

Age at menarche and age at natural menopause in East Asian women: a genome-wide association study

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Received: 25 November 2015 / Accepted: 14 July 2016 / Published online: 14 September 2016
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Abstract Age at menarche (AM) and age at natural menopause (ANM) are complex traits with a high heritability. Abnormal timing of menarche or menopause is associated with a reduced span of fertility and risk for several age-related diseases including breast, endometrial and ovarian cancer, cardiovascular disease, and osteoporosis. To identify novel genetic loci for AM or ANM in East Asian women and to replicate previously identified loci primarily in women of European ancestry by

genome-wide association studies (GWASs), we conducted a two-stage GWAS. Stage I aimed to discover promising novel AM and ANM loci using GWAS data of 8073 women from Shanghai, China. The Stage II replication study used the data from another Chinese GWAS ($n = 1230$ for AM and $n = 1458$ for ANM), a Korean GWAS ($n = 4215$ for AM and $n = 1739$ for ANM), and de novo genotyping of 2877 additional Chinese women. Previous GWAS-identified loci for AM and ANM were also

Electronic supplementary material The online version of this article (doi:10.1007/s11357-016-9939-5) contains supplementary material, which is available to authorized users.

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evaluated. We identified two suggestive menarcheal age loci tagged by rs79195475 at 10q21.3 ($\beta = -0.118$ years, $P = 3.4 \times 10^{-6}$) and rs1023935 at 4p15.1 ($\beta = -0.145$ years, $P = 4.9 \times 10^{-6}$) and one menopausal age locus tagged by rs3818134 at 22q12.2 ($\beta = -0.276$ years, $P = 8.8 \times 10^{-6}$). These suggestive loci warrant a further validation in independent populations. Although limited by low statistical power, we replicated 19 of the 98 menarche loci and 5 of the 20 menopause loci previously identified in women of European ancestry in East Asian women, suggesting a shared genetic architecture for these two traits across populations.

Keywords Menarche · Menopause · Genome-wide association · Single nucleotide polymorphism

Introduction

Age at menarche (AM) and age at natural menopause (ANM) indicate the beginning and end of a woman's normal reproductive life. Menarche and menopause out of the common range have been associated with risk for various diseases, including breast cancer (Velie et al. 2005; Vogel 2008), endometrial and ovarian cancer (Hinds and Price 2010; Cramer 2012), cardiovascular disease (Cui et al. 2006), and osteoporosis (Qiu et al. 2013; Parker et al. 2014). It has been well recognized that genetic makeup influences both AM (Sharma 2002; Anderson et al. 2008) and ANM (de Bruin et al. 2001; Murabito et al. 2005; Morris et al. 2011). Recent genome-wide association studies (GWASs) among women of European ancestry have identified at least 106 genetic loci for AM (He et al. 2009; Ong et al. 2009; Perry et al. 2009; Sulem et al. 2009; Elks et al. 2010; Perry et al. 2014) and 21 loci for ANM (He et al. 2009; Stolk et al. 2009; Stolk et al. 2012). However, only 24 AM loci and 9 ANM loci have been replicated in women of either Asian or African ancestry and no novel loci have been identified in these non-European populations (Liu et al. 2009; Chen et al. 2012; Shen et al. 2013; Spencer et al. 2013; Tanikawa et al. 2013; Delahanty et al. 2013; Carty et al. 2013; Demerath et al. 2013; Pyun et al. 2014; Chen et al. 2014).

We report here results of a meta-analysis of GWAS for AM and ANM in Chinese and Korean populations, aiming to replicate the AM and ANM loci identified in European populations and to discover novel genetic loci.

Methods

Study design

This GWAS included two stages. The Stage I study was a meta-analysis of results of AM and ANM association using data generated from the Shanghai Genome-Wide Association Studies (SGWAS), which included female participants of the Shanghai Breast Cancer Genetics Study (SBCS) (Zheng et al. 2009), the Shanghai Endometrial Cancer Study (SECS) (Long et al. 2012), the Shanghai Type II Diabetes Studies (ST2DS) (Shu et al. 2010), the Shanghai Colorectal Cancer Study (SCRCS) (Jia et al. 2013; Zhang et al. 2014), and other ancillary studies using the Shanghai Women's Health Study (SWHS) samples (Petersen et al. 2010; Abnet et al. 2010). All these studies, except for the SBCS and SECS, drew women from the SWHS, a population-based cohort study (Zheng et al. 2005). We defined a suggestive locus as a genomic region wherein one or more single-nucleotide polymorphisms (SNPs) showed $P < 1 \times 10^{-4}$ in Stage I and the region was >1 Mb away from GWAS locus discovered in women of European ancestry. One or two highly correlated SNPs ($r^2 > 0.6$) in each suggestive novel locus were selected for a replication study using data from three additional studies in Chinese and Korean women (see description in Stage II). Index SNPs or their best proxies of AM or ANM loci identified by previous GWAS in European ancestry women were also evaluated in the Stage II study. Basic information on all participating studies is summarized in Table 1.

Stage I samples and genotyping

A total of 8073 Chinese women from the SGWAS were included in Stage I (Table 1). These studies were population-based and applied similar study protocols to collect blood or buccal cell samples and relevant exposure information. Interviews were conducted in person by trained, retired medical personnel, and anthropometrics, including height, weight, and circumferences of the waist and hips were measured by trained interviewers according to standard protocols. AM, to the nearest year, was ascertained by participant recall during the interview. We used the World Health Organization's definition of menopause, i.e., the cessation of menstruation for ≥ 12 months, to determine menopausal status. Information on age at which menopause occurred and the reasons for its occurrence (natural menopause,

Table 1 Characteristics of participants in the association of age at menarche and natural menopause

Study (acronym)	Population	Primary trait	Age at menarche		Age at natural menopause	
			<i>n</i>	Mean (SD)	<i>n</i>	Mean (SD)
Stage I: Initial Shanghai GWAS meta-analysis						
The Shanghai Breast Cancer Study (SBCS)	Chinese	Breast cancer cases	2697	14.5 (1.7)	821	50.0 (3.6)
		Healthy controls ^a	1987	14.7 (1.8)	684	49.1 (4.0)
The Shanghai Endometrial Cancer Study (SECS)	Chinese	Endometrial cancer cases	827	14.5 (1.7)	457	50.4 (3.4)
The Shanghai Type II diabetes Studies (ST2DS)	Chinese	T2D cases	1030	14.7 (1.8)	496	49.3 (3.5)
The Shanghai Colorectal Cancer Study (SCRCS)	Chinese	CRC cases	487	15.1 (1.8)	328	49.2 (3.6)
		Healthy controls	730	15.2 (1.8)	531	49.5 (3.4)
Other Shanghai cancer studies	Chinese	Other cancer cases ^b	315	15.3 (1.7)	239	48.9 (3.7)
Stage I total			8073		3556	
Stage II: replication studies						
Nutrition and Health of Aging Population in China (NHAPC)	Chinese	Aging	1230	15.9 (2.1)	1458	48.9 (4.0)
The Seoul Breast Cancer Study (SeBCS)	Korean	Breast cancer cases	2164	14.7 (1.7)	745	48.4 (5.5)
		Healthy controls	2051	15.2 (1.8)	994	49.2 (4.6)
Shanghai Women's Health Study (SWHS)	Chinese	Healthy women ^c	2877	15.1 (1.8)	1911	49.0 (4.1)
Stage II total			8322		5108	
Stage I and II total			16,395		8664	

^aNote shared controls in GWAS in Stage I; most samples were selected from the Shanghai Women's Health Study (Zheng et al., 2005)

^bThese include 219 cases from the Shanghai Upper Gastrointestinal Cancer Study (SUGICS), 46 cases from the Shanghai Pancreatic Cancer Study (SPCS), and 50 cases with other cancers

^cNot included in Stage I GWAS, with genotype data available for several genetic variants noted in Table 2

hysterectomy or ovariectomy, or other treatment-induced menopause) were obtained in survey interviews. ANM was determined by subtracting the birth date from the date of the last natural menstrual period. Women with a non-natural cause of menopause (i.e., menopause brought on by surgery, radiation treatment, or chemotherapy) were excluded. The details on study design and participant characteristics for the SGWAS and the SWHS have been described elsewhere (Zheng et al. 2005; Wen et al. 2014). Written informed consent was obtained from all participants prior to interview, and the study protocols were approved by the institutional review boards of all institutions involved in the study.

Genotyping methods and quality control (QC) in the SGWAS were previously described in detail (Zheng et al. 2009; Wen et al. 2014). In brief, genotyping was performed using either Affymetrix or Illumina SNP arrays. For the present study, QC procedures included the removal of SNPs with minor allele frequency (MAF) of <5 %, Hardy-Weinberg equilibrium (HWE) *P* values of <1 ×

10⁻⁵ and samples with >5 % missing genotypes, outliers from multidimensional scaling analyses based on pairwise identity-by-state (IBS), or duplicates and first-degree relatives based on identity-by-descent (IBD) analysis. After QC filtering, genotype imputation was performed by each participating study using MACH 1.0 (Li et al. 2010) with the Genetic Investigation of ANthropometric Traits (GIANT) all-reference panel (excluding monomorphic and singleton sites) from the 1000 Genomes Project phase 1, release v3. A total of 4,633,105 SNPs with an imputation score of *R*_{sq} >0.5 and with MAF >5 % were included in final AM or ANM association analysis.

Stage I statistical analysis

A linear regression model, adjusted for age, disease status (e.g., cancer or type II diabetes), and the first ten principal components from population structure analysis using linkage disequilibrium (LD)-pruned (pairwise *r*² <0.2) common SNPs with MAF >0.3, was used to

evaluate association between SNPs and AM or ANM in each subset of the GWAS using mach2qtl software (Li et al. 2009; Li et al. 2010). Fixed-effect inverse-variance weighting meta-analysis was performed with summary statistics of beta (per-allele effect on AM or ANM) and standard error from all GWAS using the METAL software (Willer et al. 2010). The presence of heterogeneity across studies was tested with Cochran's Q statistics implemented in METAL. The inflation factor was modest ($\lambda = 1.009$) using all SNPs, suggesting little evidence of population stratification in our studies.

Stage II samples, genotyping, and association with AM or ANM

The Seoul Breast Cancer Study (SeBCS) is a hospital-based case-control study conducted in two teaching hospitals in Seoul (Cho et al. 2009; Kim et al. 2012). Included in this project were 2164 incident breast cancer patients recruited between 2001 and 2007. In-person interviews were conducted to collect information on known breast cancer risk factors, including AM and ANM, and anthropometrics by using a protocol and questionnaire. Controls were 2051 women selected from a large urban cohort that is participating in the Korea Genome Epidemiology Study (KoGES), which is an ongoing cohort study seeking to understand the causes and risk factors of disease in Korea. These controls were frequency-matched to cases on age in 5-year intervals. Information on AM and ANM, demographics, and other lifestyle factors were collected using a protocol similar to the SeBCS. DNA from these samples was genotyped using Affymetrix human SNP array 6.0. A QC procedure similar to the one used in the Stage I GWAS was conducted in this study, except for the removal of SNPs with a HWE $P < 1 \times 10^{-4}$. Finally, SNPs with genotype imputation $R_{sq} > 0.5$ were analyzed for AM or ANM association using a linear regression model with age, disease status, and the first five population structure informative principal components as covariates.

The Nutrition and Health of Aging Population in China (NHAPC) is a population-based study, focused on investigating the association of environmental and genetic factors with metabolic diseases. A total of 1638 women aged 50–70 years participated in the original study. Details of the study design and inclusion/exclusion criteria have been described elsewhere (Ye et al. 2007). Data on demographic variables including AM ($n = 1230$) and ANM ($n = 1458$) were collected using a standardized questionnaire.

Genotyping was performed using Illumina Human660W arrays. SNPs with MAF $< 1\%$ and subjects with a genotype call rate of $< 97\%$ were removed. Genotype imputation was performed using IMPUTE v2.3.0 with all-phased 1000 Genome haplotypes version 3 as reference (Howie et al. 2009). AM or ANM association was conducted using SNPTEST v2.4.1 (Marchini et al. 2007). The association models included age, region of residence (Beijing or Shanghai), and the first five population structure informative principal components as covariates.

Two suggestive AM- and one ANM-associated SNPs from Stage I were further examined by genotyping using the Sequenom MassARRAY iPLEX platform (Agena Bioscience, San Diego, CA, USA) in an additional 2877 healthy women from the SWHS. SNP association with AM or ANM was measured under a linear regression model adjusted for age using SAS software, version 9.3 (SAS Institute Inc., Cary, NC, USA).

Stage II meta-analysis

Summary results of AM or ANM association were obtained for each study included in Stage II and conformed to the association direction according to effective alleles revealed in Stage I and then meta-analyzed using METAL (Willer et al. 2010). Results from all samples were obtained through METAL analysis, combining summary results from Stages I and II. The presence of heterogeneity between cohorts for the effect sizes of risk alleles was investigated using Cochran's Q test statistic as implemented in METAL (Willer et al. 2010).

Binomial sign test

To evaluate consistency of the direction of association for the AM- or ANM-associated GWAS SNPs previously identified in women of European ancestry in our study of women with East Asian ancestry, we performed a binomial sign test. Under the null hypothesis that none of these SNPs/loci are associated with AM or ANM in East Asian populations, half of these evaluated SNPs/loci would be expected to have an association in the same direction as that of European-ancestry populations. We also conducted a binomial sign test to evaluate the probability of the number of observed significant results. Under the null hypothesis, five percent of evaluated GWAS SNPs would be expected by chance to be associated with the study phenotype at $P < 0.05$ and in the same direction as those previously reported.

Results

GWAS of AM and ANM

We identified 37 SNPs at 23 suggestive loci associated with AM and 32 SNPs at 26 suggestive loci associated with ANM at $P < 1 \times 10^{-4}$ from the Stage I study (Supplementary Tables S1 and S2). The association direction of these SNPs was consistent across the GWAS sample sets (Tables S1 and S2). In Stage II, however, none of these suggestive SNPs, except for rs79195475, were significantly associated with AM or ANM in either the healthy Chinese women or Korean women set at $P < 0.05$ (Tables S1 and S2). In the combined samples, two intergenic SNPs, rs1023935 at 4p15.1 and rs79195475 at 10q21.3, were consistently associated with AM. The intron 3 SNP rs3818134 in the *SFII* gene (NM_014775) at 22q12.2 was associated with ANM, with a combined P of 8.8×10^{-6} (Table 2).

Replication of previous AM GWAS loci in East Asian women

A total of 110 independent index SNPs at 98 GWAS loci identified from previous studies in women of European ancestry were evaluated in East Asian women; of them, 82 (74.5 %) SNPs showed a highly significant concordance of association in the same direction as that found in European-ancestry women ($P = 2.5 \times 10^{-7}$, binomial sign test; Supplementary Table S3). As shown in Table 3, 22 independent index SNPs representing 19 GWAS loci for AM were nominally replicated in the current study ($P < 0.05$ and in the same association direction), a finding highly unlikely to be attributed to chance ($P = 2.5 \times 10^{-8}$, binomial sign test). The SNP at the Lin-28 Homolog B gene (*LIN28B*) locus, rs7759938, had the strongest association ($P = 3.5 \times 10^{-8}$; Table 3 and Supplementary Table S3). Another independent signal (tagged by rs10453225) within *TMEM38B* and the SNP rs1400974 downstream of the *SATB2* also survived Bonferroni correction for multiple comparisons ($P < 4.1 \times 10^{-4}$, Table 3).

Replication of previous ANM GWAS loci in East Asian women

Table 4 summarizes the results of 21 independent ANM-associated index/proxy SNPs at 20 GWAS loci previously identified in European-ancestry women.

Table 2 SNPs showing strong associations (with combined $P < 1 \times 10^{-5}$ and in same association direction across three data sets) with puberty and natural menopause timing in East Asian women

SNP	Chr	Base position ^c	Nearby gene	Alleles ^d	EAF ^e	SGWAS (8073/3556) ^a		Stage II (8322/5108) ^a		Combined (16395/8664) ^b	
						Beta(SE)	P	Beta (SE)	P	Beta (SE)	$P_{_het}$
Age at menarche											
rs79195475	10	66964843	intergenic	T/C	0.75	-0.135 (0.032)	2.2×10^{-5}	-0.089 (0.042)	0.035	-0.118 (0.026)	3.4×10^{-6}
rs1023935	4	35150284	intergenic	T/C	0.89	-0.200 (0.044)	4.9×10^{-6}	-0.084 (0.046)	0.066	-0.145 (0.032)	4.9×10^{-6}
Age at natural menopause											
rs3818134	22	31926725	<i>SFII</i>	T/C	0.36	-0.454 (0.089)	3.5×10^{-7}	-0.108 (0.087)	0.214	-0.276 (0.062)	8.8×10^{-6}

SNP single-nucleotide polymorphism, SGWAS the Shanghai Genome-Wide Association Studies, Chr chromosome, EAF effective allele frequency, SE standard error, $P_{_het}$ meta-analysis using METAL, $P_{_het}$ P value from between-study heterogeneity test

^aNumbers in parentheses indicate sample size for age at menarche and age at natural menopause, respectively

^bMeta-analysis using the inverse-variance-weighted models in METAL

^cChromosome position based on NCBI human genome build 37 from the 1000 Genomes Project

^dShown as effect allele/other allele

^eEffect allele frequencies estimated from 6541 samples in breast cancer, endometrial, and T2D GWAS from the Shanghai cohort

Table 3 Nominally replicated GWAS-identified single-nucleotide polymorphisms for age at menarche in East Asian women

Locus	SNP	Chr	Base position ^a	Nearby gene	Alleles ^b	EAF	SGWAS (<i>n</i> = 8073)			Stage II (<i>n</i> = 5445)			Combined (<i>n</i> = 13,518)		
							Beta(SE)	<i>P</i>		Beta(SE)	<i>P</i>		Beta(SE)	<i>P</i>	<i>P</i> _{het}
1	rs2274465	1	44121557	<i>KDM4A</i>	C/G	0.74	0.053 (0.031)	0.083	0.068 (0.039)	0.078	0.059 (0.024)	0.014	0.765		
2	rs466639	1	165394882	<i>RXRG</i>	T/C	0.16	-0.063 (0.037)	0.093	-0.054 (0.044)	0.229	-0.059 (0.029)	0.040	0.874		
3	rs1400974	2	199638690	<i>SATB2</i>	A/G	0.43	0.083 (0.028)	0.003	0.068 (0.034)	0.046	0.077 (0.021)	3.3×10^{-4}	0.726		
4	rs11715566	3	117562436	<i>IGSF11</i>	T/C	0.61	0.097 (0.028)	4.6×10^{-4}	0.037 (0.034)	0.283	0.073 (0.022)	7.0×10^{-4}	0.168		
5	rs10938397	4	45182527	<i>GNPDA2</i>	A/G	0.70	0.068 (0.029)	0.021	0.033 (0.037)	0.365	0.054 (0.023)	0.018	0.464		
6	rs3733631	4	104641103	<i>TACR3</i>	C/G	0.32	0.054 (0.029)	0.063	0.047 (0.036)	0.194	0.051 (0.022)	0.024	0.879		
7	rs17171818	5	137725003	<i>JMJD1B</i>	T/C	0.59	-0.075 (0.027)	0.006	0.008 (0.034)	0.826	-0.043 (0.021)	0.045	0.058		
8 ¹	rs2153127	6	105348544	<i>LIM28B</i>	T/C	0.44	0.068 (0.028)	0.013	0.056 (0.038)	0.145	0.064 (0.022)	0.004	0.791		
8 ²	rs7759938	6	105378954	<i>LIM28B</i>	T/C	0.70	-0.165 (0.030)	3.2×10^{-8}	-0.072 (0.039)	0.064	-0.131 (0.024)	3.5×10^{-8}	0.057		
9 ¹	rs10816359	9	108757670	<i>TMEM38B</i>	T/G	0.76	0.038 (0.032)	0.229	0.088 (0.042)	0.036	0.057 (0.025)	0.026	0.346		
9 ²	rs10453225	9	108920220	<i>TMEM38B</i>	T/G	0.48	-0.086 (0.027)	0.001	-0.063 (0.033)	0.058	-0.077 (0.021)	2.4×10^{-4}	0.589		
9 ³	rs10739221	9	109060830	<i>TMEM38B</i>	T/C	0.62	-0.069 (0.028)	0.015	-0.006 (0.036)	0.865	-0.045 (0.022)	0.043	0.173		
10	rs10980921	9	114279912	<i>ZNF483</i>	T/C	0.76	-0.025 (0.032)	0.425	-0.203 (0.061)	9.2×10^{-4}	-0.062 (0.028)	0.026	0.010		
11	rs1874984	10	1731871	<i>ADARB2</i>	C/G	0.47	0.06 (0.029)	0.036	0.027 (0.035)	0.440	0.047 (0.022)	0.035	0.472		
12	rs2063730	11	78048524	<i>GAB2</i>	A/C	0.60	-0.048 (0.028)	0.085	-0.054 (0.035)	0.120	-0.051 (0.022)	0.021	0.902		
13	rs10895140	11	101436721	<i>TRPC6</i>	A/G	0.60	-0.028 (0.028)	0.307	-0.108 (0.034)	0.002	-0.06 (0.022)	0.005	0.070		
14	rs11215400	11	115052635	<i>CADMI</i>	A/C	0.88	-0.055 (0.042)	0.196	-0.071 (0.045)	0.112	-0.063 (0.031)	0.042	0.785		
15	rs12915845	15	89042467	<i>DETI</i>	T/C	0.14	-0.028 (0.039)	0.484	-0.121 (0.049)	0.014	-0.064 (0.031)	0.038	0.141		
16	rs246185	16	14395432	<i>MKL2</i>	T/C	0.45	-0.051 (0.029)	0.077	-0.080 (0.037)	0.032	-0.062 (0.023)	0.007	0.536		
17	rs1129700	16	29918034	<i>KCTD13</i>	T/C	0.59	0.042 (0.031)	0.168	0.154 (0.045)	5.4×10^{-4}	0.078 (0.025)	0.002	0.039		
18	rs12607903	18	3817134	<i>DLGAPI</i>	T/C	0.56	-0.075 (0.029)	0.010	-0.057 (0.037)	0.126	-0.068 (0.023)	0.003	0.706		
19	rs652260	19	7900562	<i>EVI5L</i>	T/C	0.58	0.050 (0.028)	0.074	0.050 (0.034)	0.141	0.050 (0.021)	0.020	0.995		

SNP single-nucleotide polymorphism, *Chr* chromosome, *EAF* effective allele frequency, *SE* standard error, *P*_{METAL} *P* value from meta-analysis using METAL, *P*_{het} *P* value from between-study heterogeneity test

1, 2, and 3 superscript numbers in the column of "Locus" indicate independent signals at the same genetic locus

Table 4 Evaluation of GWAS-identified single-nucleotide polymorphisms for age at natural menopause in East Asian women

Locus	SNP	Chr	Base position ^a	Nearby gene	Alleles ^b	EAF	SGWAS (<i>n</i> = 3556)			Stage II (<i>n</i> = 3197)			Combined (<i>n</i> = 6753)			Dir
							Beta (SE)	<i>P</i>		Beta (SE)	<i>P</i>		Beta (SE)	<i>P</i>	<i>P</i> _{METAL}	
1	rs4246511	1	39380385	<i>RHBDL2</i>	T/C	0.39	0.247 (0.089)	0.006	0.303 (0.115)	0.008	0.268 (0.071)	1.4×10^{-4}	0.699	+		
2	rs1635501	1	242040775	<i>EXO1</i>	T/C	0.23	-0.025 (0.1)	0.807	-0.051 (0.128)	0.689	-0.035 (0.079)	0.661	0.869	-		
3	rs2303369	2	27715416	<i>FNDCA</i>	T/C	0.87	0.065 (0.123)	0.597	0.117 (0.162)	0.471	0.084 (0.098)	0.391	0.801	-		
4	rs10183486	2	171990971	<i>TLKI</i>	T/C	0.93	-0.029 (0.157)	0.855	-0.557 (0.222)	0.012	-0.204 (0.128)	0.111	0.052	+		
5	rs7606918	2	172895449	<i>MAP1D</i>	A/G	0.13	-0.097 (0.127)	0.443	0.308 (0.172)	0.074	0.045 (0.102)	0.657	0.058	+		
6	rs4693089	4	84373622	<i>HEL308</i>	A/G	0.67	-0.107 (0.089)	0.230	-0.218 (0.115)	0.058	-0.149 (0.071)	0.035	0.447	+		
7	rs890835	5	175956271	<i>RNF44</i>	A/C	0.76	-0.111 (0.099)	0.259	-0.063 (0.126)	0.615	-0.093 (0.078)	0.231	0.764	-		
8	rs365132	5	176378574	<i>UIMC1</i>	T/G	0.48	0.229 (0.086)	0.008	0.125 (0.108)	0.245	0.189 (0.067)	0.005	0.450	+		
9	rs2153157	6	10897488	<i>SYCP2L</i>	A/G	0.32	0.165 (0.089)	0.065	-0.068 (0.115)	0.551	0.077 (0.071)	0.276	0.109	+		
10	rs1046089	6	31602967	<i>BAT2</i>	A/G	0.60	-0.04 (0.086)	0.640	-0.101 (0.166)	0.543	-0.053 (0.076)	0.487	0.745	+		
11	rs2517388	8	37977732	<i>ASH2L</i>	T/G	0.65	0.014 (0.091)	0.876	-0.165 (0.115)	0.150	-0.055 (0.071)	0.443	0.220	+		
12	rs12294104	11	30382899	<i>Intergene</i>	T/C	0.90	0.052 (0.158)	0.742	-0.202 (0.257)	0.431	-0.018 (0.134)	0.896	0.399	+		
13	rs2277339	12	57146069	<i>PRIMI</i>	T/G	0.23	0.181 (0.116)	0.118	0.290 (0.167)	0.082	0.216 (0.095)	0.023	0.592	+		
14	rs3736830	13	50306221	<i>KPNA3</i>	C/G	0.63	-0.032 (0.088)	0.718	0.050 (0.113)	0.657	-0.001 (0.070)	0.991	0.568	+		
15	rs4886238	13	61113739	<i>TDRD3</i>	A/G	0.96	-0.086 (0.23)	0.708	-0.041 (0.324)	0.899	-0.071 (0.188)	0.705	0.909	-		
16	rs7333181	13	112221297	<i>Intergene</i>	A/G	0.96	-0.147 (0.266)	0.580	0.194 (0.349)	0.580	-0.022 (0.212)	0.917	0.438	+		
17	rs2307449	15	89863928	<i>POLG</i>	T/G	0.36	0.210 (0.087)	0.015	0.006 (0.113)	0.955	0.134 (0.069)	0.050	0.153	+		
18	rs10852344	16	12016919	<i>Intergene</i>	T/C	0.85	-0.065 (0.122)	0.598	0.027 (0.162)	0.867	-0.031 (0.098)	0.749	0.651	+		
19 ¹	rs11668344	19	55833664	<i>TMEM150B</i>	A/G	0.09	0.659 (0.145)	5.6×10^{-6}	0.247 (0.218)	0.257	0.533 (0.121)	1.0×10^{-5}	0.115	+		
19 ²	rs12461110	19	56320663	<i>NLRP11</i>	A/G	0.71	-0.246 (0.092)	0.008	0.157 (0.136)	0.248	-0.119 (0.076)	0.117	0.014	+		
20	rs16991615	20	5948227	<i>MCM8</i>	A/G	0.99	-2.783 (1.745)	0.111	-0.655 (1.425)	0.646	-1.506 (1.103)	0.172	0.345	-		

SNP single-nucleotide polymorphism, *Chr* chromosome, *EAF* effective allele frequency, *SE* standard error, *P*_{METAL} *P* value from meta-analysis using METAL, *P*_{het} *P* value from between-study heterogeneity test, *Dir* allelic association direction compared to that from previous GWAS (“+” denotes same and “-” denotes opposite)

1, 2, and 3 superscript numbers in the column of “Locus” indicate independent signals at the same genetic locus

Sixteen SNPs showed a same direction association in East Asian women as that found in European ancestry women ($P = 0.027$ for binomial sign test). Five SNPs were nominally replicated (Table 4), showing a probability that is significantly higher than what would be expected by chance at the $P < 0.05$ level ($P = 0.003$, binomial sign test). The association for rs4246511 near the *RHBDL2* gene and an intronic SNP rs11668344 in *TMEM150B* remained significant after Bonferroni correction for 21 independent SNPs evaluated in East Asian women ($P < 0.0024$).

