Statistical Analysis Plan:

Association of Premature Natural and Surgical Menopause with Incident Cardiovascular Risk Factors and Cardiovascular Disease

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I. Specific aims

1. To examine the association of premature menopause, defined as menopause prior to age 40 (per recent guidelines), with incidence of diverse cardiovascular diseases, and to compare the risks associated with premature natural vs. surgical menopause.
    a. **Hypothesis:** Menopause prior to age 40 is associated with hazard of diverse cardiovascular conditions, after adjustment for conventional cardiovascular risk factors.
    b. **Hypothesis:** Premature surgical menopause is associated with greater cardiovascular risk than premature natural menopause.
2. To examine the association of premature menopause with the development of cardiovascular risk factors (hypertension, hyperlipidemia, and type 2 diabetes).
    a. **Hypothesis:** Premature menopause is associated with accelerated development of these conventional cardiovascular risk factors.
3. To examine the association of alternate menopausal age thresholds with the development of cardiovascular risk factors and cardiovascular disease.
    a. **Hypothesis:** Progressively lower age at menopause is associated with progressive increase in cardiovascular risk.

II. Research strategy

a. Significance:

Although cardiovascular disease is the leading cause of death among women in the U.S. and worldwide,[1] sex-specific risk factors for cardiovascular disease in women

remain under-recognized and incompletely understood.[2] Most women in North America experience menopause between age 40 and 58, with a mean age at menopause of 51 years.[3,4] Up to 10% of women undergo menopause before age 45,[5] and 1% experience menopause before age 40 (frequently labeled primary ovarian insufficiency when occurring spontaneously).[3,6-8] Although "premature menopause" has been variably defined in the literature with typical cutoffs ranging from 45-48 years, earlier age at menopause and shorter duration of reproductive lifespan (i.e., time from menarche to menopause) have been associated with increased risk of coronary artery disease and, less consistently, with increased risk of stroke.[9-15] Additionally, a study examining a subset of the Women's Health Initiative cohort found a modest association between reproductive duration and risk of incident heart failure.[16]

Based on these data and others,[9,12] recent updates to the ACC/AHA cholesterol[17] and primary prevention[18] guidelines endorse using a history of premature menopause, defined in the guidelines as menopause prior to age 40, to refine cardiovascular risk assessments and guide prescription of statin medications for asymptomatic, middle-aged women with intermediate risk of atherosclerotic cardiovascular disease (ASCVD). However, robust data are limited on the development of cardiovascular risk factors among women with menopause before age 40 and the long-term risk of both ASCVD and non-ASCVD cardiovascular diseases in this population. To date, one meta-analysis including 10 studies with 190,000 women assessed the risk of ASCVD associated with natural menopause prior to age 40 and found modestly elevated hazard of coronary artery disease but not of stroke.[12] Further, data are inconsistent on whether cardiovascular

disease risk differs between women with premature natural and surgical menopause,[19] though recent large studies have not shown a difference in risk.[9,10]

