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A polymorphism of apolipoprotein E (APOE) gene is associated with age at natural menopause in Caucasian females

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Abstract

Objective—The present study aimed to investigate possible association of the apolipoprotein E (APOE) gene polymorphisms with age at natural menopause (ANM) in Caucasian females.

Design—Four SNPs (including two replacements, SNP3 Cys112Arg and SNP4 Arg158Cys) were genotyped in 253 randomly selected unrelated Caucasian women having experienced natural menopause. The comprehensive statistical analyses focusing on the association of the APOE gene and some environmental factors with ANM were conducted.

Results—Alcohol consumption was a significantly predictor of earlier natural menopause ($P < 0.05$). One SNP (rs769450) was significantly associated with ANM according to both population based and the transmission disequilibrium test (TDT) analyses ($P = 0.007$ and 0.046 , respectively). However, no association was observed between APOE $\epsilon 2, \epsilon 3, \epsilon 4$ and ANM.

Conclusions—Genetic variation in the APOE gene may influence the variation in ANM in Caucasian women.

Keywords

age at natural menopause (ANM); association; APOE; polymorphism

Introduction

Natural menopause status is considered as an important cornerstone which is related to women's psychological and physical well-being. The timing of natural menopause may serve as a marker for the process of reproductive ageing and as an indicator of postmenopausal

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women health risks. Earlier menopause was reported to increase the risk of several female age-related diseases, such as ovarian cancer [1,2], osteoporosis [3,4], and cardiovascular disease [5], whereas later menopause contributes to the higher risk of breast [6] and endometrial cancer [7]. Thus, knowing the determinants of age at natural menopause (ANM) may help to foresee possible postmenopausal health complications.

Similar to other complex traits (e.g., osteoporosis, obesity, etc.), ANM is determined by both environmental and genetic factors [8–14]. Large volume of data has been accumulated about various environmental factors contributing to ANM, including smoking [8], oral contraceptive use [9], breastfeeding and alcohol consumption [10]. Several studies have reported that genetic factors could explain a large proportion of ANM variation [11–14]. The heritability of ANM was estimated to vary between 49–52% [13] and 63% [14]. To date, a number of studies have been conducted to identify genetic determinants of ANM using linkage [15,16] and candidate genes association approaches [17–22]. However, the causal genetic variants and associated physiological mechanisms underlying the variation of ANM need further studying.

One of the consequences of menopause is an average increase in total cholesterol [23]. Allelic variation of apolipoprotein E (APOE) has been shown to influence the level of serum lipids, especially in postmenopausal women [24,25]. It has been found that the effect of menopause on LDL-cholesterol level significantly varied according to the APOE genotype [26]. In addition, some previous studies reported a possible role of APOE in human reproduction, specifically association of allele $\epsilon 3$ with the highest fertility [27–28]. All these data suggest that APOE may contribute to the onset of menopause.

APOE has been recognized as an important candidate gene for many postmenopausal human diseases. Numerous studies have demonstrated the association between the APOE polymorphisms and Alzheimer's disease [29], osteoporosis [30], breast cancer [31], atherosclerosis [32], and coronary disease [33]. However, only few studies about association between APOE polymorphisms and ANM have been reported thus far [20,34].

The present study examines possible association of the APOE polymorphisms with ANM in postmenopausal Caucasian women.

Material and methods

Subjects

This study was approved by the Creighton University Institutional Review Board. All the study subjects signed informed-consent documents before entering the project. The subjects came from an expanding database being created for studies to search for genes underlying the risk of osteoporosis and obesity, which is underway in the Osteoporosis Research Center (ORC) of Creighton University. A random sample of 260 Caucasian females with natural menopause was included in the present study. All the study subjects signed informed-consent documents before entering the project. All of participants are US Caucasians of European origin and came from our previous studies [10,35]. Information about ANM, age at menarche (AAM), current smoking habits, breastfeeding, number of pregnancies (parity), and alcohol consumption before menopause was recorded for each subject by nurse-administered questionnaire.

