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Association analyses suggest multiple interaction effects of the methylenetetrahydrofolate reductase polymorphisms on timing of menarche and natural menopause in white women

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Abstract

OBJECTIVE

To investigate whether polymorphisms of the methylenetetrahydrofolate reductase gene (MTHFR) are associated with age at menarche and age at natural menopause in Caucasian women.

METHODS

In a cross-sectional study, in total 305 randomly selected unrelated Caucasian women were genotyped for 6 SNPs of the *MTHFR* gene (including one common replacement, rs1801133). This sample was comprehensively analyzed for association of the SNPs with age at menarche. Then a subsample of 210 women who experienced natural menopause was analyzed for association of the *MTHFR* gene with age at natural menopause.

RESULTS

Duration of breastfeeding was a significant predictor of earlier natural menopause ($P < 0.05$). No individual SNPs were associated with either age at menarche or age at natural menopause. However, three significant ($P < 0.05$) SNP/SNP interaction effects (rs2066470/rs1476413, rs2066470/rs4846049, and rs17037390/rs4846049) on the onset

of menarche were determined. Three haplotypes were significantly associated with age at menopause ($P < 0.05$). Four SNPs (rs2066470, rs17037390, rs1801133, and rs4846048) indicated significant interaction effects with various lifestyle factors on age at natural menopause.

CONCLUSIONS

The results of our study suggests that the *MTHFR* gene may influence the onset of menarche and natural menopause. This effect is probably due to the multiple SNP/SNP and SNP/environment interactions. More independent studies are needed to further clarify the possible contribution of this gene to the timing of menarche and menopause.
Keywords: age at menarche, age at natural menopause, association, MTHFR, polymorphisms, haplotypes

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Introduction

Menarche and menopause are two key physiological events in female life, which mark respectively the lower and upper limits of a reproductive period. In addition to this, the onsets of menarche and menopause affect female well-being in later life. In particular, early age at menarche (AM) was associated with the higher risk for endometrial [1], breast [2,3], and ovarian [4,5] cancers, psychological problems [6], and obesity [7,8], while the later menarche may increase the risk of osteoporosis [9,10] and preeclampsia [11]. Age at natural menopause (ANM) is associated with numerous postmenopausal health problems, including osteoporosis [12,13], cardiovascular disease [14], ovarian [15,16], breast [17] and endometrial cancer [18], to name a few. Therefore, identifying the factors, which determine AM and ANM, may potentially help in preventing these health complications.

Both AM and ANM are complex traits and are thus determined by multiple environmental and genetic factors as well as their interactions [19–21]. Various studies estimate a contribution of genetic factors to the variation of these traits about 45–74% for AM [22–24] and 63–74% [22,25] for ANM.

Research on genetic factors underlying AM and ANM has attracted an increased interest in recent years. As a result, several genes and genomic regions were reported as the candidates (see, for example, [26–29].

The methylenetetrahydrofolate reductase gene (*MTHFR*) may be a potential addition to this list. This gene encodes for an enzyme, which catalyzes irreversible reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a substrate for homocysteine remethylation to methionine. Biological role of the encoded protein is likely related to its involvement into homocysteine metabolism. Individuals who carry missense mutations, which result in the decreased activity of the enzyme, develop severe hyperhomocysteinemia, homocystinuria, motor dysfunction, various neurological and vascular problems [30]. Plasma homocysteine levels are affected by menses and menopause [31–33] and may also be influenced by the *MTHFR* polymorphisms [34,35]. Folate was shown to have a modulatory effect on carcinogenesis [36]. A common polymorphism of the *MTHFR* gene, C677T (rs1801133), was suggested to play a role in susceptibility of women to various cancers of the reproductive organs, namely cervical[37,38], ovarian[39], breast [39,40], and endometrial [41,42]. The T allele of this polymorphism was also implicated in the increased fracture risk in postmenopausal women [43,44]. All these data suggest that the *MTHFR* gene may be associated with AM and/or ANM. In the present study, we analyzed 6 single nucleotide

polymorphisms of the *MTHFR* gene for their association with AM and ANM in a sample of Caucasian females.