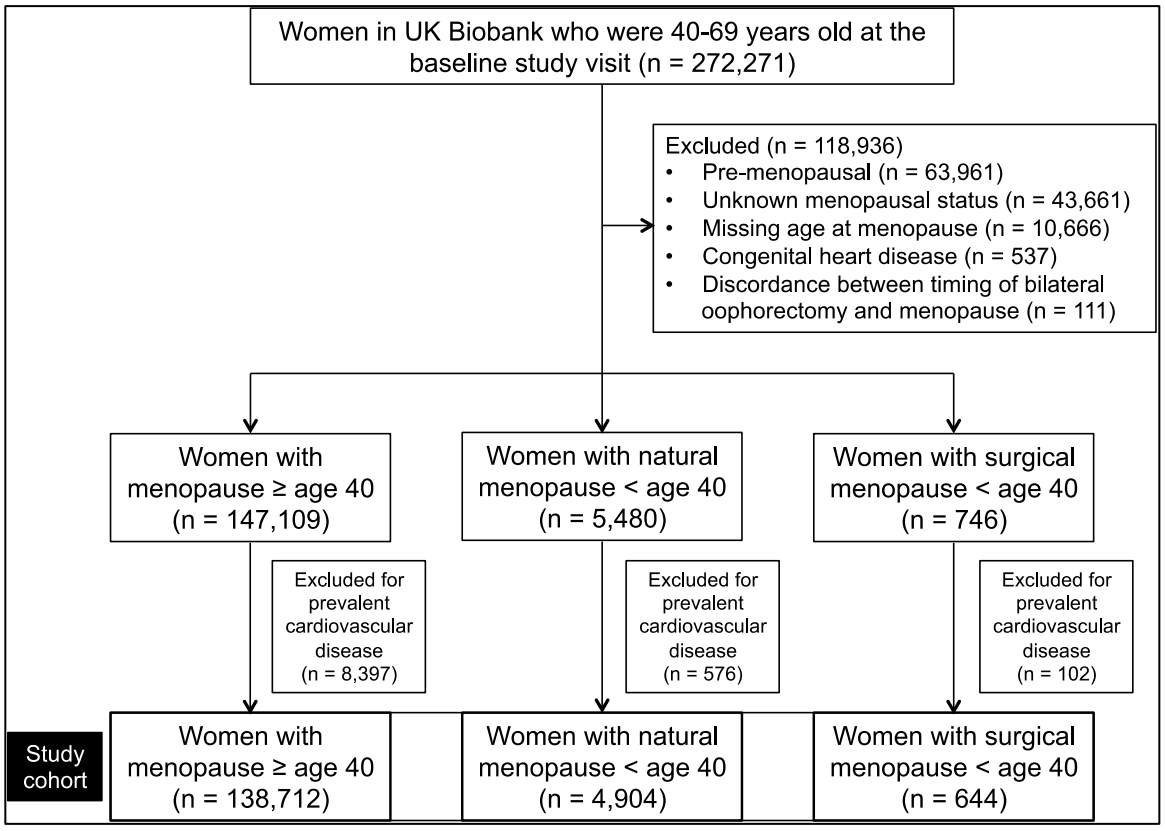
b. Innovation: The UK Biobank, a very large (~500,000 adult residents of the United Kingdom), prospective, observational cohort study, provides the largest cohort of postmenopausal women amassed to date with detailed data on reproductive history. Because the cohort is well phenotyped at baseline, we are able to capture and adjust for comprehensive prevalent comorbidities/risk factors, vital signs and body mass index, medication use (including detailed ascertainment of prior use of hormone replacement therapy [HRT]), and laboratory biomarkers. Further, we are able to examine incident hypertension, hyperlipidemia, and type 2 diabetes mellitus (T2DM), which to date have not been robustly studied in relation to premature menopause, and a greater diversity of cardiovascular outcomes than have been previously studied, spanning ASCVD, heart failure, valvular heart disease, atrial fibrillation, and venous thromboembolism.

c. Approach:

i. Study design: The UK Biobank is a prospective, observational, population-based cohort study, with median 7 (range 0-10.6) year follow-up.

ii. Study population: As summarized in the figure below, women aged 40-69 years at study enrollment will be considered for inclusion. Women will be excluded from the study sample if they are pre-menopausal, have unknown menopausal status or missing age at menopause, or have discordance between reported timing of bilateral oophorectomy and age at menopause. Further, women will be excluded if they have

congenital heart disease or if they already have any of the 8 cardiovascular diagnoses under study at enrollment (i.e., women with prevalent cardiovascular disease will be excluded).



iii. Exposures and their measurement: For the primary analysis, the exposures will be premature natural menopause, defined as age at menopause <40 years without accompany bilateral oophorectomy, and premature surgical menopause, defined as bilateral oophorectomy < age 40. In secondary analyses, alternate menopausal age thresholds will additionally be studied (menopause <30 years, <35 years, <45 years, <50 years). Age at menopause and history of bilateral oophorectomy were systematically collected by survey at study enrollment.

iv. Outcomes and their measurement: Because many cardiovascular conditions are not independent, the primary outcome will be a composite of incident coronary artery

disease, heart failure, aortic stenosis, mitral regurgitation, atrial fibrillation, ischemic stroke, peripheral artery disease, and venous thromboembolism, to be analyzed in a time-to-first diagnosis fashion. Secondary outcomes will be incident hypertension, hyperlipidemia, and T2DM, as well as individual components of the composite primary outcome. Outcomes will be ascertained by the appearance of a qualifying ICD code in the subject's medical record, which is linked to the UK Biobank study record.

v. Confounders and their measurement: Conventional cardiovascular risk factors (e.g., chronic hypertension, hyperlipidemia, diabetes, smoking) were captured at enrollment by participant self-report and/or ICD code. Additionally, medication use was captured at enrollment. All women were additionally asked about history of hormone-replacement therapy use, including date of starting and stopping therapy among users. Finally, participants reported whether they had any prior history of cancer at enrollment.

vi. Analysis plan: Baseline characteristics among the three groups in the primary analysis (premature natural menopause, premature surgical menopause, and menopause ≥ age 40) will be compared using ANOVA for continuous variables and the Pearson's chi-squared test for categorical variables.

