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Genetic loci associated with age at menopause and bone mineral density in Southern Chinese women: a replication study

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Purpose: A meta-analysis of genome-wide association studies in Europeans identified 19 loci associated with age at menopause. This study aimed to validate these loci in Southern Chinese women and their association with bone mineral density.
Methods: This was a replication study on 609 women who had participated in the Hong Kong Osteoporosis Study. Archived DNA was genotyped using the Sequenom iPLEX platform. 14 single-nucleotide polymorphisms that had been reported to be associated with age at menopause or bone mineral density in European populations were examined using univariate linear and logistic regression analyses.
Results: Of the 14 genotyped loci, only rs11668344, rs365132, and rs10183486 were associated with age at menopause in Southern Chinese women, with effect sizes of -0.847 (p=0.014), 0.524 (p=0.008), and -1.300 (p=0.028), respectively. Two of them (rs11668344 and rs365132) were associated with early menopause, with the odds ratio being 1.975 (p=0.048) and 0.639 (p=0.032), respectively. Each unit increase in the genetic risk score composed of these two single nucleotide polymorphisms was associated with an odds ratio of 1.62 (p=0.006) for early menopause. rs10183486 was also associated with bone mineral density at the lumbar spine (p=0.048) and femoral neck (p=0.039).
Conclusions: rs11668344, rs365132, and rs10183486 are associated with age at menopause and bone mineral density in Southern Chinese women.

Keywords: Bone density; Menopause; Menopause, premature; Polymorphism, single nucleotide

Introduction

Menopause is a major life event of women that marks the end of a reproductive life. The age at menopause (AAM) varies widely across different populations, with a median of 49 to 52 years. Premature or early menopause can be associated with health implications such as increased risks of cardiovascular diseases[1-4], cardiovascular death[5], stroke[3,6], osteoporosis[4], and all-cause mortality[7], with shortened life expectancy in general. Later AAM is associated with increased risks of cancers of the breast, endometrium, and ovary[8,9].

AAM is a complex trait determined by intricate interactions between a number of genetic and environmental factors. Heritability estimates ranging from 31% to 87% have been reported[10]. Identification of the genetic factors influencing AAM can contribute to understanding of the underlying mechanisms of the ovarian ageing process and the pathogenetic linkage to other health conditions that are associated with early or late AAM.

A meta-analysis of 22 genome-wide association studies (GWASs) in women of European descent identified 19 loci that were associated with AAM[11]. Whether these loci are associated with AAM in the Southern Chinese

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population remains unknown. The current study aimed to validate these loci in Southern Chinese women.

Methods

This study was a secondary analysis of the Hong Kong Osteoporosis Study[12]. In brief, women of Southern Chinese ethnicity who were not receiving medical treatment for osteoporosis or other medications that might affect bone mineral metabolism were recruited from roadshows and health talks in various districts in Hong Kong between 1995 and 2010. The recruitment procedure, inclusion and exclusion criteria have been reported previously[12,13]. This study and the original study were approved by the Institutional Review Board of The University of Hong Kong / Hospital Authority Hong Kong West Cluster. Those who were post-menopausal at recruitment with archived DNA and data on AAM and bone mineral density (BMD) were included in this secondary study.

Early menopause was defined as AAM <45 years. Women with early menopause were compared with those with AAM ≥50 years, which was the median AAM in our cohort.

The archived DNA samples were genotyped for the 20 genetic loci (Table 1), using the Sequenom iPLEX system (Sequenom, San Diego, CA, USA). For quality control, we included single nucleotide polymorphisms (SNPs) with minor allele frequency of ≥0.01, Hardy-Weinberg equilibrium p value of ≥0.0025, and call rate of ≥90%. Of the SNPs, one failed in minor allele frequency (rs16991615), three failed in Hardy-Weinberg equilibrium (rs4246511, rs12294104, rs2307449), and four failed in call rate (rs4246511, rs12294104, rs4886238, rs2307449); these were excluded from analysis.

BMD was measured at the L1 to L4 lumbar spine and femoral neck using dual-energy X-ray absorptiometry, which was calibrated daily, with in vivo precision of 1.2% at the lumbar spine and 1.5% at the femoral neck.

Associations of the minor allele of the GWAS-significant loci with AAM and BMD at baseline were analysed using the univariate linear regression model. Association of the minor allele of the GWAS-significant loci with occurrence of early menopause was analysed using the univariate logistic regression model. Additive genetic model was used in the analyses. A one-sided p value of <0.05 was considered statistically significant if the direction of association was the same. The unweighted genetic risk score was calculated by summing the number of significant at-risk alleles associated with early menopause (ie, the minor allele of rs10183486 and rs11668344, and the major allele of rs365132), or the number of significant at-

Table 1. Significant single-nucleotide polymorphisms (SNP) in genome-wide association study

Chr	SNP	Physical position	Nearest gene(s)	Major/minor allele		Beta (European studies)[11]	Missingness	Minor allele frequency
				This study	European studies[11]			
1	rs1635501	240107398	*EXO1*	T/C	T/C	-0.164	0.003	0.192
2	rs2303369	27568920	*FNDC4*	C/T	C/T	-0.175	0.002	0.161
2	rs10183486	171699217	*TLK1*	C/T	C/T	-0.196	0.062	0.017
2	rs7606918	172603695		A/G	A/G	-0.228	0.007	0.118
4	rs4693089	84592646	*HEL308*	G/A	A/G*	0.228	0.011	0.329
5	rs890835	175888877	*RNF44*	C/A	C/A	0.177	0.009	0.201
5	rs365132	176311180	*UIMC1*	G/T	G/T	0.287	0.007	0.478
6	rs2153157	11005474	*SYCP2L*	A/G	G/A*	0.165	0.01	0.346
8	rs2517388	38096889	*ASH2L*	G/T	T/G*	0.262	0.005	0.379
12	rs2277339	55432336	*PRIM1*	T/G	T/G	-0.380	0.001	0.199
13	rs3736830	49204222	*KPNA3*	G/C	C/G*	-0.180	0.011	0.426
16	rs10852344	11924420	*TNFRSF17, RUNDC2A, GSPT1*	C/T	T/C*	0.168	0.007	0.212
19	rs11668344	60525476	*TMEM150B*	A/G	A/G	-0.416	0.012	0.083
19	rs12461110	61012475	*NLRP11*	G/A	G/A	-0.158	0.007	0.327

* Minor allele was different between data from Stolk et al[11] and our cohort

risk alleles associated with early menopause (ie, the minor allele of rs11668344 and the major allele of rs365132). Statistical analysis was carried out using PLINK v1.07 and SPSS (Windows version 22; IBM Corp, Armonk [NY], US). Power calculation confirmed that our study was well-powered (≥80%) to detect SNP with an effect size of 1% with an additive genetic model, assuming the alpha was 0.05 (1-sided).

Results

A total of 609 participants were included, with median age 63 years (25th to 75th percentile, 57-70 years), median height 1.52 m (25th to 75th percentile, 1.48-1.57 m), median weight 53.6 kg (25th to 75th percentile, 47.5-61 kg), and median AAM 50 years (25th to 75th percentile, 47-52 years).

The studied SNPs were not in linkage disequilibrium. Three of the genotyped loci, namely rs10183486, rs11668344, and rs365132, showed significant association with AAM, with effect sizes (beta) of the minor allele being -1.300 (p=0.028), -0.847 (p=0.014), and 0.524 (p=0.008), respectively (Table 1). The unweighted genetic risk score of these three loci explained 2.4% of the variance in AAM, and each unit increase in genetic risk score was associated with a beta of -0.699 (SE=0.188, p<0.001).

The GWAS-significant loci were compared between those with early AAM (n=55) and those with AAM ≥50 years (n=353). The minor alleles of rs11668344 and rs365132 were associated with increased odds of 1.98 (95% CI=1.01-3.88, p=0.048) and decreased odds of 0.64 (95% CI=0.42-0.96, p=0.032) of early menopause, respectively (Table 2). The genetic risk score composed of these two SNPs showed a significant association with early menopause; each unit increase in genetic risk score was associated with an odds ratio of 1.62 (95% CI=1.15-2.28, p=0.006).

Of the three significantly replicated loci, only rs10183486 was associated with BMD at the lumbar spine and femoral neck, with a beta of -0.044 (SE=0.022, p=0.048) and -0.031 (SE=0.015, p=0.039), respectively (Table 3).

Discussion

To our knowledge, this is the first report on the replication of the genetic loci in association with AAM in

Table 2. Association between significant single-nucleotide polymorphisms (SNP) with age at menopause in Hong Kong Chinese

SNP	Age at menopause			Age at menopause of <45 vs ≥50 years (reference)	
	Beta	SE	p value	Odds ratio (95% confidence interval)	p value
rs1635501	-0.227	0.281	0.21	1.181 (0.706-1.976)	0.526
rs2303369	-0.216	0.302	0.237	1.294 (0.773-2.168)	0.327
rs10183486	-1.300	0.677	0.028	2.490 (0.897-6.915)	0.080
rs7606918	-0.342	0.336	0.154	1.679 (0.952-2.961)	0.073
rs4693089	-0.105	0.232	0.325	1.047 (0.691-1.584)	0.829
rs890835	0.163	0.269	0.273	0.855 (0.513-1.424)	0.547
rs365132	0.524	0.218	0.008	0.639 (0.424-0.963)	0.032
rs2153157	-0.113	0.224	0.308	1.001 (0.666-1.505)	0.997
rs2517388	0.072	0.225	0.375	0.932 (0.610-1.423)	0.744
rs2277339	0.074	0.261	0.389	0.974 (0.608-1.562)	0.913
rs3736830	-0.105	0.226	0.321	1.297 (0.858-1.959)	0.218
rs10852344	0.248	0.261	0.171	0.607 (0.347-1.061)	0.080
rs11668344	-0.847	0.383	0.014	1.975 (1.005-3.882)	0.048
rs12461110	0.229	0.237	0.168	1.028 (0.669-1.577)	0.901
Genetic risk score*	-0.699	0.188	<0.001	1.62 (1.15-2.28)	0.006

* Refers to the number of significant at-risk alleles associated with early age at menopause (ie, the minor allele of rs10183486 and rs11668344, and the major allele of rs365132), or the number of significant at-risk alleles associated with occurrence of early menopause (ie, the minor allele of rs11668344 and the major allele of rs365132) of individual subjects

Table 3. Association between significantly replicated single-nucleotide polymorphisms (SNP) with bone mineral density (BMD) after adjusting for age, sex, height, and weight

SNP	Lumber spine BMD			Femoral neck BMD		
	B	SE	p value	B	SE	p value
rs10183486	-0.044	0.022	0.048	-0.031	0.015	0.039
rs365132	0.002	0.006	0.708	-0.002	0.004	0.670
rs11668344	-0.011	0.011	0.291	-0.007	0.007	0.353

the Southern Chinese population. Our results demonstrated considerable disparity between the genetic factors that determine AAM in the European and Southern Chinese populations. Only three of the genetic loci, namely rs11668344, rs365132, and rs10183486, were common determinants in the two populations. In a replication study on the GWAS-identified SNPs for AAM conducted in Shanghai, only two of our replicated genetic loci, namely rs11668344 and rs365132, were common determinants, and another SNP (rs7246479) related to the former (*TMEM150B*) gene was reported instead.[14] In the Shanghai study, six other significant SNPs were reported but they were not significant in our cohort. Another replication study using GWAS data in the Shanghai and Korean populations replicated five of the loci, namely rs365132, rs11668344, rs4246511, rs2307449, and rs12461110[15]; the first two concurred with our findings in Southern Chinese.

The rs10183486 and rs11668344 loci had the largest effect sizes among those studied. The rs10183486 locus is in the intron of the *TLK1* gene, which codes for a nuclear serine-threonine kinase involved in DNA repair mechanisms and regulation of chromatin assembly[16]. The rs11668344 locus is in the intron region of the *TMEM150B* gene, which encodes a transmembrane protein called DRAM-3 which is ubiquitously expressed in all tissues and may modulate damage-related autophagy[17]. The rs365132 loci is associated with the *UIMC1* gene, which encodes a protein that interacts with BRCA1 and oestrogen receptor-alpha and regulates G2/M phase check-point control of the cell cycle. This protein is hence involved in DNA damage repair and replication[18]. Apart from association with AAM[11], these three SNPs have not been associated with any direct actions on physiological processes in the human ovary. Their underlying mechanisms associated with AAM are unknown. It is possible that the actions of these genetic factors in modulating DNA repair, cell cycle control, and inflammatory processes may interact synergistically to influence ovarian ageing processes.

The absolute effect size of each gene locus in our Southern Chinese population was much smaller than that in European populations[11]. The difference probably arises from ethnic difference in the minor allele frequency and different exposure to various environmental factors. Despite the small effect size of these gene loci and that each unit increase in the unweighted genetic risk score only explained 2.4% of the variance in AAM, the presence of one of the three at-risk alleles resulted in a 62% increase in the odds of early menopause. The synergy of these loci in interaction with various environmental factors is expected to modulate the AAM of each woman.

Only one replicated SNP, rs10183486, was significantly associated with BMD at both the lumbar spine and femoral neck. The minor allele of rs10183486 was associated with both early AAM and lower BMD, conforming with the common understanding that early AAM is considered a predisposing factor to accelerated loss in BMD. Nonetheless, further longitudinal studies with osteoporosis as the outcome measure are needed for validation. Genetic diversity between Southern Chinese and Europeans was great, because allele frequencies of most the SNPs in our population differed from those reported in the European populations[11], and some SNPs in the minor allele in one population was the major allele in the other.

The main limitation of our study was the small sample size. We preliminarily explored how the genetic loci in the European populations were replicated in our population. Larger-scale studies are needed because of the considerable difference between our population and both the European and Shanghai populations. In addition, data were lacking on environmental factors (such as smoking, diet) and past medical history (such as previous ovarian surgery or gonadotoxic treatments), which might interact with the genetic factors in determining AAM. Such gene-environment interactions are worth exploring in larger studies. Furthermore, this study was designed

as a replication study, and hence could not address other significant SNPs in our population that were not significant in Europeans.

Conclusion

Three of the genotyped loci (rs11668344, rs365132, and rs10183486) are associated with AAM in Southern Chinese women. Further investigation is needed to determine the mechanisms of how these associated genes act towards ovarian ageing, the occurrence of menopause, and the association with postmenopausal osteoporosis.

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Declaration

The authors have no conflict of interest to disclose.

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