Original article

Premenopausal cardiovascular disease and age at natural menopause: A pooled analysis of over 170,000 women

Dongshan Zhu[1], Hsin-Fang Chung[1], Nirmala Pandeya[1,2], Annette J. Dobson[1], Rebecca Hardy[3], Diana Kuh[3], Eric J. Brunner[4], Fiona Bruinsma[5], Graham G. Giles[5,6], Panayotes Demakakos[7], Jung Su Lee[8], Hideki Mizunuma[9], Kunihiko Hayashi[10], Hans-Olov Adami[11,12], Elisabete Weiderpass[11,13,14,15], Gita D. Mishra[1]

[1] The University of Queensland, School of Public Health, Brisbane, Queensland, Australia

[2] Department of Population Health, QIMR Berghofer Medical Research Institute, Brisbane, Queensland, Australia

[3] Medical Research Council Unit for Lifelong Health and Ageing at UCL, London, UK

[4] Department of Epidemiology and Public Health, University College London, London, UK

[5] Cancer Epidemiology and Intelligence Division, Cancer Council Victoria, Melbourne, Victoria, 3004, Australia

[6] Centre for Epidemiology and Biostatistics, Melbourne School of Population and Global Health, The University of Melbourne, Melbourne, Victoria 3010, Australia

[7] Department of Epidemiology and Public Health, University College London, London, UK

[8] Department of Public Health, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8654, Japan

[9] Fukushima Medical Center for Children and Women, Fukushima Medical University, Fukushima, 960-1295, Japan

[10] School of Health Sciences, Gunma University, Maebashi City, Gunma 371-0044, Japan

[11] Department of Medical Epidemiology and Biostatistics, Karolinska Institutet, Stockholm, Sweden

[12] Clinical Effectiveness Research Group, Institute of Health and Society, University of Oslo, Oslo, Norway

[13] Genetic Epidemiology Group, Folkhälsan Research Center, Faculty of Medicine, University of Helsinki, Helsinki, FI-00290, Finland

[14] Department of Community Medicine, Faculty of Health Sciences, University of Tromsø, The Arctic University of Norway, Tromsø, 9019, Norway

[15] Department of Research, Cancer Registry of Norway, Institute of Population-Based Cancer Research, Oslo, 0304, Norway

Corresponding author:
Dongshan Zhu, MD
288 Herston Road (corner of Herston Rd and Wyndham St)
School of Public Health, University of Queensland,
Brisbane, Queensland 4006, Australia
TEL: +61-7-3365-5224, FAX: +61-7-3365-5540
Email: dongshan.zhu@uq.net.au

ABSTRACT

Background

Early menopause is associated with an increased risk of subsequent cardiovascular disease (CVD). Few studies have investigated the converse. We examined whether premenopausal CVD events are associated with early age at menopause.

Methods

We pooled the individual data of 177 131 women from nine studies. We used multinomial logistic regression models to estimate multivariable relative risk ratios (RRR) and 95% confidence intervals (CI) for the associations between age at onset of premenopausal CVD events -including coronary heart disease (CHD) and stroke - and age at natural menopause.

Results

Altogether 1561 (0.9%) premenopausal participants reported CVD events (including 1130 CHD and 469 stroke) at a mean age of 41.3 years. Compared with women without any premenopausal CVD events, women who experienced a first CVD event before age 35 years had a 2-fold risk of menopause before age 45 years (early menopause); adjusted RRR (95%CI) of 1.92 (1.17, 3.14) for any CVD, 1.86 (1.01, 3.43) for CHD and 2.17 (1.43, 3.30) for stroke. Women who experienced a first premenopausal CVD event after age 40 years underwent a natural menopause at the expected age (around 51 years). These associations were robust to adjustment for smoking status, BMI, educational level, race/ethnicity, age at menarche, parity, hypertension and family history of CVD.

Conclusions

For premenopausal women, a first CVD event before age 35 years is associated with a doubling of the risk of an early menopause, while a first CVD event occurred after 35 years indicates a normal menopause at around 51 years. Shared genetic and environmental factors (such as smoking), as well as compromised vasculature following CVD events, may contribute to this outcome.

Keywords Premenopausal · Cardiovascular disease · Age at menopause · Pooled analysis

INTRODUCTION

Menopause, defined as cessation of menstrual bleeding for at least 12 months, marks the end stage of reproductive ageing [1]. Average age at menopause is 51.4 years in high-income countries [2, 3]. Early menopause, i.e., occurring before the age of 45 years, affects approximately 5% of women [4] and entails increased risk of non-fatal and fatal cardiovascular disease (CVD) and of all-cause mortality [5-9].

The reduction in circulating estrogen concentration during the menopausal transition is accompanied by unfavorable changes to CVD risk factors such as body fat distribution, blood pressure, and blood lipid levels [10-16] and, is considered, thereby, to trigger vascular ageing [17]. However, this model has been challenged by the finding of no CVD risk reduction, and possibly even an increased risk [18], following exogenous menopausal hormone therapy (MHT). This inconsistency led us to consider the converse model, i.e., that cardiovascular damage itself is a driving factor in the process of ovarian ageing. This model is indirectly supported by two studies. In the Framingham Heart Study, Kok et al. found premenopausal cardiovascular risk factors were associated with younger age at menopause [19, 20]. Another study reported women who experienced early natural menopause were more often smokers, had diabetes, and had higher average body mass index (BMI) [21]. If premenopausal CVD risk factors are associated with women's age at natural menopause, the question that follows is whether premenopausal CVD events might also be linked to reproductive ageing and early age at natural menopause. To date, no study has examined this question directly. As premenopausal CVD events are rare, a study with a large sample size is required to answer this question with adequate precision.

To this end we pooled participant-level data from multiple studies in the International collaboration for a Life course Approach to reproductive health and Chronic disease Events (InterLACE) [22, 23]. We examined the association between premenopausal CVD events and age at natural menopause with detailed adjustment for confounding by race/ethnicity, education, BMI, smoking, hypertension, family history of CVD and other reproductive factors.

METHODS

Study participants

InterLACE combines 25 observational, mostly longitudinal cohort studies with data on women's health. A more detailed description of the InterLACE collaboration has been published previously [23, 22]. In brief, participating studies collected retrospective as well as prospective data on key reproductive, sociodemographic, lifestyle and disease outcome variables using self-reported surveys.

