 1-year decrease in expected age at natural menopause.

[b] a composite end point of cardiovascular disease events defined as myocardial infarction, stroke, congestive heart failure and death from coronary heart disease

[c] Hazard Ratio