1.a. Full Title: SNPs associated with coronary artery disease and type 2 diabetes as determinants for age at natural menopause

b. Abbreviated Title (Length 26 characters): CAD & T2D SNPs and age at menopause

2. Writing Group: N. Charlotte Onland-Moret, M. Voorhuis, Ching-Ti Liu, Ellen Demerath, Nora Franceschini, Yvonne T. van der Schouw, Joanne M. Murabito

Collaboration within former CARe Consortium with addition of Women’s Health Initiative and EPICNL.

I, the first author, confirm that all the coauthors have given their approval for this manuscript proposal. NCOM [please confirm with your initials electronically or in writing]

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**3. Timeline:** Analyses will begin immediately upon approval of this proposal.

**4. Rationale:**

**Background**

Age at natural menopause (ANM) is a complex trait and believed to be highly heritable, with heritability estimates ranging from 31% to 78% \(^1,2\). This has led to the publication of many genetic studies investigating ANM. Despite the huge effort, the number of ANM-loci is still modest. Genome-wide association studies provided 17 newly identified SNPs until now \(^3-5\). However, these hits did not provide clear evidence on the biological mechanisms that leads to menopause.

Women with early menopause are at an increased risk of cardiovascular disease later in life \(^6,7\). This increased CVD risk has generally been ascribed to a deprivation of estrogens leading to unfavourable cardiovascular risk profile, but results have been conflicting \(^8,9\). Furthermore, we showed previously that indeed an unfavourable cardiovascular risk profile (higher total cholesterol levels, increase in blood pressure, and increase in relative weight) accelerates menopause, rather than the reverse \(^10\). Following this line of reasoning SNPs that determine the cardiovascular risk profile may also predict age at natural menopause. Therefore, we propose to study whether SNPs that are known to be associated with cardiovascular disease are also associated with age at natural menopause.
Similarly, early menopause is associated with an increased risk for diabetes. On the other hand it has also been suggested that early diabetes might be associated with an earlier age at natural menopause. Thus, it is interesting to study whether SNPs that are known to be associated with the risk of type 2 diabetes are also associated with age at natural menopause.

Recently, a meta-analysis of age at natural menopause was conducted in over 9000 women of European ancestry from four CARE cohorts (ARIC, CHS, FHS, MESA) and three collaborating cohorts (EPIC-NL, KORA3, and Whitehall II) using the IBC chip data. SNPs in the previously reported chromosome 19 locus(26;27) were significantly associated with age at natural menopause. No other significant associations were identified.

5. Main Hypothesis/Study Questions:
We hypothesize that a focused search for genetic variants associated with age at menopause that is limited to SNPs that are already known to play a role in determining the cardiovascular risk profile will increase power to detect associations. The research hypothesis is whether 53 candidate SNPs are associated with age at natural menopause.

6. Design and analysis (study design, inclusion/exclusion, outcome and other variables of interest with specific reference to the time of their collection, summary of data analysis, and any anticipated methodologic limitations or challenges if present).

Design: We will perform a cross sectional study on candidate SNPs from the IBC array and age at menopause. Data from the CARe consortium will be used in these analyses complemented with data from the Women’s Health Initiative and the EPIC-NL study. In total we will have around 12000 women available for the analyses (794 from ARIC). All data have been uploaded already for the chipwide SNP main analyses and this proposal will primarily be a look-up of the association results for the listed SNPs. Should these analyses yield interesting results, we might want to decide on additional analyses such as adjustments for age at baseline, BMI (for the T2D SNPs) and smoking (for the CAD SNPs).

Subjects: Because we are examining SNPs identified in European ancestry women only, we will include only white participants in this analysis, and restrict the analysis to those with relevant genotype and phenotypic data.

Variables: Menopausal Status, Age at menopause, Type of menopause, Hysterectomy, Number of ovaries removed, Hormone replacement therapy use, Oral contraceptive use, Body mass index, Smoking status, Parity, Diabetes, Coronary artery disease status, Alcohol intake

SNPs:

Table 1. SNPs (or proxy SNPs) associated with T2D on IBC chip

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Table 2. SNPs (or proxy SNPs) associated with CAD on IBC chip

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Inclusion: All white ARIC subjects with available genotype and phenotype data
Exclusions: Non-white participants, due to low N (<1%) in HGS
Exposure: Candidate SNPs passing QC from the IBC chip
SNP chip that have been previously identified in GWAS for Coronary artery disease or type 2 diabetes (NSNPs~53)

Models:

Primary analysis:
We will test for association using linear regression analyses without adjustments for relatedness. All analyses will be adjusted for the first three principal components.
Should these analyses yield interesting results, we might want to decide on additional analyses such as adjustments for age at baseline, BMI (for the T2D SNPs) and smoking (for the CAD SNPs)
All association analyses will be performed in PLINK. Pooling of the study specific results will be performed using METAL.

Covariates: Age, BMI, smoking, first three principal components.

Statistical significance: The analyses will be adjusted for multiple testing. Given the fact that we will test 53 SNPs we will use a threshold of $10^{-3}$ for statistical significance.

7.a. Will the data be used for non-CVD analysis in this manuscript? __X__ Yes __ No
b. If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = “CVD Research” for non-DNA analysis, and for DNA analysis RES_DNA = “CVD Research” would be used?
___ Yes ___ No
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. Will the DNA data be used in this manuscript? __X__ Yes __ No
b. If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = “No use/storage DNA”?
___X__ Yes ___ No

9. The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status.
ARIC Investigators have access to the publications lists under the Study Members Area of the web site at: http://www.cscc.unc.edu/ARIC/search.php
___X__ Yes _______ No

10. What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?

1474. Genome-wide association study of age at natural menopause and related phenotypes (Demerath et al.) This study does not examine CAD and DM SNPs in association with ANM.
1682. Genetic determinants of age at menarche and natural menopause in diverse populations from the PAGE study (Heiss et al). This project interrogates previous ANM SNPs in different populations, not CAD and DM SNPs, as proposed here.
1139. Interaction of lipid gene polymorphisms and menopausal transition in LDL, HDL, TG, and TC levels (Gramenz et al). This project uses ANM as an interaction term—focus is on lipid and lipoprotein outcomes.
All other papers are either non-genetic analyses involving ANM, or use ANM only as a covariate.

11. a. Is this manuscript proposal associated with any ARIC ancillary studies or use any ancillary study data? ___X___ Yes ___ No

11.b. If yes, is the proposal ___X___ A. primarily the result of an ancillary study (list number)*2006.03 (Stampede and Geneva genotype funding in Caucasians) and 2007.02 (CARe, genotyping in African Americans).
___ B. primarily based on ARIC data with ancillary data playing a minor role (usually control variables; list number(s))* __________ __________

*ancillary studies are listed by number at http://www.cscc.unc.edu/aric/forms/

12. Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.

References: