ApoE genotypes are associated with age at natural menopause in Chinese females

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Abstract Ages at natural menarche and menopause are influenced by several genetic factors. This study aimed to investigate the possible relationship between the apolipoprotein E (ApoE) genotype and the age at menarche and natural menopause in Chinese females. In the current study, 398 (elderly group, aged 47–80 years) and 825 (young group, aged 15–25 years) Chinese females were enrolled under informed content. Ages at natural menarche and menopause were obtained by questionnaires. ApoE genotypes were identified by restriction fragment length polymorphism analysis. In the elderly group, the number of pregnancies and live births and breastfeeding were associated with the age at

menopause ($P=0.008$, $P=0.002$, and $P=0.023$, respectively). One-way ANOVA analysis revealed that the ApoE genotype was significantly associated with age at natural menopause (ANM; $P=0.010$). Compared with ApoE $\epsilon 3/3$ carriers, ApoE $\epsilon 3/4$ females showed a 1.8-year delay in ANM ($P=0.002$). Single ApoE allele-positive/allele-negative analysis also showed that the age at menopause of ApoE $\epsilon 4$ carriers was delayed compared with those who were not carriers ($P=0.023$). In the young group, no statistical difference was found in the age of menarche between the carriers of ApoE $\epsilon 3/3$ and $\epsilon 3/4$. Single ApoE allele-positive/allele-negative analysis showed that the age at menarche in ApoE $\epsilon 4$ carriers was slightly earlier than in those who were not carriers ($P=0.048$). Meanwhile, univariate association analysis revealed that the ApoE genotypes were not significantly associated with the age at menarche using age as a covariate in the pooled group (young+elderly) ($P=0.143$). We demonstrated that the ApoE genotype is significantly linked to the age at natural menopause.

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Introduction

Menarche and menopause are two major cornerstones in women's psychological and physiological well-being. Early onset of natural menopause is associated

with an increased risk for age-related diseases, such as cardiovascular disease (van der Schouw et al. 1996) and osteoporosis (Harlow and Signorello 2000), whereas early onset of menarche and later menopause are associated with a higher risk of breast cancer (Kampert et al. 1988; Hulka 1997; Velie et al. 2005). Thus, investigating the determinants of the age at natural menarche and menopause may be important to postmenopausal women's health.

Although age at menarche and menopause, as complex traits, is influenced by multiple environmental factors, such as nutrition, exercise, socioeconomic conditions, and psychosocial stimuli (Treloar and Martin 1990), genetic factors also play a significant role in contributing to the timing of menarche and menopause. Twin studies have demonstrated a high heritability for age at menarche (53–74%) (Chie et al. 1997) and menopause (63%) (Snieder et al. 1998). Recently, several genome-wide association studies have identified chromosome regions that may harbor genes associated with menarche and menopause (Anderson et al. 2008; He et al. 2009a; Sulem et al. 2009; Elks et al. 2010). However, the specific genetic determinants remain unclear.

Apolipoprotein E (ApoE) has been recognized as an important candidate gene for variation in reproductive occurrences such as age at menarche and menopause (Finch and Sapolsky 1999). There are three isoforms of ApoE in humans: $\epsilon 2$, $\epsilon 3$, and $\epsilon 4$ (Weisgraber 1994). Of the three common human ApoE isoforms, inheriting ApoE $\epsilon 4$ greatly increases the risk for several age-related diseases, such as Alzheimer's disease (Strittmatter et al. 1993), atherosclerosis (Davignon et al. 1988), and other diseases. ApoE also plays a central role in plasma lipoprotein metabolism and lipid transport within tissues (Utermann et al. 1977). In steroidogenic tissues, ApoE is the most important supplier of the cholesterol precursor for steroid hormone production (Mahley and Rall 2000). Thus, ApoE may play a role in the regulation of the steroid hormone system and influence human reproduction (Finch and Sapolsky 1999). However, only a few studies have reported an association between ApoE polymorphisms and age at natural menopause (ANM) (Koochmeshgi et al. 2004; Tempfer et al. 2005; van Disseldorp et al. 2008; He et al. 2009b). The present study aimed to investigate the possible association of ApoE genotype with age at menarche and menopause in Chinese females.