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Materials and methods

Participants

All of participants came from our previous studies [45;46]. The protocol of the present study was approved by the Institutional Review Board of Creighton University. Information about the following reproductive history and lifestyle factors was collected from subjects through a nurse-administered questionnaire: number of pregnancies, length of breastfeeding, smoking and alcohol consumption habits, use of hormonal contraceptives. Informed consent was obtained from each study subject prior to entering the project. The exclusion criteria for the subjects were detailed elsewhere [47]. In brief, they included, among the others, chronic diseases of vital organs (brain, lung, heart, liver, kidney), systemic metabolic diseases (diabetes, hypo- and hyperparathyroidism, hyperthyroidism, etc.), and malnutrition conditions (chronic diarrhea, chronic ulcerative colitis, etc.), etc. The assessment of the exclusion criteria was conducted through nurse-administered questionnaires and/or medical records. Women with ANM below 40 years were also excluded from the analyses as probably having experienced premature ovarian failure. A total of 305 otherwise healthy Caucasian females of European origin were included in the study. For the association analysis of AM and ANM, two subsamples of unrelated women (305 and 210 subjects, respectively) were selected from the total sample. The size of the ANM sample was smaller due to exclusion of females with surgical menopause.
AM was defined as the age of first menstrual bleeding less the birth date (in years rounded to the tenth decimal); ANM was calculated as the age at the last menstrual period (years) followed by 12 consecutive months without menses. The data about the subject samples are given in Table 1.

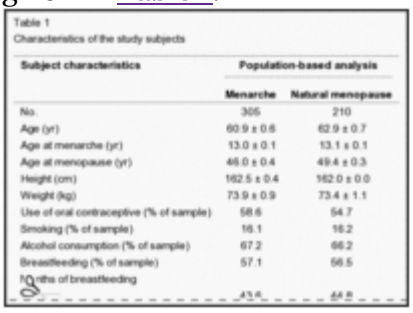

Table 1
Characteristics of the study subjects

Subject characteristics	Population-based analysis	
	Menarche	Natural menopause
No.	305	210
Age (yr)	60.9 ± 0.6	62.9 ± 0.7
Age at menarche (yr)	13.0 ± 0.1	13.1 ± 0.1
Age at menopause (yr)	46.0 ± 0.4	49.4 ± 0.3
Height (cm)	162.5 ± 0.4	162.0 ± 0.0
Weight (kg)	73.9 ± 0.9	73.4 ± 1.1
Use of oral contraceptive (% of sample)	58.6	54.7
Smoking (% of sample)	16.1	16.2
Alcohol consumption (% of sample)	67.2	66.2
Breastfeeding (% of sample)	57.1	56.5
[illegible]nths of breastfeeding	[illegible]	[illegible]

Genotyping

Genomic DNA was isolated from blood buffy coat using a commercial kit (Gentra Systems, Inc. Minneapolis, MN, USA). The SNPs were genotyped using Integrated BeadArray System (Illumina Inc.).

Statistical analyses

The χ^2-test was applied to check the Hardy Weinberg equilibrium of all SNPs. In addition, Mendelian consistency of genotype data was verified using PedCheck [48]. The effects of the lifestyle factors and the*MTHFR* gene polymorphisms on AM and ANM were estimated by linear regression models.
For two SNPs, rs2066470 and rs17037390, the minor allele homozygotes were of too low frequencies, so that they were combined with heterozygotes in a single group. In such a case, two groups (with or without the minor allele) were analyzed instead of the three (minor allele homozygote, heterozygote, and major allele homozygote). The subjects were also categorized according to the number of pregnancies and months of breastfeeding (Table 1). The association of the SNPs and lifestyle factors with AM and ANM was examined by both stepwise multiple regression and univariate analysis of variance. In the univariate analysis, each marker was investigated independently. The analyses were performed using SPSS (v. 16.0.1, Inc. Chicago, IL) and the PLINK software [49], available at http://pngu.mgh.harvard.edu/~purcell/plink/.

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Results

Study participants' characteristics

The total sample used in the study consisted of 305 subjects. The mean AM of the subjects was 13.0 ± 0.1 years and the mean ANM was 46.0 ± 0.4 years. The relatively low ANM in the total sample was due to the inclusion of subjects with surgical menopause. The age at surgical menopause for this population is about 40 years [50]. The ANM in the subsample of postmenopausal women was higher (49.4 ± 0.3 years, Table 1) but still lower than the average ANM for the US female population (about 51 years). This may be due to the interpopulation differences in ANM across the USA. On the other hand, the median ANM (50 years) of our subsample was very similar to the mean ANM for the US female population. The parameters of skewness and kurtosis of the ANM for the subsample were −0.020 ± 0.169 and 0.830 ± 0.337, respectively. The former falls within the range of normal distribution, the latter slightly deviates from that. Altogether, these results suggested that our sample was not biased.
The multiple regression analysis showed that, among the lifestyle factors considered, smoking (P = 0.03), alcohol consumption (P = 0.03) and length of breastfeeding (P = 0.03) had significant effect on ANM in this sample. These variables were used as covariates in the subsequent association analysis. No lifestyle factors were used as covariates for the AM association analyses, as they all occurred presumably after menarche. Length of breastfeeding seems to be a high risk factor for earlier natural menopause: women having breastfed for more than one year have about 2.2 times (95% CI = 1.1–4.6) higher risk to pass menopause before the average ANM for the population, i.e., 49 years, than those who have never breastfed. All studied polymorphisms indicated no deviation from HWE (Table 2).