Discussion

Although limited in statistical power, in this study of up to 16395 East Asian women, we identified two suggestive novel AM loci and replicated 19 of the 98 independent loci for AM, which were previously reported in European ancestry women. In the analysis of up to 8664 East Asian women, we identified one novel suggestive locus and replicated 5 of the 20 independent loci for ANM.

To our knowledge, this study is the first meta-analysis of GWASs in women from two East Asian countries to search for novel genetic loci associated with AM and ANM. Our study also offered a unique opportunity to assess shared genetic determinants of the AM or ANM loci between women of European ancestry and women of East Asian ancestry.

Several limitations in our study deserve mention. First, the relatively small sample size in our study offered a limited statistical power to detect novel genetic loci for these two complex reproductive phenotypes or to replicate most of the previously reported GWAS loci that have a small per-allele effect. For example, under an additive inheritance mode, with allele frequency of 0.25 and per-allele effect of 0.123 year-change on AM, our study with 16,395 women has only 46 % power to discover the novel locus 10q21.3 represented by rs79195475 at the genome-wide significance level ($P < 5.0 \times 10^{-8}$). Power of 13,518 women with available imputed genotype data ranged from 9 to 100 % to replicate GWAS loci with MAF of 0.05–0.50 and reported per-allele effect between 0.03 and 0.12 years. Second, self-reported AM or ANM may suffer from measurement errors, which further lowered the statistical power of our study. Finally, heterogeneity was observed between studies or sample sets in this two-stage GWAS, although effective allele frequencies are generally comparable across the sample sets

(Tables S1 and S2). We have applied study-specific analyses and adjusted for population stratification in our study to overcome this limitation.

This study highlights a possible novel AM locus near the *NKX2-1* gene. Notably, this promising locus at the *NKX2-1* gene (Table S1) was also associated with AM in the Japanese population ($P = 7.4 \times 10^{-6}$) (Tanikawa et al. 2013). Moreover, meta-analysis of SGWAS data and available summary results of four Japanese GWASs confirmed this novel genome-wide significant AM locus in East Asian women (rs2076751: beta of allele A = -0.105 years, standard error = 0.017, $P = 1.3 \times 10^{-9}$).

Our meta-analysis of summary results from GWAS datasets (not including data of the 2877 samples genotyped using the Sequenom MassARRAY platform) shows a significant association of rs79195475 with AM (beta = -0.145 years, standard error = 0.030, $P = 1.0 \times 10^{-6}$). However, the per-allele effect on AM in the 2877 additional healthy Chinese women from the same population in Shanghai, China, was very low (beta = -0.012 years, standard error = 0.054, not shown in Table 2), thus substantially attenuating the association effect from the GWASs and resulting in significant between-study heterogeneity ($P = 0.020$). The suggestive variant rs79195475 resides in an intergenic region at 10q21.3, which is 707 kb away from the closest gene *CTNNA3* encoding catenin (cadherin-associated protein), alpha 3. This protein plays a role in cell-cell adhesion in muscle tissue. *CTNNA3* gene mutations are thought to cause arrhythmogenic right ventricular cardiomyopathy (van Hengel et al. 2013), and an intron SNP rs12251332 in the *CTNNA3* gene has been implicated in heart failure-related serum pyroglutamine level change (Yu et al. 2013). However, whether the suggestive AM locus tagged by rs79195475 is involved in regulation of *CTNNA3*, the biological mechanism for its association with AM remains unknown. The second suggestive AM locus, tagged by rs1023935, is mapped to a gene desert region at 4p15.1. The per-T allele effect in the GWAS ranged from -0.100 in the SeBCS to -0.200 years in the SGWAS; again, its effect size was relatively small (beta = -0.035) in the 2877 SWHS women (not shown in Table 2).

For ANM, we identified a suggestive locus at 22q12.2 tagged by rs3818134 within intron 3 of the *SFII* gene. Notably, each additional copy of the major T allele decreased 0.454 ± 0.089 years in menopause age in 3556 women participants in the SGWAS, but only 0.063 ± 0.139 years among 1911 unrelated healthy women from the SWHS (not shown in Table 2). The spindle assembly-associated Sfi1 homolog protein encoded by *SFII*

regulates the dynamic structure of centrosome-associated fibers via its interaction with centrin EF-hand protein 2 (Martinez-Sanz et al. 2010). No genetic variants in the *SFII* gene region have been previously reported to be associated with any human disease or trait, including ANM. Therefore, further studies are warranted to investigate this suggestive ANM locus around the *SFII* gene and functionally support its biological connection to ANM.