There were 177 750 women who had reported their age at natural menopause and provided information on pre- or post-menopausal CVD events (yes/no) and their age at onset of the CVD event. Because we focused on early premenopausal CVD events, women who experienced premenopausal CVD events after age 50 years (the average age at menopause in this study) were excluded (n=619). The final sample consisted of the 177 131 women who had either experienced no premenopausal CVD event (the reference group) or had experienced a premenopausal CVD event before age 50 years, and had complete data on key covariates at baseline including BMI, smoking status, education level, race/ethnicity, and parity. Consequently, nine studies were included in the analyses (Table 1).

Outcome and exposure variables

Age at natural menopause was the outcome variable and was defined as the time when a woman has experienced 12 consecutive months of amenorrhea which was not due to surgery (such as bilateral oophorectomy or hysterectomy). For some women, use of MHT and oral contraceptive pills (OCPs) made it difficult to ascertain their menopausal status; hence MHT or OCP users were excluded unless their age at natural menopause had been reported and the assumption of only post-menopausal MHT use could be made. Age at menopause was categorised as <45 (early menopause), 45-49, 50-51 (reference category), 52-53, and 54 years and above (late menopause), according to the clinical recommendation [4] and also as defined in our previous papers [24, 25].

CVD events were acertained by self-report or/and hospital diagnosis, and were defined as the occurrence of coronary heart disease (CHD, including heart attack and angina) or stroke (including ischemic strokes and haemorrhagic strokes). The exposure variable was the age at onset of premenopausal CVD events, and was categorized as < 35, 35-39, and ≥ 40 years. We used 35 years as a cut-off point because patients with CVD onset before age 35 years were referred as "very young CVD" and might be genetic predisposed [26, 27]. Also, these CVD events fall into the optimal period of childbearing age [28, 29]. Women who experienced no premenopausal CVD event were used as the reference group.

Covariates

BMI, smoking status, years of education, race/ethnicity/region, parity and age at menarche collected at baseline were used as covariates. BMI was categorised according to World Health Organization (WHO) criteria as <18.5 kg/m^2, 18.5 to 24.9 kg/m^2, 25 to 29.9 kg/m^2 and ≥30 kg/m^2. Smoking status was categorised as current,

former, or never smokers. Years of education was categorised as follows: ≤10, 11-12, and >12 years. Race/ethnicity/region was combined into one with four categories: Caucasian, Asian, African American/Black, and other. Parity was grouped as no children, one child, two, and three or more children. Age at menarche was divided into 5 categories as ≤11, 12, 13, 14, and 15 years or more.

Statistical analysis

We used multinomial (polytomous) logistic regression models to examine the associations between age at onset of premenopausal CVD events and age at natural menopause. CVD events were analysed both as a composite event and for CHD and stroke separately. For the outcome variable, women with an age at menopause of 50-51 years were used as the reference group, while for the exposure variable, women who had not experienced premenopausal CVD were the reference group. All models were adjusted for BMI, smoking status, education level, race/ethnicity and parity.

Multivariable relative risk ratios (RRR) [30] and 95% confidence intervals (95% CI) were used to quantify the association between age at onset of premenopausal CVD events and age at menopause. Because age at menarche is a potential confounder of the CVD-menopause association, it was later included in the model. For this analysis only eight studies were included because age at menarche was not available for the WHITEHALL II study.

We conducted several sensitivity analyses to test the robustness of our findings. First, to address the validity of the self-reported CVD events, we only included CVD cases that had a hospital record of diagnosis. Second, because the UK Biobank data contributed more than 50% of the total premenopausal CVD cases, we conducted an analysis excluding this study to assess its dominance. Third, women who experienced

postmenopausal CVD events may have had unfavourable CVD risk profile before menopause, which might have led to an earlier menopause.[19] Thus, we excluded them from the reference group. Fourth, to guarantee the temporal direction from premenopausal CVD events to menopause, we performed an analysis by only including premenopausal CVD events which occurred at least two years before menopause. Fifth, smoking and BMI are two important factors that may influence age at menopause [31, 24]. We thus analysed the combined effects of premenopausal CVD events and smoking status, premenopausal CVD events and BMI levels on age at menopause. Sixth, because a previous study had found an association between premenopausal blood pressure and earlier age at menopause [19], we also adjusted for hypertension status before the premenopausal CVD event in the four studies with available information (MCCS, WLH, JNHS, and UK Biobank). Last, we adjusted for family history of CVD using the five studies (MCCS, NHSD, WHITEHALL II, JNHS, and UK Biobank) with relevant information.

We used the SURVEYLOGISTIC procedure in SAS software (SAS Version 9.4, SAS Institute Inc, 2008.) with the generalized logit link to adjust for the clustering of data within studies, and to obtain robust standard errors. For all hypothesis tests we used the two-sided 5% level of significance.

Ethics

Each study in the InterLACE consortium has been undertaken with ethical approval from the Institutional Review Board or Human Research Ethics Committee at each participating institution, and all participants provided consent for that study.

RESULTS

Study characteristics

Overall, nine studies (177 131 women) had data on premenopausal CVD events. The majority of women were white (85.0%). The mean age (standard deviation, SD) at baseline was 57.8 (7.1) years and ranged from 45.0 (3.5) to 60.1 (9.4) years within studies. Over half of the participants were born between 1940 and 1949 (Table 1). There were 1561 women with premenopausal CVD events (including 1130 CHD and 469 stroke). The overall prevalence of premenopausal CVD was 0.9%. The overall mean age at natural menopause was 50.3 (4.4) years and the mean age at first premenopausal CVD event was 41.3(8.2) years (median 44.0 years, interquartile range 38.0-47.0years). The mean age at natural menopause by age categories of premenopausal CVD <35, 35-39, and ≥40 years were 49.2 (5.3), 49.4 (4.3) and 51.4 (3.3) years respectively. Early age at natural menopause was more common for women with premenopausal CVD events occurring before the age of 35 years than for other groups (Table 2, Figure 1).

Association between premenopausal CVD events and age at menopause

Compared with women who experienced no premenopausal CVD events, women experiencing a first event before the age of 35 years had around a 2-fold increased risk of early age (<45 years) at menopause with adjusted RRR (95%CI) 1.92 (1.17, 3.14) for CVD, 1.86 (1.01, 3.43) for CHD and 2.17 (1.43, 3.30) for stroke. There was a significant increasing trend of the associations between premenopausal CHD (<35 years) and earlier age at menopause (P-trend<0.001), while the trend with premenopausal stroke was not significant (P-trend>0.05). Women experiencing first premenopausal CVD events when they were aged more than 35 or 40 years were less likely to experience either earlier (45-49 years) or later age at natural menopause (52 years or more) (Table 3), i.e., they were more likely to experience natural menopause at around 51 years of age (Table 2). For women who experienced premenopausal

stroke before age 35 years, a statistically significant association was also found with late age at menopause (≥54 years) (1.45, 1.10-1.91) (Table 3).

Sensitivity analyses

When only CVD events with a hospital record of diagnosis were included in the analysis, we found results in a similar direction to those from the main analysis. Nevertheless, the association between premenopausal CHD events (<35 years) and early menopause, and the association between premenopausal stroke (<35 years) and late menopause were attenuated and no longer statistically significant (Table 4). Results were also similar when the UK Biobank study was excluded (Table 5) or when women who had experienced a postmenopausal CVD event were excluded from the reference group (Table S1). By including only premenopausal CVD events which occurred at least two years before menopause, similar results were observed (Table S 2). After analysing the combined effect with smoking and BMI, we found the significant associations between CVD events <35 years and early menopause were mainly observed in ever smokers and in women who were normal weight (Table S3 and S4). Similar results were also obtained when the analysis was further adjusted for hypertension prior to CVD (Table S5). After the adjustments for family history of CVD, only the association with CVD events was statistically significant, although the point estimates were not changed (Table S6).

DISCUSSION

Our results show that compared with women who had not experienced any premenopausal CVD event, women experiencing CHD or stroke before age 35 years had twice the risk of having an early menopause (<45 years) rather than a late menopause (≥54 years), while women who first experienced premenopausal CVD

events at age 40 years or older were more likely to have menopause at the average age of 50 to 51years.

The very young premenopausal CVD events

Coronary atherosclerosis begins at a young age with an estimated prevalence of 28% under 30 years of age [32]. The prevalence and extent of lesions increases rapidly during the 15 to 34 year age span [33]. Patients with symptomatic CVD onset before 35 years are at times referred as "very young CVD" [26, 27]. Around 1.5% of all documented CHD cases occur among individuals less than 35 years of age, predominantly in males [27, 34]. Younger patients have relatively few traditional risk factors such as diabetes mellitus, hypertension, and hyperlipidemia although smoking and family history of CVD have been found to be common [26, 35, 34]. Within the InterLACE consortium the prevalence of family history of CVD was also significantly higher for women with premenopausal CVD events than those without (78% vs. 60%) suggesting an inherited genetic predisposition to CVD in young cases.

Mechanisms underlying the link between premenopausal CVD events and age at menopause

Genetics plays an important role in age at natural menopause, with estimates of heritability ranging from 31% to 87% [36]. The genetic regions associated with premature or early-onset menopause may also tie to the occurrence of CVD [11]. Thus, our observation of a significant association between "very young CVD" and early menopause may arise due to shared genetic factors. Single nucleotide polymorphisms in several vascular-function-related genes are significantly associated also with age at menopause [36]. The coagulation Factor V Leiden gene, the methylene tetrahydrofolate reductase gene and the Apolipoprotein E gene have all

been linked to earlier age at menopause [37-40], whereas the coagulation factor VII gene is related to delayed menopause [41].

An interplay between genetic and environmental factors that may expedite the compromise of vascular health and advance ovarian ageing is also conceivable [37], as well as shared environmental factors. Smoking, for example, is common in those who experience very young CVD events and is also associated with early menopause [26, 34, 42]. Smokers carrying single nucleotide polymorphisms CYP3A4*1B and CYP1B1*3 have a greater risk of menopause commencement compared with those not carrying these variants [43]. Smoking also induces the expression of the apoptosis-promoting gene Bcl2-associated X protein in oocytes leading to an increased rate of oocyte apoptosis, and thus earlier ovarian failure [44].

Vascular and ovarian ageing are connected [45]. Coronary disease occurring at a young age may carry a long-term adverse influence on the vasculature [35]. Vascular damage, in turn, may accelerate ovarian ageing and thus lead to early menopause [36, 45]. Additionally, fertility often starts declining at age 35 years [28, 29]. Hence, CVD events that occur before age 35 years fall into the optimal period of childbearing age (the average age at onset of premenopausal CVD in those aged ≤ 35 years' was 27.0 years in our study) [29]. It is possible that CVD occurring at optimal reproductive age may affect maternal vascular health in the long term and accelerate the process of reproductive ageing. Although we found no studies evaluating the relationship between damage in large vessels and ovarian ageing, microvascular complications in women with type 1 diabetes have been suggested to accelerate ovarian ageing [46, 26]. Our study also found that premenopausal stroke had a stronger association with early menopause than CHD suggesting that a damaged cerebrovascular system is a more

sensitive marker of ovarian ageing. Further studies are needed to verify this proposition.

Smoking leads to early menopause and overweight/obese has been linked to later menopause [31, 24]. Our results showed the significant associations between CVD events <35 years and early menopause were mainly observed in ever smokers and women with normal weight. In addition, the significant association between premenopausal stroke before age 35 years and late menopause (≥54 years) were only observed in women with overweight or obesity, which has been associated with both stroke and late menopause [24].

We also found that women with first CHD or stroke after age 35 years were less likely to experience either earlier (45-49 years) or later natural menopause (52 years or more). The average age at menopause in this group was 51.4 years (Table 2), similar to the mean age at menopause reported in high-income countries [2, 3]. Thus, they were more likely to experience menopause at the 'usual' age. .The median age of premenopausal CVD in this group was 46 years, which means the downstream effect of vascular damage on ovarian ageing is limited. Ovarian ageing in this group to a large extent reflects natural ageing.

Strengths and limitations

To the best of our knowledge, the link between premenopausal CVD events and timing of menopause has remained untested [47]. The strengths of the current study include participant-level data from nine studies which provided sufficient number of CVD cases to examine in detail the association between premenopausal CVD and the multiple categories of age at menopause.

317 Several limitations need also to be acknowledged. First, around 47% of
318 premenopausal CVD events were self-reported without validation by hospital records.
319 This may have led to some degree of misclassification but findings were reassuringly
320 consistent in the sensitivity analysis that used only hospital ascertained cases. Second,
321 we used the BMI and smoking status values reported at baseline as covariates, which
322 may not reflect their values proximal to the onset of premenopausal CVD. Women
323 with early CVD may have modified their lifestyle resulting in changed BMI and
324 smoking status before menopause. On the other hand, over 50% of women
325 experienced premenopausal CVD events when aged in their mid-forties or later. For
326 these women, we assume that the misclassification in reported BMI or smoking is
327 limited. For women who had experienced very young CVD (<35 years), their BMI
328 level prior to CVD events might not have been an important risk factor. In two birth
329 cohort studies (NSHD and NCDS) in the InterLACE consortium that also collected
330 BMI and smoking at younger age, the average BMI before 35 years (26-35 years) was
331 22.4-24.4 kg/m^2, and the concordance in smoking status between age 26 years and
332 baseline age (mid age) was 84%. Third, due to the limited number of cases, we were
333 unable to perform subgroup analysis between sub-types of CHD (angina and heart
334 attack) and age at menopause. Also, most studies did not collect specific types of
335 stroke, so we could not separate the hemorrhagic strokes from ischemic strokes. The
336 associations between different sub-types of stroke with age at menopause may differ
337 due to their dissimilar biological mechanisms. However, approximately 87% strokes
338 are ischemic [48]. Thus, we believe the bias caused by hemorrhagic strokes was
339 limited. Data that were collected from four countries might have heterogeneity among
340 them. However, after performing country-specific random-effects meta-analysis, we
341 found no significant heterogeneity between studies ($p>0.05$) (data not shown). Last,

because the majority of women were white (Caucasian), our results may need to be verified in other race/ethnicities.

Conclusions

Premenopausal CVD before age 35 years is associated with a higher risk of menopause before age 45 years, while premenopausal CVD after 35 years indicates a normal menopause at around 51 years. Shared genetic and environmental factors (such as smoking), as well as compromised vasculature after CVD events may contribute to this health outcome. Further studies that include measures of vascular damage are needed to examine its possible relationship with age at natural menopause. Additionally, women experiencing a CVD event prior to age 35 years should be alerted for their future high possibility of having early menopause.

Acknowledgments

The data on which this research is based were drawn from 9 observational studies. The research included data from the ALSWH, the University of Newcastle, Australia, and the University of Queensland, Australia. We are grateful to the Australian Government Department of Health for funding and to the women who provided the survey data. MCCS was supported by VicHealth and the Cancer Council, Victoria, Australia. WLHS was funded by a grant from the Swedish Research Council (Grant number 521-2011-2955). NSHD has core funding from the UK Medical Research Council (MC UU 12019/1). NCDS is funded by the Economic and Social Research Council. ELSA is funded by the National Institute on Aging (Grants 2RO1AG7644 and 2RO1AG017644-01A1) and a consortium of UK government departments. The Whitehall II study has been supported by grants from the Medical Research Council. Baseline survey of the JNHS was supported in part by a Grant-in-Aid for Scientific

Research (B: 14370133, 18390195) from the Japan Society for the Promotion of Science, and by the grants from the Japan Menopause Society. This research has been conducted using the UK Biobank resource under application 26629.

All studies would like to thank the participants for volunteering their time to be involved in the respective studies. The findings and views in this paper are not necessarily those of the original studies or their respective funding agencies.

Author's contribution

GDM and DZ conceptualized the study. GDM interpreted the results, and revised the manuscript critically. DZ analysed and interpreted data, and drafted the manuscript. HFC and NP harmonised the data and revised the manuscript. AJD, RH, DK, EJB, FB, GGG, PD, JSL, HM, KH, HOA, EW provided study data and revised the manuscript.

Funding

InterLACE project is funded by the Australian National Health and Medical Research Council project grant (APP1027196). GDM is supported by Australian National Health and Medical Research Council Principal Research Fellowship (APP1121844). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

References

1. Harlow SD, Gass M, Hall JE, Lobo R, Maki P, Rebar RW et al. Executive summary of the Stages of Reproductive Aging Workshop+ 10: addressing the unfinished agenda of staging reproductive aging. The Journal of Clinical Endocrinology & Metabolism. 2012;97(4):1159-68.
2. Morabia A, Costanza MC. International variability in ages at menarche, first livebirth, and menopause. World Health Organization Collaborative Study of Neoplasia and Steroid Contraceptives. Am J Epidemiol. 1998;148(12):1195-205.
3. Gold EB, Crawford SL, Avis NE, Crandall CJ, Matthews KA, Waetjen LE et al. Factors related to age at natural menopause: longitudinal analyses from SWAN. Am J Epidemiol. 2013;178(1):70-83. doi:10.1093/aje/kws421.
4. Shifren JL, Gass ML, NAMS Recommendations for Clinical Care of Midlife Women Working Group. The North American Menopause Society recommendations for clinical care of midlife women. Menopause (New York, NY). 2014;21(10):1038-62. doi:10.1097/GME.0000000000000319.
5. Muka T, Oliver-Williams C, Kunutsor S, Laven JS, Fauser BC, Chowdhury R et al. Association of Age at Onset of Menopause and Time Since Onset of Menopause With Cardiovascular Outcomes, Intermediate Vascular Traits, and All-Cause Mortality: A Systematic Review and Meta-analysis. JAMA Cardiol. 2016;1(7):767-76. doi:10.1001/jamacardio.2016.2415.
6. Lisabeth LD, Beiser AS, Brown DL, Murabito JM, Kelly-Hayes M, Wolf PA. Age at natural menopause and risk of ischemic stroke: the Framingham heart study. Stroke. 2009;40(4):1044-9. doi:10.1161/strokeaha.108.542993.
7. Rocca WA, Grossardt BR, Miller VM, Shuster LT, Brown RD, Jr. Premature menopause or early menopause and risk of ischemic stroke. Menopause (New York, NY). 2012;19(3):272-7. doi:10.1097/gme.0b013e31822a9937.
8. Atsma F, Bartelink ML, Grobbee DE, van der Schouw YT. Postmenopausal status and early menopause as independent risk factors for cardiovascular disease: a meta-analysis. Menopause (New York, NY). 2006;13(2):265-79. doi:10.1097/01.gme.0000218683.97338.ea.
9. Gong D, Sun J, Zhou Y, Zou C, Fan Y. Early age at natural menopause and risk of cardiovascular and all-cause mortality: A meta-analysis of prospective observational studies. International journal of cardiology. 2016;203:115-9. doi:10.1016/j.ijcard.2015.10.092.
10. Matthews KA, Kuller LH, Sutton-Tyrrell K, Chang YF. Changes in cardiovascular risk factors during the perimenopause and postmenopause and carotid artery atherosclerosis in healthy women. Stroke. 2001;32(5):1104-11.
11. McNally E. Reproductive Aging and Cardiovascular Disease Risk. JAMA Cardiol. 2016;1(7):778. doi:10.1001/jamacardio.2016.2638.
12. Woodard GA, Brooks MM, Barinas-Mitchell E, Mackey RH, Matthews KA, Sutton-Tyrrell K. Lipids, menopause, and early atherosclerosis in Study of Women's Health Across the Nation Heart women. Menopause (New York, NY). 2011;18(4):376-84. doi:10.1097/gme.0b013e3181f6480e.
13. Park JK, Lim YH, Kim KS, Kim SG, Kim JH, Lim HG et al. Changes in body fat distribution through menopause increase blood pressure independently of total body fat in middle-aged women: the Korean National Health and Nutrition Examination Survey 2007-2010. Hypertens Res. 2013;36(5):444-9. doi:10.1038/hr.2012.194.
14. Tchernof A, Poehlman ET. Effects of the menopause transition on body fatness and body fat distribution. Obes Res. 1998;6(3):246-54.
15. Son MK, Lim NK, Lim JY, Cho J, Chang Y, Ryu S et al. Difference in blood pressure between early and late menopausal transition was significant in healthy Korean women. BMC Womens Health. 2015;15:64. doi:10.1186/s12905-015-0219-9.
16. Moreau KL, Hildreth KL. Vascular Aging across the Menopause Transition in Healthy Women. Adv Vasc Med. 2014;2014. doi:10.1155/2014/204390.

17. Hardy R, Lawlor DA, Kuh D. A life course approach to cardiovascular aging. Future Cardiol. 2015;11(1):101-13. doi:10.2217/fca.14.67.
18. Writing Group for the Women's Health Initiative Investigators. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: principal results from the Women's Health Initiative randomized controlled trial. Jama. 2002;288(3):321-33.
19. Kok HS, van Asselt KM, van der Schouw YT, van der Tweel I, Peeters PH, Wilson PW et al. Heart disease risk determines menopausal age rather than the reverse. J Am Coll Cardiol. 2006;47(10):1976-83. doi:10.1016/j.jacc.2005.12.066.
20. Bittner V. Menopause and cardiovascular risk cause or consequence? J Am Coll Cardiol. 2006;47(10):1984-6. doi:10.1016/j.jacc.2006.02.032.
21. Wellons M, Ouyang P, Schreiner PJ, Herrington DM, Vaidya D. Early menopause predicts future coronary heart disease and stroke: the Multi-Ethnic Study of Atherosclerosis. Menopause (New York, NY). 2012;19(10):1081-7. doi:10.1097/gme.0b013e3182517bd0.
22. Mishra GD, Anderson D, Schoenaker DA, Adami H-O, Avis NE, Brown D et al. InterLACE: a new international collaboration for a life course approach to women's reproductive health and chronic disease events. Maturitas. 2013;74(3):235-40.
23. Mishra GD, Chung H-F, Pandeya N, Dobson AJ, Jones L, Avis NE et al. The InterLACE study: Design, data harmonization and characteristics across 20 studies on women's health. Maturitas. 2016;92:176-85.
24. Zhu D, Chung HF, Pandeya N, Dobson AJ, Kuh D, Crawford SL et al. Body mass index and age at natural menopause: an international pooled analysis of 11 prospective studies. Eur J Epidemiol. 2018;33(8):699-710. doi:10.1007/s10654-018-0367-y.
25. Mishra GD, Pandeya N, Dobson AJ, Chung HF, Anderson D, Kuh D et al. Early menarche, nulliparity and the risk for premature and early natural menopause. Hum Reprod. 2017. doi:10.1093/humrep/dew350.
26. Christus T, Shukkur AM, Rashdan I, Koshy T, Alanbaei M, Zubaid M et al. Coronary Artery Disease in Patients Aged 35 or less - A Different Beast? Heart Views. 2011;12(1):7-11. doi:10.4103/1995-705X.81550.
27. Wolfe MW, Vacek JL. Myocardial infarction in the young. Angiographic features and risk factor analysis of patients with myocardial infarction at or before the age of 35 years. Chest. 1988;94(5):926-30.
28. Medicine PCotASfR. Age-related fertility decline: a committee opinion. Fertility and sterility. 2008;90(5):S154-S5.
29. Te Velde E, Dorland M, Broekmans F. Age at menopause as a marker of reproductive ageing. Maturitas. 1998;30(2):119-25.
30. Borooah VK. Logit and probit: Ordered and multinomial models. vol 138. Sage; 2002.
31. Zhu D, Chung HF, Pandeya N, Dobson AJ, Cade JE, Greenwood DC et al. Relationships between intensity, duration, cumulative dose, and timing of smoking with age at menopause: A pooled analysis of individual data from 17 observational studies. PLoS Med. 2018;15(11):e1002704. doi:10.1371/journal.pmed.1002704.
32. Tuzcu EM, Kapadia SR, Tutar E, Ziada KM, Hobbs RE, McCarthy PM et al. High prevalence of coronary atherosclerosis in asymptomatic teenagers and young adults: evidence from intravascular ultrasound. Circulation. 2001;103(22):2705-10.
33. Strong JP, Malcom GT, McMahan CA, Tracy RE, Newman WP, 3rd, Herderick EE et al. Prevalence and extent of atherosclerosis in adolescents and young adults: implications for prevention from the Pathobiological Determinants of Atherosclerosis in Youth Study. JAMA. 1999;281(8):727-35.
34. Kalimuddin M, Ahmed N, Badiuzzzaman M, Ahmed MN, Dutta A, Banik D et al. AMI in very young (aged⩽ 35 years) Bangladeshi patients: Risk factors & coronary angiographic profile. Clinical Trials and Regulatory Science in Cardiology. 2016;13:1-5.