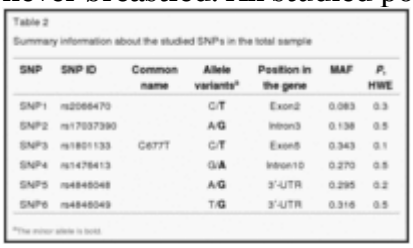

Table 2
Summary information about the studied SNPs in the total sample

SNP	SNP ID	Common name	Allele variants[a]	Position in the gene	MAF	P_{HWE}
SNP1	rs2066470		C/T	Exon2	0.083	0.3
SNP2	rs17037390		A/G	Intron3	0.138	0.5
SNP3	rs1801133	C677T	C/T	Exon5	0.343	0.1
SNP4	rs1476413		G/A	Intron10	0.270	0.5
SNP5	rs4846048		A/G	3′-UTR	0.295	0.2
SNP6	rs4846049		T/G	3′-UTR	0.316	0.5

[a]The minor allele is bold.

Table 2
Summary information about the studied SNPs in the total sample

SNP association analyses

The analyses did not reveal any association between the individual SNPs of the *MTHFR* gene and AM. However, three significant pairwise SNP interactions were determined: SNP1/SNP4 ($P = 0.02$), SNP1/SNP6 ($P = 0.02$), and SNP2/SNP6 ($P = 0.04$). Similar to AM, no association between the individual *MTHFR* polymorphisms and ANM was determined. Interaction effects between the SNPs were not detected either. However, several haplotypes were found to be significantly associated with ANM (Table 4). Interestingly, all these haplotypes included SNP4 (rs1476413) located in an intron of *MTHFR*. An average contribution of each haplotype to the overall variation of ANM in the studied population is about 2% (Table 4, coefficient R^2). Some haplotypes (e.g., A and C) are quite common in the population. The rarest haplotypes (B and D) seem to be associated with the lowest ANM: their carriers enter menopause, on average, about 2.7 years earlier than the noncarriers (Table 4, coefficient β). Several SNPs indicated significant interaction with some lifestyle factors (Table 5). Specifically, both SNP1 and SNP2 showed to interact with smoking, length of breastfeeding, and AM. SNP3, which results in a common replacement of alanine with valine in position 222 of the respective protein, indicates nearly significant interaction with smoking and duration of breastfeeding. Overall, the *MTHFR* gene seems to strongly mediate the effect on the lifestyle factors on ANM.

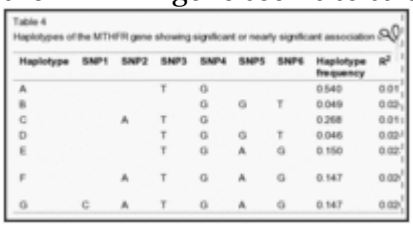

Table 4

Haplotypes of the MTHFR gene showing significant or nearly significant association with ANM

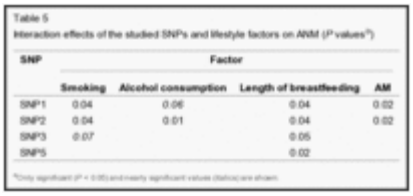

Table 5

Interaction effects of the studied SNPs and lifestyle factors on ANM (*P* values[a])

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Discussion

The present study firstly reports possible effects of the *MTHFR* gene on AM and ANM. These effects are of different nature. While no direct association of any independent SNP with the above traits was determined, several haplotypes associated with ANM, plus several SNP-SNP and SNP-environment interaction effects on the traits were identified. Principal health implications for the *MTHFR* gene are based on its key role in folate and homocysteine metabolism as well as DNA synthesis. Therefore, the *MTHFR* gene has been a subject of numerous studies for possible association of its polymorphisms with various disorders, particularly those associated with folate and homocysteine status (see, for review, [51–53]). Among all known SNPs of the *MTHFR*gene, two replacement polymorphisms, C677T (rs1801133, SNP3) and A1298C (rs1801131) have been most commonly used as the markers in the above studies. The C→T substitution at position 677

(and Ala→Val replacement at amino acid 222, respectively) results in a thermolabile enzyme with reduced activity [54]. Recently, direct association of this allele with low plasma folate level was reported [55]. Some evidence exists that this polymorphism is associated with a number of postmenopausal disorders, including osteoporosis [43;56] and Alzheimer's disease [57].

