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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

**Pengyuan Liu, PhD**[1], **Yan Lu, PhD**[1], **Robert R. Recker, MD**[2], **Hong-Wen Deng, PhD**[3], and **Volodymyr Dvornyk, PhD**[4,*]

[1] Department of Surgery, Washington University in St. Louis, Campus Box 8109, 660 S. Euclid Ave, St. Louis, MO 63110, USA

[2] Osteoporosis Research Center and Department of Biomedical Sciences, Creighton University, Omaha, NE 68131, USA

[3] School of Medicine, University of Missouri-Kansas City, Kansas City, MO 64108, USA

[4] School of Biological Sciences, University of Hong Kong, Pokfulam Road, Hong Kong SAR, PR China

## 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

*Corresponding author: Dr. Volodymyr Dvornyk, School of Biological Sciences, The University of Hong Kong, Pokfulam Road, Hong Kong SAR, P.R. China, Tel.: (852) 2299 0611, Fax: (852) 2559 9114, dvornyk@hku.hk.

## 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], ovarian39, 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.

## 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.

### 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 $\chi^2$-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/.

## 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).

### 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.

## 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.

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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## References

1. Xu WH, Xiang YB, Ruan ZX, et al. Menstrual and reproductive factors and endometrial cancer risk: Results from a population-based case-control study in urban Shanghai. Int J Cancer 2004;108:613–619. [PubMed: 14696129]
2. Hsieh CC, Trichopoulos D, Katsouyanni K, Yuasa S. Age at menarche, age at menopause, height and obesity as risk factors for breast cancer: associations and interactions in an international case-control study. Int J Cancer 1990;46:796–800. [PubMed: 2228308]
3. Petridou E, Syrigou E, Toupadaki N, Zavitsanos X, Willett W, Trichopoulos D. Determinants of age at menarche as early life predictors of breast cancer risk. Int J Cancer 1996;68:193–198. [PubMed: 8900427]
4. Jordan SJ, Webb PM, Green AC. Height, age at menarche, and risk of epithelial ovarian cancer. Cancer Epidemiol Biomarkers Prev 2005;14:2045–2048. [PubMed: 16103459]
5. Chiaffarino F, Pelucchi C, Parazzini F, et al. Reproductive and hormonal factors and ovarian cancer. Ann Oncol 2001;12:337–341. [PubMed: 11332145]
6. Harlow BL, Cohen LS, Otto MW, Spiegelman D, Cramer DW. Early life menstrual characteristics and pregnancy experiences among women with and without major depression: the Harvard study of moods and cycles. J Affect Disord 2004;79:167–176. [PubMed: 15023491]
7. Laitinen J, Power C, Jarvelin MR. Family social class, maternal body mass index, childhood body mass index, and age at menarche as predictors of adult obesity. Am J Clin Nutr 2001;74:287–294. [PubMed: 11522550]
8. Wang W, Zhao LJ, Liu YZ, Recker RR, Deng HW. Genetic and environmental correlations between obesity phenotypes and age at menarche. Int J Obes 2006;30:1595–1600.
9. Gerdhem P, Obrant KJ. Bone mineral density in old age: the influence of age at menarche and menopause. J Bone Miner Metab 2004;22:372–375. [PubMed: 15221497]
10. Ito M, Yamada M, Hayashi K, Ohki M, Uetani M, Nakamura T. Relation of early menarche to high bone mineral density. Calcif Tissue Int 1995;57:11–14. [PubMed: 7671158]
11. Rudra CL, Williams MA. BMI as a modifying factor in the relations between age at menarche, menstrual cycle characteristics, and risk of preeclampsia. Gynecol Endocrinol 2005;21:200–205. [PubMed: 16316840]
12. Harlow BL, Signorello LB. Factors associated with early menopause. Maturitas 2000;35:3–9. [PubMed: 10802394]
13. Kritz-Silverstein D, Barrett-Connor E. Early menopause, number of reproductive years, and bone mineral density in postmenopausal women. Am J Public Health 1993;83:983–988. [PubMed: 8328621]
14. Jacobsen BK, Knutsen SF, Fraser GE. Age at natural menopause and total mortality and mortality from ischemic heart disease: the Adventist Health Study. J Clin Epidemiol 1999;52:303–307. [PubMed: 10235170]
15. Cramer DW. Epidemiologic aspects of early menopause and ovarian cancer. Ann N Y Acad Sci 1990;592:363–375. [PubMed: 2197952]
16. Schildkraut JM, Cooper GS, Halabi S, Calingaert B, Hartge P, Whittemore AS. Age at natural menopause and the risk of epithelial ovarian cancer. Obstet Gynecol 2001;98:85–90. [PubMed: 11430962]
17. Velie EM, Nechuta S, Osuch JR. Lifetime reproductive and anthropometric risk factors for breast cancer in postmenopausal women. Breast Dis 2005;24:17–35. [PubMed: 16917137]
18. Purdie DM, Green AC. Epidemiology of endometrial cancer. Best Pract Res Clin Obstet Gynaecol 2001;15:341–354. [PubMed: 11476557]