(a) To evaluate the association between premature menopause and incident hypertension, hyperlipidemia, and T2DM, we will construct Cox proportional hazard models for each of these conditions, adjusted for age at enrollment, race, and the prevalent hypertension, hyperlipidemia and T2DM statuses not under consideration in each model to account for potential reverse causation. In these models, premature natural menopause, premature surgical menopause, and no premature menopause will be a single, non-ordered, categorical variable with no premature menopause as the reference

group. In secondary models to directly compare the hazards associated with premature natural and surgical menopause, premature natural menopause will serve as the reference group. To further account for reverse causation, we will run additional secondary models eliminating women with incident cardiovascular diagnoses during the study period. The proportional hazards assumption will be tested using Schoenfeld residuals, with additional stratified models run as needed to satisfy the assumption.

(b) To evaluate the association between premature menopause and incident cardiovascular disease, Cox models will assess time to first cardiovascular diagnosis (primary outcome) and incident coronary artery disease, heart failure, aortic stenosis, mitral regurgitation, atrial fibrillation, ischemic stroke, peripheral artery disease, and venous thromboembolism. As above, premature natural menopause, premature surgical menopause, and no premature menopause will constitute a categorical variable with no premature menopause as the reference group. Sparse models will adjust for age and race. Fully adjusted models will incorporate prevalent diabetes, ever-smoking, body mass index, systolic blood pressure, anti-hypertensive medication, non-HDL cholesterol, cholesterol-lowering medication, C-reactive protein, and ever-use of HRT.

(c) In a series of secondary models, we will incorporate multiple aspects of HRT use, including current HRT use, duration of HRT use, and delayed initiation of HRT following menopause (in analyses of the Women's Health Initiative and other large studies of HRT, these latter two factors have been associated with increased cardiovascular risk among HRT users). We will assess whether these aspects of HRT use are significantly associated with cardiovascular disease risk in our sample and whether

incorporating these factors changes the observed associations between premature menopause and cardiovascular disease.

(d) Because the menopausal age threshold of 40 years endorsed in recent guidelines is somewhat arbitrary, we will explore the association of alternate menopausal age thresholds (<30 years, <35 years, <45 years, and <50 years) with incident cardiovascular disease. These models will follow the same approach as (b) above.

vii. Sample size/power calculation: Our sample sizes are determined by the available dataset, which includes 4,904 women with premature natural menopause and 644 women with premature surgical menopause (see above figure), compared to a reference group of 138,712 women without premature menopause. Assuming that ≥1 incident cardiovascular diagnosis will occur in 5% of women in our sample during follow-up, we have >99% power to detect a hazard ratio of 2.0 for time to first cardiovascular diagnosis in both premature natural and premature surgical menopause groups at an alpha level of 0.05.

III. Strengths and limitations

Strengths of this proposal include leveraging the UK Biobank to create the largest cohort of postmenopausal women to date to study the relationship between menopausal age and cardiovascular risk. The size and extensive phenotyping of the cohort enable us to adjust comprehensively for comorbidities and to study novel cardiovascular outcomes not previously addressed in the literature. Furthermore, the question of cardiovascular risk associated with premature menopause is timely given recent cholesterol and prevention guideline updates.

Limitations of the study include the possibility of misclassification given self-reported age at menopause and long duration since menopause for many women, although women were prompted to indicate if they did not know their menopausal age. Misclassification would be expected to bias our estimated effects toward the null. Given that women enrolled over a three-decade range of ages and women with prevalent cardiovascular disease will be excluded, magnitude of effect may be underestimated, particularly in older age strata. Further, a "healthy participant" bias has been noted in the UK Biobank,[20] and it is possible sicker women with a history of premature menopause may have been less likely to enroll. In women with prevalent cardiovascular risk factors, we are unable to ascertain whether the risk factors predated menopause or developed subsequently; the baseline risk factor profile of subjects at enrollment and our analysis of incident hypertension, hyperlipidemia, and T2DM will provide insight into the importance or unimportance of this limitation.

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