Further support for the possible association of the *MTHFR* gene with AM and ANM comes from the data about effect of menses and menopause on plasma homocysteine status. Although there is no direct evidence about influence of menarche on plasma homocysteine level (simply due to the lack of such studies), several studies reported that this level significantly varies between the phases of the menstrual cycle [32;33]. In turn, menopause was shown to decrease plasma homocysteine concentration [31;58-60]. Also, folate and homocysteine were implicated in human reproductive health and, in particular, subfertility [61;62].

In addition to menopause, plasma homocysteine level is elevated by various environmental factors, including smoking [63], alcohol intake [64], and breastfeeding [65;66], which all were shown to influence ANM (Table 3). Our results indicate that the *MTHFR* polymorphisms probably interact with the above environmental factors in their effect on ANM (Table 5). The mechanism of this interaction is unknown, but it is likely related to the maintenance of plasma homocysteine and folate status. The previously reported data about an interaction effect of alcohol with the C677T polymorphism on total plasma homocysteine level [67] are in further support of this assumption. On the other hand, it should be noted that the data about smoking and alcohol consumption used in the present study did not specify dose and duration of these factors. Therefore, the obtained results about interaction of the *MTHFR*polymorphisms with these lifestyle factors may be biased to some extent.

Table 3

Associations of lifestyle factors with ANM

Factor	*P* value	Effect
Smoking	**0.03**	0.158
Alcohol consumption	**0.03**	0.159
No. of pregnancies	0.31	0.080
Length of breastfeeding	**0.03**	−0.165
Use of oral contraceptives	0.62	−0.038
Age at menarche	0.32	−0.072

P value <0.05 are shown in bold.

Table 3

Associations of lifestyle factors with ANM

There is increased evidence that health problems, which are associated with AM or ANM, may have a shared genetic basis with these traits. For example, late menarche is a known risk factor for low bone mass [68]. Recent study by Guo et al. [19] suggested that the observed significant phenotypic correlation between bone mineral density(BMD) and AM is likely determined by shared genetic factors. Later, Pan et al. [69] identified several genomic regions, which may harbour genes contributing to both osteoporosis and menarche. Likewise, such shared regions were also determined for BMD and ANM [70]. There are also ample data about association of BMD candidate genes with AM and ANM (e.g., [50;71-74]). The*MTHFR* gene may be an addition to this list, as it was previously reported to contribute to BMD and higher risk of fractures [43;75]. The question whether AM and ANM have shared genetic basis remains unresolved. Some studies report positive phenotypic correlation between these traits [76;77], while the others do not [22;78]. Results of genetic linkage and association studies suggest that this basis may be shared partially. Specifically, several genomic regions were

reported to harbor QTLs differently for AM and ANM [27,79–81], while one region, 11q23, was identified as linked to both AM [27] and ANM [28]. Also, several genes seem to be associated with both these traits [82–84]. Our results about potential contribution of *MTHFR* to AM and ANM provide further support for the shared genes for both traits. However, to what extent this basis is shared needs further investigation.

Several caveats for our findings should also be acknowledged. First, our study has limited statistical power to detect interactions due to the small sample size; therefore the observed significant interactions should be treated with caution. Second, six SNPs and several lifestyle factors were analyzed in the analysis, which may raise a multiple testing problem. Although adjustments for multiple testing are rare in studies where a modest number of candidate markers are genotyped, this may nonetheless result in a risk of false discoveries. On the other hand, in our study, all six SNPs in the *MTHFR* gene were in high linkage disequilibrium, and tests were highly correlated. Thus, the risk of false discoveries seems to be small. In such a case, simple correction for multiple testing may result in further decrease of power to detect real effects. This study firstly reports a possible effect of the *MTHFR* gene on timing of menarche and menopause. In addition to the well-known missense functional polymorphism, C677T (rs1801133), several other non-replacement nucleotide polymorphisms, which may be associated with AM and/or ANM, were identified. Further replication studies in independent samples of larger scale and different ethnicities are needed to validate the results of the present study and to better understand the relationships between the *MTHFR* gene and onset of menarche and menopause.

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Acknowledgments

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