19. Guo Y, Zhao LJ, Shen H, Guo Y, Deng HW. Genetic and environmental correlations between age at menarche and bone mineral density at different skeletal sites. Calcif Tissue Int 2005;77:356–360. [PubMed: 16362457]
20. Loesch DZ, Huggins R, Rogucka E, Hoang NH, Hopper JL. Genetic correlates of menarcheal age: a multivariate twin study. Ann Hum Biol 1995;22:470–490. [PubMed: 8815776]
21. de Bruin JP, Bovenhuis H, van Noord PA, et al. The role of genetic factors in age at natural menopause. Hum Reprod 2001;16:2014–2018. [PubMed: 11527915]
22. Snieder H, MacGregor AJ, Spector TD. Genes control the cessation of a woman's reproductive life: a twin study of hysterectomy and age at menopause. J Clin Endocrinol Metab 1998;83:1875–1880. [PubMed: 9626112]
23. Sharma K. Genetic basis of human female pelvic morphology: a twin study. Am J Phys Anthropol 2002;117:327–333. [PubMed: 11920368]
24. van den Akker OB, Stein GS, Neale MC, Murray RM. Genetic and environmental variation in menstrual cycle: histories of two British twin samples. Acta Genet Med Gemellol (Roma) 1987;36:541–548. [PubMed: 3454517]
25. Murabito JM, Yang Q, Fox C, Wilson PW, Cupples LA. Heritability of age at natural menopause in the Framingham Heart Study. Obstet Gynecol Surv 2005;60:656–657.
26. Dvornyk V, Liu Y, Lu Y, et al. Effect of menopause on gene expression profiles of circulating monocytes: a pilot in vivo microarray study. J Genet Genomics 2007;34:974–983. [PubMed: 18037134]
27. Guo Y, Shen H, Xiao P, et al. Genomewide linkage scan for quantitative trait loci underlying variation in age at menarche. J Clin Endocrinol Metab 2006;91:1009–1014. [PubMed: 16394082]
28. Murabito JM, Yang Q, Fox CS, Cupples LA. Genome-wide linkage analysis to age at natural menopause in a community-based sample: the Framingham Heart Study. Fertil Steril 2005;84:1674–1679. [PubMed: 16359963]
29. Pan F, Xiao P, Guo Y, et al. Chromosomal regions 22q13 and 3p25 may harbor quantitative trait loci influencing both age at menarche and bone mineral density. Hum Genet 2008;123:419–427. [PubMed: 18379822]
30. Sibani S, Christensen B, O'Ferrall E, et al. Characterization of six novel mutations in the methylenetetrahydrofolate reductase (MTHFR) gene in patients with homocystinuria. Hum Mutat 2000;15:280–287. [PubMed: 10679944]
31. Hak AE, Polderman KH, Westendorp IC, et al. Increased plasma homocysteine after menopause. Atherosclerosis 2000;149:163–168. [PubMed: 10704628]
32. Tallova J, Tomandl J, Bicikova M, Hill M. Changes of plasma total homocysteine levels during the menstrual cycle. Eur J Clin Invest 1999;29:1041–1044. [PubMed: 10583452]
33. Tallova J, Bicikova M, Hill M, Tomandl J, Valentova D. Homocysteine during the menstrual cycle in depressive women. Eur J Clin Invest 2003;33:268–273. [PubMed: 12641547]
34. Somekawa Y, Kobayashi K, Tomura S, Aso T, Hamaguchi H. Effects of hormone replacement therapy and methylenetetrahydrofolate reductase polymorphism on plasma folate and homocysteine levels in postmenopausal Japanese women. Fertil Steril 2002;77:481–486. [PubMed: 11872199]
35. Weisberg IS, Jacques PF, Selhub J, et al. The 1298A→C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine. Atherosclerosis 2001;156:409–415. [PubMed: 11395038]
36. Kim YI. Folate, colorectal carcinogenesis, and DNA methylation: lessons from animal studies. Environ Mol Mutagen 2004;44:10–25. [PubMed: 15199543]
37. Rao GG, Kurien A, Gossett D, Griffith WF, Coleman RL, Muller CY. A case-control study of methylenetetrahydrofolate reductase polymorphisms in cervical carcinogenesis. Gynecol Oncol 2006;101:250–254. [PubMed: 16297972]
38. Piyathilake CJ, Macaluso M, Johanning GL, Whiteside M, Heimburger DC, Giuliano A. Methylenetetrahydrofolate reductase (MTHFR) polymorphism increases the risk of cervical intraepithelial neoplasia. Anticancer Res 2000;20:1751–1757. [PubMed: 10928104]
39. Gershoni-Baruch R, Dagan E, Israeli D, Kasinetz L, Kadouri E, Friedman E. Association of the C677T polymorphism in the MTHFR gene with breast and/or ovarian cancer risk in Jewish women. Eur J Cancer 2000;36:2313–2316. [PubMed: 11094304]