Materials and methods

Subjects

Subjects in the elderly group were those who took part in normal physical examination in the First Affiliated Hospital of Anhui Medical University. The subjects in the young group were Anhui business vocational college student. In all, 398 postmenopausal women aged 45–80 years and 825 young subjects aged 15–25 years were included in the study. A well-trained endocrinologist who was blind to the ApoE genotype information and statistics interviewed the subjects with a unified questionnaire. The questionnaire included information about education, employment, habits, history of menstruation and reproduction, menopausal symptoms, and other details. Those who had liver or kidney disease, an ovariectomy, hormone therapy, or cancers such as hysteromyoma, cervical carcinoma, or breast cancer, were excluded. Menarche was defined as the age at the first menstrual period (years), and natural menopause was defined as age at the last menstrual period (years) without having menstrual periods for at least 12 consecutive months. The study was approved by the medical ethics committee of Anhui Medical University. Each subject provided written informed consent prior to entering the study.

ApoE genotyping

DNA was isolated using a blood genome DNA extraction kit and analyzed for the ApoE genotype by restriction fragment length polymorphism (Hixson and Vernier 1990; Wang et al. 2002). The 227-bp fragment was amplified by polymerase chain reaction (PCR) using the following primers: 5'-TC CAAGGAGCTGCAGGC GGCGCA-3' and 5'-ACAGAATTTCGCCCCGGCCTGTACACTGCCA-3'. Two single nucleotide polymorphism (SNP) sites (SNP ID: rs429358, rs7412, in exon4) were included in the fragment, which divide human ApoE into three isoforms ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$). After amplification, 15 μ l of the PCR product was digested with CfoI units and separated with a 1.2% polyacrylamide gel. The authors who performed the ApoE genotyping were blind to questionnaire information and statistics.

Hormone measurement

The blood of the participants was obtained between 8:00 and 9:00 a.m. Serum was separated from the blood samples and stored at -80°C until measurement. 17β -Estradiol, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels were measured by highly sensitive radioimmunoassays using a commercial kit (BNIBT, Beijing) at the Department of Endocrinology of the First Affiliated Hospital of Anhui Medical University. Intra- and inter-assay coefficients of variation were 3.2% and 5.4% for 17β -estradiol, 3.0% and 6.1% for LH, and 3.3% and 5.6% for FSH, respectively. The sensitivity limits for serum levels of 17β -estradiol, FSH, and LH were 2 pg/ml, 0.2 mIU/ml, and 2 mIU/ml, respectively. Excluding 32 early postmenopausal subjects (1 to 2 years since the cessation of menses) and 12 hemolytic samples, half of the 354 serum samples were selected for hormone measurement according to the blood collection odd numbering sequence. The hormone measurement was performed before ApoE genotyping.

Statistical analysis

The genotypic distribution of ApoE SNPs (112SNP and 158SNP) and alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) was examined against the Hardy–Weinberg equilibrium (H-W-E) by χ^2 tests. The general and menstrual characteristics among different ApoE genotypes were described as the means $\pm$ SD and compared between groups by one-way ANOVA and Student's *t* test. The influence of lifestyle parameters and personal history on age at natural menopause was assessed by Student's *t* test (breastfeeding and use of oral contraceptives), one-way ANOVA (number of pregnancies and live births), and Pearson's correlation analysis (age at menarche and body mass index (BMI)). Furthermore, the association between ApoE genotypes with ANM was compared by univariate analysis with lifestyle parameters and personal history as covariates, which were significantly associated with age at menopause. Lifestyle parameters and personal history among different ApoE genotype were assessed by chi-square (ApoE $\epsilon 3/3$ as reference). The nonparametric Mann–Whitney *U* test was used to compare association between positive/negative ApoE single alleles with menarche and menopause age. Cumulative

incidence analysis was conducted to analyze the age at menarche and menopause among different ApoE carriers. A *p* value ≤ 0.05 was considered to be statistically significant. All statistical analyses were performed using SPSS version 13 with the exception of the cumulative incidence analyses performed using NCSS 2007.