35. Cole JH, Miller JI, 3rd, Sperling LS, Weintraub WS. Long-term follow-up of coronary artery disease presenting in young adults. J Am Coll Cardiol. 2003;41(4):521-8.
36. Voorhuis M, Onland-Moret NC, van der Schouw YT, Fauser BC, Broekmans FJ. Human studies on genetics of the age at natural menopause: a systematic review. Hum Reprod Update. 2010;16(4):364-77. doi:10.1093/humupd/dmp055.
37. van Asselt KM, Kok HS, Peeters PH, Roest M, Pearson PL, te Velde ER et al. Factor V Leiden mutation accelerates the onset of natural menopause. Menopause (New York, NY). 2003;10(5):477-81. doi:10.1097/01.GME.0000056040.51813.1A.
38. Tempfer CB, Riener EK, Keck C, Grimm C, Heinze G, Huber JC et al. Polymorphisms associated with thrombophilia and vascular homeostasis and the timing of menarche and menopause in 728 white women. Menopause (New York, NY). 2005;12(3):325-30.
39. Liu P, Lu Y, Recker RR, Deng HW, Dvornyk V. Association analyses suggest multiple interaction effects of the methylenetetrahydrofolate reductase polymorphisms on timing of menarche and natural menopause in white women. Menopause (New York, NY). 2010;17(1):185-90. doi:10.1097/gme.0b013e3181aa2597.
40. He LN, Recker RR, Deng HW, Dvornyk V. A polymorphism of apolipoprotein E (APOE) gene is associated with age at natural menopause in Caucasian females. Maturitas. 2009;62(1):37-41. doi:10.1016/j.maturitas.2008.10.011.
41. van Disseldorp J, Broekmans FJ, Peeters PH, Fauser BC, van der Schouw YT. The association between vascular function-related genes and age at natural menopause. Menopause (New York, NY). 2008;15(3):511-6. doi:10.1097/gme.0b013e31814cec52.
42. Parente RC, Faerstein E, Celeste RK, Werneck GL. The relationship between smoking and age at the menopause: A systematic review. Maturitas. 2008;61(4):287-98. doi:10.1016/j.maturitas.2008.09.021.
43. Butts SF, Sammel MD, Greer C, Rebbeck TR, Boorman DW, Freeman EW. Cigarettes, genetic background, and menopausal timing: the presence of single nucleotide polymorphisms in cytochrome P450 genes is associated with increased risk of natural menopause in European-American smokers. Menopause (New York, NY). 2014;21(7):694-701. doi:10.1097/GME.0000000000000140.
44. Dechanet C, Anahory T, Mathieu Daude JC, Quantin X, Reyftmann L, Hamamah S et al. Effects of cigarette smoking on reproduction. Hum Reprod Update. 2011;17(1):76-95. doi:10.1093/humupd/dmq033.
45. Yarde F. Advanced Ovarian Ageing: Studies on Fertility and Vascular Health: Utrecht University; 2014.
46. Sjoberg L, Pitkaniemi J, Harjutsalo V, Haapala L, Tiitinen A, Tuomilehto J et al. Menopause in women with type 1 diabetes. Menopause (New York, NY). 2011;18(2):158-63. doi:10.1097/gme.0b013e3181ef3af0.
47. Manson JE, Woodruff TK. Reproductive Health as a Marker of Subsequent Cardiovascular Disease: The Role of Estrogen. JAMA Cardiol. 2016;1(7):776-7. doi:10.1001/jamacardio.2016.2662.
48. Rosamond W, Flegal K, Furie K, Go A, Greenlund K, Haase N et al. Heart disease and stroke statistics--2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Circulation. 2008;117(4):e25-146. doi:10.1161/CIRCULATIONAHA.107.187998.

Table 1. Characteristics of women in each study of the InterLACE consortium [a]

Study	Country	N	Age at baseline, Mean (SD)	Age at last follow-up, Mean (SD)	Women's year of birth (%)				
					<1930	1930-1939	1940-1949	1950-1959	1960+
Australian Longitudinal Study on Women's Health (ALSWH)	Australia	7061	47.6 (1.4)	63.2 (3.3)	.	.	74.8	25.2	.
Melbourne Collaborative Cohort Study (MCCS)	Australia	12 814	58.7 (7.2)	67.9 (7.6)	35.6	42.6	19.8	2.0	.
Women's Lifestyle and Health Study (WLHS)	Sweden	10 659	45.0 (3.5)	55.8 (3.7)	.	.	77.0	22.7	0.3
MRC National Survey of Health and Development (NSHD) [b]	UK	631	47.0	53.9	.	.	100	.	.
National Child Development Study (NCDS) [b]	UK	2407	50.0	54.8	.	.	.	100	.
English Longitudinal Study of Ageing (ELSA)	UK	3595	60.1 (9.4)	68.7 (9.8)	16.4	25.6	35.8	22.1	0.2
Whitehall II study (WHITEHALL II)	UK	1460	46.0 (5.8)	64.8 (5.9)	.	46.4	46.8	6.7	.
Japan Nurse's Health Study (JNHS)	Japan	4933	54.7 (3.9)	54.7 (3.9)	.	1.5	63.6	34.2	0.7
UK Biobank (UK Biobank)	UK	133 571	59.6 (5.6)	60.1 (5.5)	.	4.0	55.4	37.5	3.0
All		177 131	57.8 (7.1)	60.5 (6.3)	2.9	7.1	54.1	33.6	2.3

[a] In this study, the dataset included women who experienced premenopausal CVD events (including CHD and stroke) and had reported their age at onset of CVD events, and women who had no premenopausal CVD event (used as reference group). All women had complete information on age at natural menopause and key covariates.
[b] NSHD (1946 British Birth Cohort) and NCDS (1958 British Birth Cohort) first collected information on women's health in 1993 (aged 47) and 2008 (aged 50), respectively, so we used 1993 and 2008 as the baseline year for the InterLACE.
Abbreviations: InterLACE, International Collaboration for a Life Course Approach to Reproductive Health and Chronic Disease Events; SD, standard deviation; CVD, cardiovascular disease; CHD, coronary heart disease.

Table 2. Average age at onset of premenopausal CVD events, average age and distribution of natural menopause by age categories of premenopausal CVD/CHD/stroke events

	Number of premenopausal CVD/CHD/stroke events	Age at premenopausal CVD/CHD/stroke event		Age at natural menopause, Mean (SD)	Distribution of age at natural menopause				
		Mean (SD)	Median (Q1, Q3)		<45	45-49	50-51	52-53	≥54
Age at onset of premenopausal CVD events									
<35	287	27.0 (7.4)	29.4 (23.0, 33.0)	49.2 (5.3)	46 (16.0)	82 (28.6)	59 (20.6)	45 (15.7)	55 (19.2)
35-39	151	37.1 (1.4)	37.0 (36.0, 38.0)	49.4 (4.3)	16 (10.6)	45 (29.8)	45 (29.8)	23 (15.2)	22 (14.6)
≥40	1123	45.5 (2.9)	46.0 (43.0, 48.0)	51.4 (3.3)	17 (1.5)	240 (21.4)	358 (31.9)	240 (21.4)	268 (23.9)
No premenopausal CVD event	-	-	-	50.3 (4.4)	16 029 (9.1)	42 803 (24.4)	42 829 (24.4)	34 766 (19.8)	39 143 (22.3)
Age at onset of premenopausal CHD events									
<35	185	27.2 (8.0)	31.0 (24.0, 33.0)	48.9 (5.0)	29 (15.7)	56 (30.3)	38 (20.5)	34 (18.4)	28 (15.1)
≥35	945	44.7 (3.7)	45.0 (42.0, 48.0)	51.3 (3.4)	21 (2.2)	207 (21.9)	300 (31.7)	191 (20.2)	226 (23.9)
No premenopausal CHD event	-	-	-	50.3 (4.4)	16 037 (9.1)	42 863 (24.4)	42 929 (24.4)	34 839 (19.8)	39 301 (22.3)
Age at onset of premenopausal stroke									
<35	114	27.5 (6.5)	28.0 (24.0, 32.0)	49.6 (5.7)	19 (16.7)	28 (24.6)	22 (19.3)	15 (13.2)	30 (26.3)
≥35	355	44.2 (3.9)	45.0 (41.0, 48.0)	50.8 (3.4)	13 (3.7)	87 (24.5)	112 (31.5)	76 (21.4)	67 (18.9)
No premenopausal stroke	-	-	-	50.4 (4.4)	15 819 (9.1)	42 152 (24.1)	42 687 (24.5)	34 434 (19.7)	39 486 (22.6)

Abbreviations: CVD, cardiovascular disease; CHD, coronary heart disease; SD, standard deviation.

1

Table 3. Unadjusted and adjusted associations between age at premenopausal CVD/CHD/stroke events and age at natural menopause [a] (n= 177 131)

	Age at natural menopause: crude RRRs (95% CI)				Age at natural menopause: adjusted RRRs (95% CI) [b]			
	<45	45-49	52-53	≥54	<45	45-49	52-53	≥54
Age at onset of premenopausal CVD events								
<35	2.07 (1.29, 3.31)	1.36 (0.94, 1.96)	0.94 (0.69, 1.27)	1.05 (0.77, 1.52)	1.92 (1.17, 3.14)	1.30 (0.91, 1.85)	0.94 (0.70, 1.27)	1.05 (0.79, 1.41)
35-39	0.95 (0.69, 1.29)	0.99 (0.72, 1.35)	0.63 (0.50, 0.80)	0.54 (0.38, 0.76)	0.88 (0.65, 1.19)	0.95 (0.71, 1.29)	0.64 (0.50, 0.81)	0.54 (0.37, 0.79)
≥40 [c]	-	0.66 (0.54, 0.80)	0.82 (0.69, 0.98)	0.84 (0.71, 1.00)		0.62 (0.51, 0.75)	0.84 (0.71, 0.99)	0.85 (0.72, 0.99)
No premenopausal CVD event	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Age at onset of premenopausal CHD events								
<35	2.02 (1.14, 3.59)	1.43 (0.85, 2.39)	1.10 (0.85, 1.43)	0.83 (0.48, 1.45)	1.86 (1.01, 3.43)	1.34 (0.80, 2.23)	1.10 (0.84, 1.45)	0.86 (0.50, 1.47)
≥35 [c]	-	0.67 (0.50, 0.91)	0.78 (0.65, 0.94)	0.84 (0.69, 1.04)		0.64 (0.47, 0.86)	0.80 (0.67, 0.95)	0.85 (0.72, 1.01)
No premenopausal CHD event	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Age at onset of premenopausal stroke								
<35	2.33 (1.53, 3.54)	1.29 (0.86, 1.93)	0.85 (0.54, 1.33)	1.48 (1.13, 1.93)	2.17 (1.43, 3.30)	1.26 (0.84, 1.90)	0.85 (0.55, 1.33)	1.45 (1.10, 1.91)
≥35 [c]	-	0.78 (0.64, 0.95)	0.84 (0.70, 1.01)	0.65 (0.57, 0.74)		0.74 (0.60, 0.93)	0.85 (0.71, 1.02)	0.65 (0.57, 0.74)
No premenopausal stroke	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00

[a] Multinomial logistic regression model was used to estimate relative risk ratio (RRR) and 95% confidence interval (95% CI). The 50-51 age category was used as reference group for age at menopause.
[b] Body mass index, smoking status, years of education, race/ethnicity/region, number of children at baseline were adjusted.
[c] The average age for premenopausal CVD event in the ≥40 years group, or premenopausal CHD and stroke in the ≥35 years group were all around 45 (Table 2), which means women with subsequent early menopause were less likely to be included in these groups. So we use a "-" to represent their effect on early menopause.
Abbreviations: CVD, cardiovascular disease; CHD, coronary heart disease.

2

1

Table 4. The associations between age at premenopausal CVD/CHD/stroke events and age at natural menopause--Only cases with hospital diagnosed record were included [a] (n= 176 265)

	Age at menopause, n (%)					Adjusted RRRs (95% CI) [b]			
	<45	45-49	50-51	52-53	>=54	<45 [c]	45-49	52-53	>=54
Age of onset of premenopausal CVD events									
<35	18 (17.0)	28 (26.4)	24 (22.6)	14 (13.2)	22 (20.8)	1.73 (1.02, 3.00)	1.11 (0.88, 1.40)	0.73 (0.47, 1.15)	0.98 (0.89, 1.08)
≥35	19 (3.2)	145 (24.6)	172 (29.2)	123 (20.9)	130 (22.1)		0.80 (0.68, 0.94)	0.89 (0.71, 1.11)	0.80 (0.70, 0.91)
No premenopausal CVD event	16 029 (9.1)	42 803 (24.4)	42 829 (24.4)	34 766 (19.8)	39 143 (22.3)	1.00	1.00	1.00	1.00
Age of onset of premenopausal CHD events									
<35	10 (16.1)	18 (29.0)	15 (24.2)	10 (16.1)	9 (14.5)	1.53 (0.65, 3.61)	1.15 (0.69, 1.89)	0.84 (0.47, 1.52)	0.65 (0.48, 0.88)
≥35	15 (3.3)	103 (22.5)	131 (28.6)	96 (21.0)	113 (24.7)		0.75 (0.61, 0.92)	0.91 (0.72, 1.16)	0.90 (0.79, 1.02)
No premenopausal CHD event	16 037 (9.1)	42 863 (24.4)	42 929 (24.4)	34 839 (19.8)	39 301 (22.3)	1.00	1.00	1.00	1.00
Age of onset of premenopausal stroke									
<35	9 (21.4)	10 (23.8)	9 (21.4)	4 (9.5)	10 (23.8)	2.37 (1.53, 3.70)	1.09 (0.42, 2.80)	0.56 (0.25, 1.27)	1.17 (0.60, 2.30)
≥35	5 (3.7)	42 (30.9)	41 (30.1)	29 (21.3)	19 (14.0)		0.98 (0.73, 1.31)	0.88 (0.64, 1.22)	0.50 (0.30, 0.80)
No premenopausal stroke	15 819 (9.1)	42 152 (24.1)	42 687 (24.5)	34 434 (19.7)	39 486 (22.6)	1.00	1.00	1.00	1.00

[a] Multinomial logistic regression model was used to estimate relative risk ratio (RRR) and 95% confidence interval (95% CI). The 50-51 age category was used as reference group for age at menopause.
[b] Body mass index, smoking status, years of education, race/ethnicity/region, number of children at baseline were adjusted.
c The average age for premenopausal CVD event in the ≥40 years group, or premenopausal CHD and stroke in the ≥35 years group were all around 45 (Table 2), which means women with subsequent early menopause were less likely to be included in these groups. So we use a "-" to represent their effect on early menopause.
Abbreviations: CVD, cardiovascular disease; CHD, coronary heart disease.

2

Table 5. The associations between age at premenopausal CVD/CHD/stroke events and age at natural menopause—After excluding UK Biobank study [a]

	Age at menopause, n (%)					Adjusted RRRs (95% CI) [b]			
	<45	45-49	50-51	52-53	>=54	<45 [c]	45-49	52-53	>=54
Age of onset of premenopausal CVD events									
<35	29 (19.3)	49 (32.7)	26 (17.3)	25 (16.7)	21 (14.0)	2.82 (1.71, 4.63)	1.67 (1.19, 2.35)	1.17 (0.76, 1.81)	1.08 (0.55, 2.10)
≥35	12 (2.1)	126 (21.7)	202 (34.8)	115 (19.8)	126 (21.7)	-	0.56 (0.45, 0.69)	0.71 (0.58, 0.87)	0.82 (0.59, 1.12)
No premenopausal CVD event	3862 (9.0)	11 534 (26.9)	10606 (24.8)	8589 (20.1)	8238 (19.2)	1.00	1.00	1.00	1.00
Age of onset of premenopausal CHD events									
<35	22 (18.2)	42 (34.7)	21 (17.4)	20 (16.5)	16 (13.2)	2.65(1.48, 4.74)	1.76 (1.22, 2.55)	1.16 (0.70, 1.94)	1.05 (0.44, 2.52)
≥35	9 (1.8)	99 (20.3)	174 (35.7)	97 (19.9)	109 (22.3)	-	0.50 (0.41, 0.63)	0.70 (0.59, 0.83)	0.83 (0.61, 1.13)
No premenopausal CHD event	3872 (9.0)	11 568 (26.9)	10 639 (24.8)	8621 (20.1)	8285 (19.3)	1.00	1.00	1.00	1.00
Age of onset of premenopausal stroke									
<35	8 (26.7)	6 (20.0)	6 (20.0)	4 (13.3)	6 (20.0)	3.42 (1.07, 10.9)	0.95 (0.25, 3.55)	0.86 (0.16, 4.44)	1.20 (0.52, 2.74)
≥35	3 (2.6)	34 (29.8)	36 (31.6)	21 (18.4)	20 (17.5)	-	0.89 (0.55, 1.42)	0.73 (0.44, 1.24)	0.65 (0.44, 0.96)
No premenopausal stroke	3651 (8.9)	10 787 (26.2)	10 329 (25.1)	8113 (19.7)	8266 (20.1)	1.00	1.00	1.00	1.00

[a] Multinomial logistic regression model was used to estimate relative risk ratio (RRR) and 95% confidence interval (95% CI). The 50-51 age category was used as reference group for age at menopause.
[b] Body mass index, smoking status, years of education, race/ethnicity/region, number of children, age at menarche at baseline.
[c] The average age for premenopausal CVD event in the ≥40 years group, or premenopausal CHD and stroke in the ≥35 years group were all around 45 (Table 2), which means women with subsequent early menopause were less likely to be included in these groups. So we use a "-" to represent their effect on early menopause.
Abbreviations: CVD, cardiovascular disease; CHD, coronary heart disease.

Figure legends

Fig. 1 Distribution of age at menopause in different age categories of premenopausal CVD/CHD/stroke events. Abbreviations: CVD, cardiovascular disease; CHD, coronary heart disease.