ARIC Manuscript Proposal #2163

PC Reviewed: 7/9/13	**Status: A**	**Priority: 2**
SC Reviewed: _____	**Status: _____**	**Priority: ____**

1.a. Full Title: **Title: Genetic determinants of reproductive traits in CHARGE: menarche and menopause timing**

b. Abbreviated Title (Length 26 characters): **reproductive and genetics**

2. Writing Group: Nora Franceschini, Ellen Demerath, Eric Boerwinkle, Megan Grove, Alanna Morrison and other interested ARIC Authors.

Other authors from the CHARGE consortium will be included.

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

First authors: Nora Franceschini and Ellen Demerath

Nora Franceschini
Department of Epidemiology
University of North Carolina Chapel Hill
Bank of America Center
137 E. Franklin St., Suite 306
CB #8050
Chapel Hill, NC 27599-8050
(919) 966-1305 (Voice)
(919) 966-9800 (Fax)
noraf@unc.edu

Corresponding/senior author (if different from first author correspondence will be sent to both the first author & the corresponding author):

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

4. Rationale: Menarche and menopause are fundamental biologic events during a woman's lifetime. The timing of both age at menarche (AAM) and age at natural menopause (ANM) is associated with a range of significant health conditions later in life that extend beyond

reproductive health to obesity[1], T2DM[2;3], osteoporosis[4-6], cardiovascular disease[7-9], breast[10] and ovarian cancer[11], and mortality.[9;12;13]

We have conducted GWAS for AAM and ANM within the international CHARGE and ReproGen consortia and have identified and replicated 32 genetic loci for AAM[14] and 17 genetic loci for ANM[15] from diverse biologic pathways. Our strongest association for AAM was in a SNP in *LIN28B* a gene associated with adult height. The allele associated with earlier AAM was associated with decreased height (girls with early menarche are shorter as women).[16] Subsequent work established that *LIN28B* has sex specific effects on childhood growth [17] and prolonged effects on body mass index (BMI) from adolescence to adulthood in women.[18] These studies demonstrate how further investigation into genetic associations may provide insights into the effects of puberty timing on human growth and obesity risk over the lifecourse. For ANM a *UIMC1* SNP[19;20] was among the top associations. The protein encoded by *UIMC1* interacts with BRCA1 and estrogen receptor α and is thought to recruit BRCA1 to DNA damage sites.[21] Many of the novel associations for ANM function in pathways important to somatic aging (DNA repair and immune response) and suggest mechanistic links between reproductive aging and senescence. Insights into the genetic basis of sexual maturation and reproductive aging may not only improve our understanding of disorders of puberty and menopause but also advance our understanding of how AAM and ANM confer risk for serious health conditions and provide new strategies for prevention and treatment.

In this study we propose to extend this work by conducting a meta-analysis of Exomechip genotyping data for AAM and ANM to identify low frequency and rare variants associated with these phenotypes that are more likely to be functional.

5. Main Hypothesis/Study Questions:

1. Common and low frequency variants in the exome are associated with menarche and menopause timing.
2. Genome wide common and low frequency variants are associated with menarche and menopause timing.

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).

Subjects and sample sizes:

Phenotype harmonization across CHARGE cohorts and collaborating studies has been already done.

Multiple cohorts participated in joint calling of the Exomechip including: AGES, ARIC, CHS, CARDIA, Rotterdam, Fam HS, FHS, HABC, JHS, MESA (CHS and MESA do not have menarche; CARDIA does not have many menopausal women; FamHS does not have reproductive traits; JHS has only AA women)

In addition the following cohorts outside the CHARGE joint calling effort are interested in participating in this project:
Estonia: n=2500
KORA F4
SardiNIA
WGHS
WHI

Finally, the European Exomechip Consortium has agreed to exchange top associations for replication.

Genotypes: All samples in CHARGE have exome chip data and 1000G imputed data. Genotype calling has been standardized across studies. Quality control has been already done.

Inclusion/exclusion Criteria: Only women with available trait will be used for analysis. For menopause, we will restrict the data to natural menopause by excluding women with hysterectomy or menopause due to chemical/drug therapy. We propose analysis of European and African ancestry individuals.

Quality Control: Quality control has been standardized across participating studies.

Primary Phenotypes
Age of menarche(14): self-reported between ages of 9 and 17 years
Age of natural menopause(15): self-reported between ages of 40 and 60 years

Covariates: adjustments for birth year, measures of population stratification and region will be done for age of menarche analysis. For age of menopause, we will only adjust for measures of population stratification and region.

Analysis: Analysis protocols have been developed by the CHARGE statistical working group for single variant analysis and burden test using gene-based units. Meta-analysis of data across studies has also been standardized.

We will implement two burden tests. First, a Variable Threshold Combined Multivariate and Collapsing count method, where the number of rare alleles is counted in each gene, then the gene is tested for association. Second, we will use SKAT for all rare variants (MAF < .05) within a gene. SKAT allows for variants with opposite directions of effect within the same gene, whereas the variable threshold combined multivariate and collapsing method does not.
Other Analysis Considerations

Genotype Annotation: this has been already provided by the CHARGE consortium

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

8.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)?

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

11.b. If yes, is the proposal

__ A. primarily the result of an ancillary study (list number*

___ 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.

Reference List

(1) Freedman DS, Khan LK, Serdula MK, Dietz WH, Srinivasan SR, Berenson GS. The relation of menarcheal age to obesity in childhood and adulthood: the Bogalusa heart study. BMC Pediatr 2003; 3:3.

(2) He C, Zhang C, Hunter DJ, Hankinson SE, Buck Louis GM, Hediger ML et al. Age at menarche and risk of type 2 diabetes: results from 2 large prospective cohort studies. Am J Epidemiol 2010; 171(3):334-344.

(3) Lakshman R, Forouhi N, Luben R, Bingham S, Khaw K, Wareham N et al. Association between age at menarche and risk of diabetes in adults: results from the EPIC-Norfolk cohort study. Diabetologia 2008; 51(5):781-786.

(4) Chevalley T, Bonjour JP, Ferrari S, Rizzoli R. Influence of age at menarche on forearm bone microstructure in healthy young women. J Clin Endocrinol Metab 2008; 93(7):2594-2601.

(5) Grainge MJ, Coupland CA, Cliffe SJ, Chilvers CE, Hosking DJ. Reproductive, menstrual and menopausal factors: which are associated with bone mineral density in early postmenopausal women? Osteoporos Int 2001; 12(9):777-787.

(6) Ito M, Yamada M, Hayashi K, Ohki M, Uetani M, Nakamura T. Relation of early menarche to high bone mineral density. Calcif Tissue Int 1995; 57(1):11-14.

(7) Cui R, Iso H, Toyoshima H, Date C, Yamamoto A, Kikuchi S et al. Relationships of age at menarche and menopause, and reproductive year with mortality from cardiovascular disease in Japanese postmenopausal women: the JACC study. J Epidemiol 2006; 16(5):177-184.

(8) de Lecinana MA, Egido JA, Fernandez C, Martinez-Vila E, Santos S, Morales A et al. Risk of ischemic stroke and lifetime estrogen exposure. Neurology 2007; 68(1):33-38.

(9) Lakshman R, Forouhi NG, Sharp SJ, Luben R, Bingham SA, Khaw KT et al. Early age at menarche associated with cardiovascular disease and mortality. J Clin Endocrinol Metab 2009; 94(12):4953-4960.

(10) Kotsopoulos J, Lubinski J, Lynch HT, Neuhausen SL, Ghadirian P, Isaacs C et al. Age at menarche and the risk of breast cancer in BRCA1 and BRCA2 mutation carriers. Cancer Causes Control 2005; 16(6):667-674.

(11) Chiaffarino F, Pelucchi C, Parazzini F, Negri E, Franceschi S, Talamini R et al. Reproductive and hormonal factors and ovarian cancer. Ann Oncol 2001; 12(3):337-341.

(12) Snowdon DA, Kane RL, Beeson WL, Burke GL, Sprafka JM, Potter J et al. Is early natural menopause a biologic marker of health and aging? Am J Public Health 1989; 79(6):709-714.

(13) Ossewaarde ME, Bots ML, Verbeek AL, Peeters PH, van der GY, Grobbee DE et al. Age at menopause, cause-specific mortality and total life expectancy. Epidemiology 2005; 16(4):556-562.

(14) Elks CE, Perry JR, Sulem P, Chasman DI, Franceschini N, He C et al. Thirty new loci for age at menarche identified by a meta-analysis of genome-wide association studies. Nat Genet 2010; 42(12):1077-1085.

(15) Stolk L, Perry JR, Chasman DI, He C, Mangino M, Sulem P et al. Meta-analyses identify 13 loci associated with age at menopause and highlight DNA repair and immune pathways. Nat Genet 2012.

(16) Gudbjartsson DF, Walters GB, Thorleifsson G, Stefansson H, Halldorsson BV, Zusmanovich P et al. Many sequence variants affecting diversity of adult human height. Nat Genet 2008; 40(5):609-615.

(17) Widen E, Ripatti S, Cousminer DL, Surakka I, Lappalainen T, Jarvelin MR et al. Distinct Variants at LIN28B Influence Growth in Height from Birth to Adulthood. Am J Hum Genet 2010.

(18) Ong KK, Elks CE, Wills AK, Wong A, Wareham NJ, Loos RJ et al. Associations between the pubertal timing-related variant in LIN28B and BMI vary across the life course. J Clin Endocrinol Metab 2011; 96(1):E125-E129.

(19) Stolk L, Zhai G, van Meurs JB, Verbiest MM, Visser JA, Estrada K et al. Loci at chromosomes 13, 19 and 20 influence age at natural menopause. Nat Genet 2009.

(20) He C, Kraft P, Chasman DI, Buring JE, Chen C, Hankinson SE et al. A large-scale candidate gene association study of age at menarche and age at natural menopause. Hum Genet 2010; 128(5):515-527.

(21) Yan J, Kim YS, Yang XP, Li LP, Liao G, Xia F et al. The ubiquitin-interacting motif containing protein RAP80 interacts with BRCA1 and functions in DNA damage repair response. Cancer Res 2007; 67(14):6647-6656.