Results

Descriptive characteristics of the study subjects

The characteristics of the elderly subjects are summarized in Table 1. The elderly group used for studying menopause included 398 subjects aged 45–80 years. Among the different ApoE genotype groups, education ($P=0.838$), BMI (kilograms per meter) ($P=0.814$), and use of oral contraceptives ($P=0.819$) were similar. Smoking and alcohol intake characteristics were also included in the questionnaire used in the present study; however, no one reported smoking or drinking habits. The mean ages at menarche and menopause were 14.79 ± 1.8 and 49.87 ± 3.7 years, respectively.

The association between lifestyle parameters and personal history with age at menopause is depicted in Table 2. The number of pregnancies, number of live births, and breastfeeding are associated with the age at menopause ($P=0.008$, $P=0.002$, and $P=0.023$, respectively). Furthermore, Pearson's correlation analysis showed no association between age at menarche and BMI with the age at menopause ($P=0.383$, $r=-0.05$ and $P=0.183$, $r=0.067$, respectively). Lifestyle factors and personal history values among the different ApoE genotypes are shown in Table 3. The risk for late menopause (after 50) was higher in ApoE $\epsilon 3/\epsilon 4$ carriers than in ApoE $\epsilon 3/\epsilon 3$ carriers ($P=0.001$, odds ratio=2.9, 95% confidence interval, 1.5–5.5).

The characteristics of the young subjects are summarized in Table 4. Young group participants were ranged in age from 14 to 25 years. The mean age at menarche was 14.04 ± 1.2 years.

The distributions of the ApoE allele frequencies in young and elderly subjects were as follows: young group, 9.0% for $\epsilon 2$, 81.2% for $\epsilon 3$, and 9.8% for $\epsilon 4$; elderly group, 8.4% for $\epsilon 2$, 84.9% for $\epsilon 3$, and 6.7% for $\epsilon 4$ (Table 5). The genotypic distribution of ApoE SNPs (112SNP and 158SNP) and alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$)

Table 1 ApoE genotypes and their relationships to clinical characteristics of elderly females

ApoE genotype	$\epsilon 2/\epsilon 3$	$\epsilon 3/\epsilon 3$	$\epsilon 3/\epsilon 4$	One-way ANOVA <i>P</i> value	<i>T</i> test (33 to 34) <i>P</i> value
Subject numbers	60	286	44		
Age	63.3±7.4	61.7±7.9	63.6±7.4		0.142
Age at menarche (year)	14.3±1.6	14.8±1.8	14.9±1.8	0.487	0.558
Menstrual periods (%)	92.0	94.92	92.5	0.638	
Number of pregnancies	3.3±1.5	3.6±1.7	3.7±1.7	0.451	0.737
Number of live births	2.2±1.2	2.1±1.0	2.3±0.9	0.347	0.142
Breastfeeding (yes) (%)	85.0	87.4	90.9	0.839	
Use of oral contraceptives (no) (%)	92.1	94.2	89.7	0.819	
BMI (kg/m ²)	23.6±2.9	23.9±2.9	23.9±3.1	0.814	0.955
Education (year)	12.5±2.0	12.8±2.0	12.6±3.0	0.838	0.740
Age at natural menopause (year)	49.8±4.2	49.7±3.6	51.5±3.2	0.010	0.002

Values are given as mean±SD; Student's *t* test and one-way ANOVA analysis were performed. Eight individuals that had genotypes of ApoE $\epsilon 2/\epsilon 2$ (one), $\epsilon 2/\epsilon 4$ (five), and $\epsilon 4/\epsilon 4$ (two) were not included in the statistical analysis

BMI body mass index

in both the young and elderly group was in Hardy–Weinberg equilibrium. The genotypic distribution of ApoE alleles for hormone measurement was also in Hardy–Weinberg equilibrium.