AAM was calculated as the age of first menstrual period, following the onset of menses minus the date of birth (in years rounded to the tenth decimal); ANM was defined as an age at the last menstrual period (years) without having menstrual periods for at least 12 consecutive months. Women with surgical menopause, a history of perimenopausal hormone therapy use before menopause and women with probable premature ovarian failure (i.e. age at menopause < 38 years, 6 subjects) were excluded from this study. The varied range for ANM in our study

followed the distribution of menopausal ages in Caucasian females [14]. Thus, finally 253 postmenopausal women with the data about ANM were involved into the study.

Genotyping

SNPs were identified by searching through public databases, such as dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>), JSNP (<http://snp.ims.u-tokyo.ac.jp/>). SNPs in the APOE gene were selected on the basis of the following criteria: (1) validation status, especially in Caucasians, (2) position in or around the gene, (3) degree of heterozygosity, i.e. minor allele frequencies (MAF) > 0.05, (4) functional relevance and importance, and (5) reported to dbSNP by various sources. Finally 4 SNPs (rs440446, rs769450, rs429358, and rs7412) were selected for the analysis.

Genomic DNA was extracted from peripheral blood using a commercial isolation kit (Gentra Systems, Inc. Minneapolis, MN, USA). DNA concentration was assessed by a DU530 UV/VIS Spectrophotometer (Beckman Coulter, Inc. Fullerton, CA, USA). The genotyping procedure and primers for the four SNP markers were detailed elsewhere [35].

Statistical analyses

Hardy-Weinberg equilibrium (HWE) analyses—The program PowerMarker [36] was used to calculate allele frequencies. The χ^2 test was adopted to check correspondence of the genotype data to the Hardy-Weinberg equilibrium.

Association analyses of the four single markers—Stepwise multiple regression was used to adjust the effects of the lifestyle factors (here including smoking, breastfeeding, alcohol consumption, pregnancies and AAM) on ANM. We also analyzed the influence of amount of alcohol consumption on ANM. To investigate association between any single SNP and ANM, we employed two complementary statistical designs. First, we used the population-based association test, which has maximal power to detect association, but is susceptible to false positive results because of population stratification [37]. Population based association between each single SNP and ANM was estimated using the nonparametric test. $P < 0.05$ was considered significant. All the statistical analyses were performed using SPSS (v. 13.0, Inc. Chicago, IL). Then we extracted 30 pedigrees (69 subjects) from the sample and used them for the family based association analysis (transmission disequilibrium test, TDT) [38]. The Haploview software [39] was utilized for the TDT.

Association analyses for APOE $\epsilon 2$, $\epsilon 3$, $\epsilon 4$ —Human APOE is a polymorphic protein with three common isoforms (APOE $\epsilon 2$, $\epsilon 3$, $\epsilon 4$), encoded by three alleles ($\epsilon 2$, $\epsilon 3$, $\epsilon 4$) of a single gene on chromosome 19q13.2. From the genotypes of SNP3 (rs429358, Cys112Arg Exon4) and SNP4 (rs7412, Arg158Cys Exon4), we obtained five distributions of the APOE genotype ($\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$, $\epsilon 4/\epsilon 4$) through various combinations of any two of the three major alleles. This is a commonly used approach in population association studies of APOE with various traits [40–42]. In this part of study, our available sample contained 243 subjects excluding 10 with missing genotype data. Then we assigned subjects to three genotype groups: genotype group $\epsilon 2$ ($\epsilon 2/\epsilon 2 + \epsilon 2/\epsilon 3 + \epsilon 2/\epsilon 4$), group $\epsilon 3$ ($\epsilon 3/\epsilon 3$) and group $\epsilon 4$ ($\epsilon 4/\epsilon 4 + \epsilon 3/\epsilon 4$). Using the nonparametric test we established the contribution of the APOE genotype to the phenotypic variation of ANM. We considered two-sided P value < 0.05 as significant. SPSS 13.0 (SPSS Inc., Chicago, IL) was used for all analyses.