By analyzing available data of the East Asian women from China and Korea, we nominally replicated 19 out of the 98 AM loci previously identified through GWAS in European ancestry women (Table 3). The effect sizes of these most-significantly associated loci at *LIN28B* and *TMEM38B* were approximately 6.8 and 4.0 weeks, respectively, comparable to those estimated from 182416 European women (Perry et al. 2014). Three independent signals (represented by SNPs rs10816359, rs10453225, and rs10739221) at the *TMEM38B* gene region and two independent signals (represented by SNPs rs2153127 and rs7759938) within the *LIN28* gene region were nominally replicated ($P < 0.05$, Table 3). The third independent signal represented by rs4946632 at the *LIN28B* locus was not replicated ($P = 0.191$, Supplementary Table S3), possibly reflecting allelic heterogeneity among women populations of different ancestry and/or the present under-powered study ($n = 13518$) or a small effect size detected by the European GWAS with a one-order larger sample size (Perry et al. 2014).

We replicated 5 of the 20 previous GWAS-identified ANM loci at $P < 0.05$. These significantly associated SNPs are mapped to the protein-coding genes of *RHBDL2*, *UIMC1*, and two closely neighboring genes, *BRSK1* and *TMEM150B*, within the same LD block, respectively. The *RHBDL2*-encoded human rhomboid-like intra-membrane serine protease could activate epidermal growth factor receptors (Adrain et al. 2011). The coding-synonymous SNP rs365132, in the ubiquitin interaction motif-containing 1 gene (*UIMC1*), is an expression-quantitative trait locus for nearby genes *FGFR4* and *ZNF346* in the cortex. *UIMC1* functions in DNA repair via interaction with BRCA1 and estrogen receptor α (Stolk et al. 2012). *BRSK1* is an AMP-activated protein kinase (AMPK)-related serine/threonine kinase, which regulates neurotransmitter release at the axonal terminals (Inoue et al. 2006). Transmembrane protein 150B (*TMEM150B*), also known as *TMEM224*, belongs to the damage-regulated autophagy modulator family, containing a conserved domain of FGF receptor-activating protein 1. Although the

abovementioned four genes are moderately expressed in human ovaries, no solid evidence indicates a role of their encoded proteins in regulating ovarian aging and thus changing age at menopause.

In conclusion, our genome-wide study of up to 16,395 women of Chinese or Korean ancestry identified two suggestive novel loci for AM and one locus for ANM. In addition, our study nominally replicated 19 AM loci and 5 ANM loci previously identified through GWAS among women of European ancestry. These findings call for larger-scale studies in non-European ancestry populations for identifying additional novel genetic loci and replicating loci for AM and ANM previously identified in women of European ancestry.

Acknowledgments This study was supported, in part, by grants from the US National Institutes of Health (grants R01CA124558, R01CA090899, R01CA070867; R01CA064277 and R01CA092585, R01CA118229, R01CA122756, R01CA137013, R01CA148667), Ingram professorship funds, and Allen Foundation funds at Vanderbilt Epidemiology Center and Division of Epidemiology; SeBCS was supported by the Basic Research Laboratory (BRL) program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2011-0001564). NHAPC was supported by research grants including the National Basic Research Program of China (2012CB524905 and 2011CB504002), Knowledge Innovation Program (KSCX2-EW-R-10), the National Natural Science Foundation of China (81321062, 81170734, 81471013, 81200581, 81202272), and the Knowledge Innovation Program of Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences (2013KIP107 and 2014KIP107). The authors wish to thank Jing He, Hui Cai, Gong Yang, Jie Wu, and Regina Courtney for their contributions to this project, as well as Nan Kennedy for editing and preparing the manuscript. We also thank the research staff of the Shanghai Breast Cancer Genetics Study, Shanghai Endometrial Cancer Genetics Study, the Shanghai Diabetes Genetics Study, the Shanghai Colorectal Cancer Study, the Nutrition and Health of Aging Population in China study, and the Seoul Breast Cancer Study. In addition, sample preparation and GWAS genotyping assays at Vanderbilt were conducted at the Survey and Biospecimen Shared Resources and the Vanderbilt Microarray Shared Resource, which are supported in part by the Vanderbilt-Ingram Cancer Center (P30CA068485).

Compliance with ethical standards

Conflict of interest The authors have no conflict of interest to declare. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or any other funding agency.

Details of ethics approval Written informed consent was obtained from all participants, and the study protocols were approved by the institutional review boards of all institutions involved in the study.

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