Table 2 Lifestyle parameters, personal history, and age at natural menopause

Parameters	<i>N</i> (%)	ANM	<i>P</i> value
Number of pregnancies			
0	4 (1.0)	45.5±8.4	0.008 ^a
1	28 (7.0)	49.0±3.1	
2	83 (20.9)	49.3±3.9	
≥3	283 (71.1)	50.2±3.5	
Number of live births			
0	9 (2.3)	48.3±6.1	0.002 ^a
1	102 (25.6)	48.8±3.3	
2	162 (40.7)	50.5±3.5	
≥3	125 (31.4)	50.0±3.7	
Breastfeeding			
Yes	347 (87.2)	50.0±3.6	0.013 ^b
No	51 (12.8)	48.7±3.9	
Use of oral contraceptives			
Ever	87 (21.9)	50.4±4.0	0.094 ^b
Never	311 (78.1)	49.7±3.5	

Values are given as mean±SD

BMI body mass index

^aOne-way ANOVA was performed

^bStudent's *t* test was performed

Association of the ApoE genotype with menarche and menopause

In the elderly group (Table 1), we found that the ApoE genotype was significantly associated with the age at natural menopause ($P=0.010$). The covariates did not make a significant contribution to the association between the ApoE genotype and ANM, i.e., the number of pregnancies ($P=0.347$), the number of live births ($P=0.707$), and breastfeeding ($P=0.118$). Age at menopause in ApoE $\epsilon 3/4$ carriers was delayed 1.8 years ($\epsilon 3/3=49.7\pm 3.6$, $\epsilon 3/4=51.5\pm 3.2$, $P=0.002$) compared with $\epsilon 3/3$ carriers. The cumulative incidence for onset age of menopause in the elderly group is shown in Fig. 1b. No association was observed between ApoE genotype and other reproductive characteristic such as age at menarche ($P=0.487$), menstrual periods ($P=0.638$), number of pregnancies ($P=0.451$), number of live births ($P=0.347$), and breastfeeding (yes) (percent) ($P=0.839$). In the young group (Table 4), age at menarche and other menstrual characteristics did not show any significant difference among different ApoE carrier groups. The cumulative incidence for onset age of menarche in the young group is showed in Fig. 1a.

In the association analysis of single ApoE alleles with age at menarche and menopause, age at menopause in ApoE $\epsilon 4$ carriers was delayed approximately 1.2 years compared with those who were not carriers

Table 3 Lifestyle factors and personal history among different ApoE genotype in elderly females

Factors	$\epsilon 2/\epsilon 3$			$\epsilon 3/\epsilon 3$		$\epsilon 3/\epsilon 4$		
	<i>N</i> (%)	<i>P</i> value	OR (95% CI)	<i>N</i> (%)	OR (95% CI)	<i>N</i> (%)	<i>P</i> value	OR (95% CI)
Age at menarche								
≤14	34 (60.7)			139 (50.4)	1	19 (44.2)		
>14	22 (39.3)	0.157	0.7 (0.4–1.2)	137 (49.6)	1	24 (55.8)	0.451	1.3 (0.7–2.4)
Age at menopause								
≤50	30 (50)			184 (64.3)	1	17 (38.6)		
>50	30 (50)	0.038	1.8 (1.0–3.2)	102 (35.7)	1	27 (61.4)	0.001	2.9 (1.5–5.5)
Number of pregnancies								
0	2 (3.3)			2 (0.7)		0		
1	3 (5.0)			22 (7.7)		2 (4.5)		
2	14 (23.3)	0.774	1.2 (0.4–3.6)	57 (19.9)	1	9 (20.5)		
≥3	41 (68.3)	0.603	0.9 (0.5–1.6)	205 (71.7)	1	33 (75.0)	0.647	1.2 (0.6–2.5)
Number of live births								
0	3 (5.0)			6 (2.1)		0		
1	11 (18.3)			81 (28.3)	1	8 (18.2)		
2	29 (48.3)	0.163	1.6 (0.8–3.3)	110 (38.4)	1	21 (47.7)	0.091	2.1 (0.9–4.9)
≥3	17 (28.3)	0.670	0.9 (0.5–1.6)	89 (31.1)	1	15 (34.1)	0.693	1.1 (0.6–2.2)
Breastfeeding								
Yes	51 (85.0)			250 (87.4)	1	40 (90.9)		
No	9 (15)	0.613	1.2 (0.6–2.7)	36 (12.6)	1	4 (9.1)	0.508	0.7 (0.2–2.1)
Use of oral contraceptives								
Ever	15 (25.0)			61 (21.3)	1	10 (22.7)		
Never	45 (75.0)	0.532	0.8 (0.4–1.6)	225 (78.7)	1	34 (77.3)	0.834	1.1 (0.5–2.3)
BMI (kg/m ²)								
≤25	44 (73.3)			198 (69.2)	1	31 (70.5)		
>25	16 (26.7)	0.529	0.8 (0.4–1.5)	88 (30.8)	1	13 (29.5)	0.870	0.9 (0.5–1.9)

For OR values, the most common ApoE genotype, $\epsilon 3/\epsilon 3$, was selected as the Ref; when the number in the group was more than 3, the OR were performed. For factors including two categories, ORs and 95% CIs are given for the bottom line of each parameter versus all others; for the number of pregnancies and births, ORs and 95% CIs are given for 2 versus (0+1) and 3 versus (0+1+2). Age at menarche: 4 missing in $\epsilon 2/\epsilon 3$, 10 missing in $\epsilon 3/\epsilon 3$, 1 missing in $\epsilon 3/\epsilon 4$. Eight individuals that had genotypes of ApoE $\epsilon 2/\epsilon 2$ (one), $\epsilon 2/\epsilon 4$ (five), and $\epsilon 4/\epsilon 4$ (two) were not included in the statistical analysis. The threshold of age at menarche and menopause was defined according to the mean age at menarche and menopause of subjects

BMI body mass index, *OR* odds ratio, *CI* confidence interval, *Ref* reference

Table 4 ApoE genotypes and their relationships to clinical characteristics of young females

ApoE genotype	$\epsilon 2/\epsilon 2$	$\epsilon 2/\epsilon 3$	$\epsilon 2/\epsilon 4$	$\epsilon 3/\epsilon 3$	$\epsilon 3/\epsilon 4$	$\epsilon 4/\epsilon 4$	One-way ANOVA <i>P</i> value
Subject numbers	6	124	13	540	136	6	
Age	18.7±1.5	19.2±1.0	18.7±1.3	19.2±1.1	19.2±1.0	19.3±0.5	
Age of menarche (year)	13.8±0.8	14.1±1.1	13.7±1.1	14.1±1.2	13.9±1.1	14.3±0.5	0.430
Menstrual periods (day)	4.8±1.2	5.1±1.0	5.1±1.0	5.2±1.0	5.3±1.2	4.7±1.0	0.353
Menstrual cycle (day)	30±0.0	29.0±2.4	29.2±1.6	29.2±2.0	29.2±2.0	29.3±1.0	0.871

Values are given as mean±SD; one-way ANOVA analysis was performed

Table 5 Frequencies of ApoE allele ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) and H-W-E test

ApoE allele	Frequency (%)	H-W-E <i>P</i> value
Young		
ε2	9.0	0.802
ε3	81.2	
ε4	9.8	
Elderly		
ε2	8.4	0.690
ε3	84.9	
ε4	6.7	

($P=0.023$) (Table 6). No significant difference was found in the age at menarche between ApoE allele carriers and non-carriers. In the young group, age at menarche in the ApoE $\epsilon 4$ carriers was slightly earlier than the non-carriers ($P=0.048$). No statistically significant difference was found in the age at menarche between ApoE allele-positive and allele-negative carriers in the pooled group (young+elderly) ($P=0.055$). Meanwhile, univariate association analysis revealed that the ApoE genotype was not significantly associated with the age at menarche using age as a covariate ($P=0.143$).

E2/LH/FSH levels of different ApoE groups

There was no difference in hormonal levels of E2 ($P=0.515$), FSH ($P=0.436$), or LH ($P=0.296$) among the different ApoE genotype groups (Fig. 2).

Discussion

In the present study, we performed analyses to examine the association between the ApoE genotype and age at menarche and age at menopause. We found that ApoE $\epsilon 4$ was strongly correlated with delayed onset of menopause in Chinese females. ApoE single allele-positive/allele-negative analysis showed a slightly earlier age at menarche in ApoE $\epsilon 4$ carriers than in non-carriers in the young group. These results suggest an important role of ApoE in reproductive functions.

As complex traits, ANM is determined by both environmental and genetic factors. A large volume of data has been accumulated about various environmental factors contributing to ANM, including smoking (Kinney et al. 2006), oral contraceptive use (Palmer et al. 2003), breastfeeding, and alcohol consumption (Dvornyk et al. 2006). In the present study, univariate analysis revealed that the number of pregnancies and live births were significantly associated with the timing of natural menopause, which is consistent with previous studies (Harlow and Signorello 2000; Hefler et al. 2005). Also consistent with the literatures, we found that breastfeeding was associated with a delayed onset of natural menopause (Zhang et al. 2002; Chang et al. 2007). Other studies have reported that breastfeeding may be a predictor of earlier natural menopause (Dvornyk et al. 2006; He et al. 2009b) or is not associated with ANM (Ozdemir and Col 2004; Long et al. 2006). This inconsistency can be partly attributed to different genetic backgrounds and environmental factors. No

Fig. 1 Cumulative incidence plots for age at menarche (a) and menopause (b) of subjects of different ApoE genotypes ($\epsilon 2/3$, $\epsilon 3/3$, $\epsilon 3/4$)

Table 6 Association analyses of each of ApoE allele ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) with menarche and menopause

ApoE allele	N (%)		P value
Young subjects			
		Menarche age	
$\epsilon 2$	143 (17.3)	14.0 $\pm$ 1.1	
None $\epsilon 2$	682 (82.7)	14.0 $\pm$ 1.2	0.795
$\epsilon 3$	800 (97.0)	14.1 $\pm$ 1.2	
None $\epsilon 3$	25 (3.0)	13.9 $\pm$ 0.9	0.493
$\epsilon 4$	155 (18.8)	13.8 $\pm$ 1.1	
None $\epsilon 4$	670 (81.2)	14.1 $\pm$ 1.2	0.048
Elderly subjects			
		Menarche age	
$\epsilon 2$	62 (16.2)	14.6 $\pm$ 1.6	
None $\epsilon 2$	321 (83.8)	14.8 $\pm$ 1.8	0.305
$\epsilon 3$	375 (97.9)	14.8 $\pm$ 1.7	
None $\epsilon 3$	8 (2.1)	13.8 $\pm$ 2.9	0.370
$\epsilon 4$	50 (13.1)	14.8 $\pm$ 2.1	
None $\epsilon 4$	333 (86.9)	14.8 $\pm$ 1.7	0.816
Young+elderly			
		Menarche age	
$\epsilon 2$	205 (17.0)	14.2 $\pm$ 1.3	
None $\epsilon 2$	1003 (83.0)	14.3 $\pm$ 1.5	0.368
$\epsilon 3$	1175 (97.3)	14.3 $\pm$ 1.4	
None $\epsilon 3$	33 (2.7)	13.8 $\pm$ 1.6	0.208
$\epsilon 4$	205 (17.0)	14.1 $\pm$ 1.5	
None $\epsilon 4$	1003 (83.0)	14.3 $\pm$ 1.4	0.055
Elderly subjects			
		Menopause age	
$\epsilon 2$	66 (16.6)	49.6 $\pm$ 4.2	
None $\epsilon 2$	332 (83.4)	49.9 $\pm$ 3.6	0.826
$\epsilon 3$	390 (98)	49.9 $\pm$ 3.7	
None $\epsilon 3$	8 (2.0)	47.9 $\pm$ 2.4	0.053
$\epsilon 4$	51 (12.8)	50.9 $\pm$ 3.4	
None $\epsilon 4$	347 (87.2)	49.7 $\pm$ 3.7	0.023

Values are given as mean $\pm$ SD. Nonparametric test analysis was performed

significant association of ANM with other personal history and lifestyle factors was observed in the present study.

In addition to environmental factors, a large number of studies have demonstrated that genetic factors play a significant role in contributing to the timing of menopause (Meyer et al. 1991; Snieder et al. 1998; Treloar et al. 1998). ApoE participates in the transport of cholesterol and other lipids by interacting with low-density lipoprotein receptors among various cells of the body (Mahley and Rall 2000). It has been reported that the ApoE genotype is associated with obesity (Long et al. 2003) and increased cholesterol levels during menopause (Hak et al. 2004), which were prevailing in females during the menopause periods (Stevenson et al. 1993; Akaoshi et al. 1996; Pavon de Paz et al. 2006). In addition, some studies have reported that the ApoE genotype is associated with differential reproductive efficiency (Gerdes et al. 1996; Corbo et al. 2004), indicating a possible role of ApoE in human reproduction. These findings are likely to support the role of ApoE in natural menopause.