Results

Sample characteristics

The basic characteristics of 253 study subjects are presented in Table 1. The mean ANM of these subjects was 49.22 ± 0.24 years, ranging from 38 to 58 years. The stepwise multiple regression analysis showed significant effect of alcohol consumption on ANM ($P = 0.028$). The univariate regression analysis indicated similar patterns for not only alcohol consumption ($P = 0.017$) but also for breastfeeding ($P = 0.042$). Women who have drunk alcohol had, on average, 1.0 years earlier menopause than those have never had. And women which had 3–6 times/week of alcohol consumption had 1.3 years earlier menopause than that of the average ANM of others who had alcohol consumption ($P = 0.034$). Likewise, breastfeeding seemed to promote 1.1 years earlier menopause. Table 3 provides a summary of the confounding factors for the ANM association analyses.

Association analyses for the single markers

The information of the four SNPs analyzed is shown in Table 2. All of them were in Hardy-Weinberg equilibrium ($P > 0.05$). The minor allele frequencies (MAFs) ranged from 0.075 to 0.437. The results of four single SNP association analyses are summarized in Table 3. SNP2 (rs769450) showed significant association with ANM in the population-based analyses ($P = 0.007$). Consistent with these results, significant association was also observed in the family-based TDT analysis, though P value was slightly lower (0.046). As shown in Figure 1, the subjects carrying genotype AA for SNP2 had, on average, ~1.93 year earlier onset of menopause than those with the GA genotype (47.90 versus 49.83 years).

Association analyses for APOE $\epsilon 2$, $\epsilon 3$, $\epsilon 4$

The distribution of the APOE allele frequencies in our 243 women were as follows: 9.47% for $\epsilon 2/\epsilon 3$, 5.35% for $\epsilon 2/\epsilon 4$, 63.37% for $\epsilon 3/\epsilon 3$, 20.58% for $\epsilon 3/\epsilon 4$ and 1.23% for $\epsilon 4/\epsilon 4$ (Table 5). Genotypic distribution was consistent with HWE ($P > 0.05$). The $\epsilon 2/\epsilon 2$ genotype was absent in our sample. No significant difference in ANM was observed for any single genotype of the five. We did not find any significant association for $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$ alleles either. Even when the subjects were partitioned into three genotype groups according to carrying/not carrying a particular allele, no significant difference for ANM between the groups was detected (Table 5).

Discussion

In the present study we performed analyses to examine association between four APOE polymorphisms and ANM. We also analyzed the probable correlation between the APOE genotypes ($\epsilon 2/\epsilon 3$, $\epsilon 2/\epsilon 4$, $\epsilon 3/\epsilon 3$, $\epsilon 3/\epsilon 4$, $\epsilon 4/\epsilon 4$) and ANM. In the single SNP analyses, we found a significant relationship between SNP2 (rs769450) and ANM.

ANM is affected by environmental and lifestyle factors. In the present study we found that alcohol consumption and breastfeeding were likely promoting the earlier onset of natural menopause. These two significant factors and other factors such as smoking and the number of pregnancies were discussed in our previous study [10]. We did not find any significant association between AAM and ANM that was consistent with the results of some previous studies [9,13,18,43,44]. However, other studies have reported positive [45] or inverse [46, 47] association between them. This inconsistency can be partly attributed to different genetic backgrounds, environmental and lifestyle factors.

Both population- and family-based analyses suggested that SNP2 (rs769450) is significantly associated with ANM. A large body of data provide support for such association. APOE is a

ligand for various cell-surface receptors including the low-density lipoprotein (LDL) receptor, the LDL-receptor related protein and the very low-density lipoprotein (VLDL) receptor [48]. It serves as a ligand for the receptor-mediated uptake of cholesterol-rich particles by hepatocytes and peripheral tissues [49]. On the one hand, APOE plays a major physiological role in the regulation of overall lipid and lipoprotein homeostasis [48,50,51]. Several studies reported association between APOE and obesity [35,52,53]. On the other hand, menopause is one of the critical periods of a woman's life during which obesity prevalence is the highest [54]. Serum lipid level increases during hormonal transition before and after menopause [55]. As a result, postmenopausal women have significantly more fat and lower lean tissue mass than premenopausal women [56]. It is thought that at least part of the higher prevalence of cardiovascular disease in postmenopausal women may be due to increased central body fatness [57]. Postmenopausal obesity is commonly associated with many physiological disorders that results in higher mortality [58]. APOE genotype also contributes to the variation in cholesterol increase with menopause [26]. In addition, it has been found that the APOE gene could determine serum testosterone and dehydroepiandrosterone levels in postmenopausal women [59]. All these results are in favour that APOE likely contributes to the development of menopause.

Previously, Tempfer et al.[34] found that SNP4 (rs7412) was significantly ($P=0.03$) associated with lower ANM in an ethnically homogenous cohort of Middle European white women. Koochmeshgi et al.[20] reported that the APOE genotype group $\epsilon 4$ predicted earlier age at menopause with the two other genotype groups ($P=0.049$) in the Iranian population. However, we did not find any significant association between the above two genotypes and ANM in our study sample. In addition to the differences in sample size, statistical methods and confounding environmental factors, the inconsistency may be mostly attributed to differences in the ethnic background of the population. The abovementioned studies used virtually monoethnic cohorts. Specifically, in the study by Tempfer et al. [34], only women of the Austrian and German ancestry were included to avoid confounding by ethnicity, while the study sample of Koochmeshgi et al. [20] consisted only of Iranian women. Although all subjects in our study identified themselves as Caucasians, they might in fact represent different ethnic subgroups, e.g., Northern or Southern Europeans, Jews, etc. Ethnicity may be an important factor contributing to the complex traits with between-ethnic differences in prevalence [60–63] and interacting with the genetic factors underlying these traits [64–66]. However, despite the inconsistencies as regards the association of the particular polymorphisms, all above results are in favor that the APOE gene is likely associated with ANM.

Like many other studies involving in reproductive history characteristics, the data of AAM and ANM were obtained by questionnaire, which might potentially incur a recalling bias. Specifically, several studies have demonstrated that accuracy of long-term recall of AAM and self-reported ANM varied between 70% and 84% [67–70].

In the present study we did not find significant association between APOE $\epsilon 2$, $\epsilon 3$, $\epsilon 4$ and ANM, though the $\epsilon 4$ allele is recognized as the most commonly identifiable genetic risk factor in some postmenopausal disorders, such as Alzheimer's disease [50], coronary artery disease [32], and low bone mass [30]. On the other hand, one SNP (rs769450) within the intron 2 of the APOE gene was significantly associated with ANM.

In summary, this study provides further evidence for that APOE gene may influence timing of menopause and, thus, potentially contribute to development of diseases associated with age of menopause. Further association studies utilizing denser markers within the APOE gene and larger sample size are required to better clarify the importance of the APOE gene polymorphisms for the onset of menopause.

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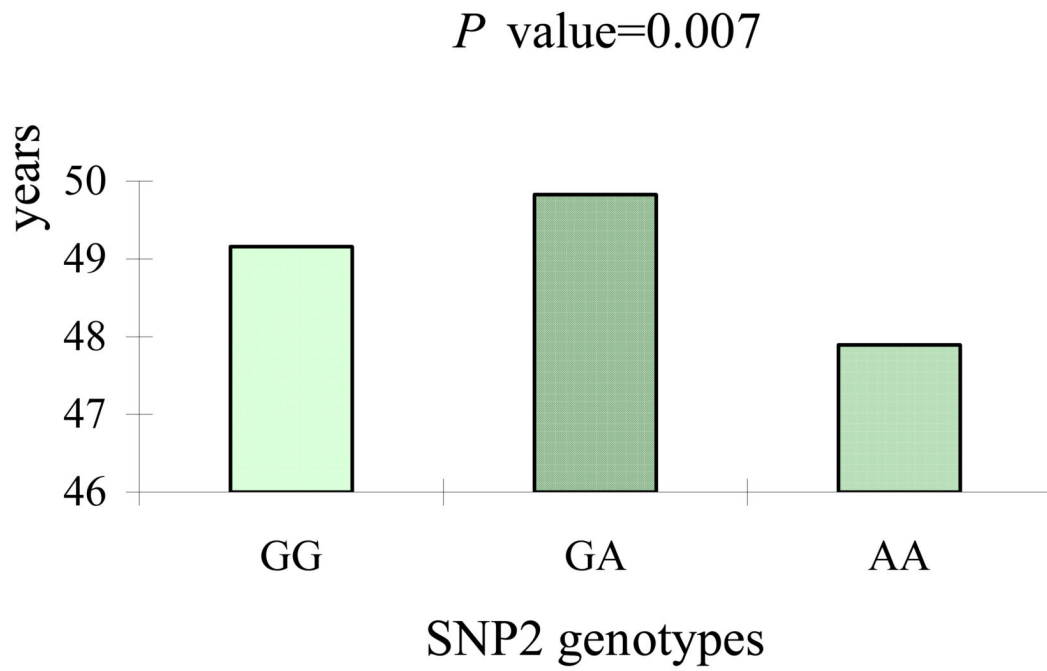


Figure 1. Differences in the ANM across genotypes of the SNP2. The subjects carrying genotype AA for SNP2 had, on average, ~1.93 year earlier onset of menopause ($P = 0.007$) than those with the GA genotype (47.90 versus 49.83 years).

Table 1

Characteristics of women involved in the study

	Number (% of total sample)	Mean \pm S.E.
Age(year)	251 (99.20)	62.25 \pm 0.62
Age at natural menopause	253	49.22 \pm 0.24
Height	182 (71.94)	1.62 \pm 0.00
Weight	185 (73.12)	72.96 \pm 1.77
Age at menarche	247 (97.63)	13.06 \pm 0.09
Smoking	39 (15.42)	
Breastfeeding	136 (53.75)	
Alcohol consumption	167 (66.01)	
<1-2/week	85	
1-2/week	25	
3-6/week	41	
7-10/week	8	
>10/week	8	
No. of pregnancies	243 (96.05)	
None	10	
1-3	120	
4-6	82	
>7	41	

Table 2

Information of SNPs for APOE

SNP	SNP ID	Genomic position ^a (bp)	Allele variants ^b	Position in the gene	MAF
SNP1	rs440446	50101007	G/C	Intron1	0.331
SNP2	rs769450	50102284	G/A	Intron2	0.437
SNP3	rs429358	50103781	T/C	Cys112Arg Exon4	0.144
SNP4	rs7412	50103919	C/T	Arg158Cys Exon4	0.075

^aPositions are based on the GenBank data, www.ncbi.nlm.nih.gov/SNP.

^bThe minor allele is bold.

GenBank accession no. NC_000019.

Table 3

Results of the univariate and multiple analyses for association between lifestyle factors and ANM

Factor	<i>P</i> value for univariate analyses	<i>P</i> value for stepwise regression
Smoking	0.139	0.993
Alcohol consumption	0.017	0.028
No. of pregnancies	0.817	1.000
breastfeeding	0.042	0.997
Age at menarche	0.768	0.998

P value <0.05 are shown in bold.

Table 4

The results of single SNPs association analyses

SNP	<i>P</i> value for Population-based analyses	<i>P</i> value for TDT analyses
SNP1	0.714	0.317
SNP2	0.007	0.046
SNP3	0.592	0.132
SNP4	0.255	0.257

P value <0.05 are shown in bold.

Table 5

Distribution of APOE genotypes and allele frequencies and results for relative association analyses

	Number of subjects (%)	<i>P</i> value
$\epsilon 2/\epsilon 3$	23 (9.47)	0.831
$\epsilon 2/\epsilon 4$	13 (5.35)	0.134
$\epsilon 3/\epsilon 3$	154 (63.37)	0.185
$\epsilon 3/\epsilon 4$	50 (20.58)	0.546
$\epsilon 4/\epsilon 4$	3 (1.23)	0.967
$\epsilon 2$ allele	36 (7.40)	0.261
$\epsilon 3$ allele	381 (78.40)	0.180
$\epsilon 4$ allele	69 (14.20)	0.195
APOE group $\epsilon 2$	36 (10.00)	0.746
Non-APOE group $\epsilon 2$	207 (90.00)	
APOE group $\epsilon 3$	154 (66.96)	0.373
Non-APOE group $\epsilon 3$	89 (33.04)	
APOE group $\epsilon 4$	53 (23.04)	0.444
Non-APOE group $\epsilon 3$	190 (76.96)	