According to phylogenetic analysis, ApoE $\epsilon 4$ was the ancestral mammalian isoform (Fullerton et al. 2000). However, many studies have demonstrated that ApoE $\epsilon 4$ is an important risk factor for age-related diseases, such as Alzheimer's disease (Strittmatter et al. 1993; Liu et al. 1999), atherosclerosis (Davignon et al. 1988), and other diseases. One may wonder why the ancient ApoE $\epsilon 4$ allele persists if it is disadvantageous. However, in relation to the much shorter lifespan of predecessors of humans (Finch 2007), these disadvantages might not have been a problem. Recently, several groups have reported possible positive functions of ApoE $\epsilon 4$, especially favoring young carriers. Mondadori et al. (2007) reported that

Fig. 2 E2/LH/FSH levels measured in the elderly group according to different ApoE genotype groups. Hormonal levels among different ApoE genotype groups showed no significant

difference. Values are given as the mean $\pm$ SEM. E2 ($P=0.594$), LH ($P=0.378$), FSH ($P=0.436$), one-way ANOVA

young ApoE $\epsilon 4$ carriers had better memory and neural efficiency. Filippini et al. found that the encoding task induced a greater hippocampal activation in $\epsilon 4$ -carriers relative to non-carriers (Filippini et al. 2009). Marchant et al. reported that possession of the $\epsilon 4$ allele confers a cognitive advantage on tasks mediated by the frontal lobe and that young carriers of the $\epsilon 4$ allele showed a larger cognitive benefit from procholinergic nicotinic stimulation (Marchant et al. 2010). Our results showed that ApoE $\epsilon 4$ is associated with a delayed age at menopause, suggesting that ApoE $\epsilon 4$ carriers may have a longer reproductive period, which is important for reproductive function, and will thus have had an advantage in ancient times.

Previously, Tempfer et al. (2005) found that ApoE 158SNP was significantly associated with ANM ($P=0.03$) and ApoE 112SNP was associated with age at menarche ($P=0.06$) in white women. Koochmeshgi et al. (2004) first reported that carriers of ApoE $\epsilon 4$ reached menopause at an earlier age ($P=0.049$) in the Iranian population. He et al. (2009b) found that one SNP (rs769450) in the intron was significantly associated with ANM; however, no association was observed between ApoE $\epsilon 2$, $\epsilon 3$, $\epsilon 4$, and ANM. van Disseldorp et al. (2008) also reported that the polymorphism in the ApoE2 gene was not associated with ANM. In the present study, we found that ApoE $\epsilon 4$ was associated with delayed menopause age. The inconsistency may be due to several factors. First, ethnic differences may have a profound influence on menarche and menopause studies (Gold et al. 2001). Second, environmental factors, life styles, and other factors also play a significant role in women's psychological and physiological well-being (Dvornyk et al. 2006). Furthermore, differences in the number of samples, statistical methods, and details of questionnaires may have influenced the data.

The reproductive characteristics presented in our study were obtained by questionnaire, potentially incurring a recall bias. Several studies have demonstrated that the accuracy of long-term recall of age at menarche and menopause varies between 70% and 84% and depends on recall years (Casey et al. 1991; den Tonkelaar 1997; Must et al. 2002). To enhance the questionnaire accuracy, elderly subjects aged from 45 to 80 were used to study the age at menopause, while young participants aged 14–25 years were used to study age at menarche.

In summary, the present study provides further evidence for the association between ApoE genotype and the age at menarche and menopause in Chinese females. We demonstrated that the age at menopause was greatly delayed in ApoE $\epsilon 4$ carriers compared with that of non-carriers. Although the number of pregnancies and live births and breastfeeding were associated with ANM, these covariates did not make a significant contribution to the association between the ApoE genotype and ANM. Taken together, these findings indicate that ApoE $\epsilon 4$ may be associated with a longer reproductive period, the underlying mechanism of which remains to be resolved.

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