40. Lin WY, Chou YC, Wu MH, et al. The MTHFR C677T polymorphism, estrogen exposure and breast cancer risk: a nested case-control study in Taiwan. Anticancer Res 2004;24:3863–3868. [PubMed: 15736423]
41. Xu WH, Shrubsole MJ, Xiang YB, et al. Dietary folate intake, MTHFR genetic polymorphisms, and the risk of endometrial cancer among Chinese women. Cancer Epidemiol Biomarkers Prev 2007;16:281–287. [PubMed: 17301261]
42. Esteller M, Garcia A, Martinez-Palones JM, Xercavins J, Reventos J. Germ line polymorphisms in cytochrome-P450 1A1 (C4887 CYP1A1) and methylenetetrahydrofolate reductase (MTHFR) genes and endometrial cancer susceptibility. Carcinogenesis 1997;18:2307–2311. [PubMed: 9450474]
43. Hong X, Hsu YH, Terwedow H, et al. Association of the methylenetetrahydrofolate reductase C677T polymorphism and fracture risk in Chinese postmenopausal women. Bone 2007;40:737–742. [PubMed: 17174622]
44. Abrahamsen B, Madsen JS, Tofteng CL, et al. A common methylenetetrahydrofolate reductase (C677T) polymorphism is associated with low bone mineral density and increased fracture incidence after menopause: longitudinal data from the Danish osteoporosis prevention study. J Bone Miner Res 2003;18:723–729. [PubMed: 12674333]
45. Dvornyk V, Long JR, Liu PY, et al. Predictive factors for age at menopause in Caucasian females. Maturitas 2006;54:19–26. [PubMed: 16213680]
46. Long JR, Liu PY, Zhang YY, et al. Interaction effects between estrogen receptor α gene, vitamin D receptor gene, age, and sex on bone mineral density in Chinese. J Hum Genet 2003;48:514–519. [PubMed: 14505233]
47. Deng HW, Xu FH, Liu YZ, et al. A whole-genome linkage scan suggests several genomic regions potentially containing QTLs underlying the variation of stature. Am J Med Genet 2002;113:29–39. [PubMed: 12400063]
48. O'Connell JR, Weeks DE. PedCheck: a program for identification of genotype incompatibilities in linkage analysis. Am J Hum Genet 1998;63:259–266. [PubMed: 9634505]
49. Purcell S, Neale B, Todd-Brown K, et al. PLINK: a tool set for whole-genome associationand population-based linkage analyses. Am J Hum Genet 2007;81:559–575. [PubMed: 17701901]
50. Dvornyk V, Long JR, Liu PY, et al. Polymorphisms of the vitamin D receptor gene predict the onset of surgical menopause in Caucasian females. Gynecol Endocrinol 2006;22:552–556. [PubMed: 17135034]
51. Iqbal MP, Frossard PM. Methylene tetrahydrofolate reductase gene and coronary artery disease. J Pak Med Assoc 2003;53:33–36. [PubMed: 12666851]
52. Lin D, Li H, Tan W, Miao X, Wang L. Genetic polymorphisms in folate-metabolizing enzymes and risk of gastroesophageal cancers: a potential nutrient-gene interaction in cancer development. Forum Nutr 2007;60:140–145. [PubMed: 17684410]
53. Schwahn B, Rozen R. Polymorphisms in the methylenetetrahydrofolate reductase gene: clinical consequences. Am J Pharmacogenomics 2001;1:189–201. [PubMed: 12083967]
54. Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. Nat Genet 1995;10:111–113. [PubMed: 7647779]
55. Chatzikyriakidou A, Vakalis KV, Kolaitis N, et al. Distinct association of SLC19A1 polymorphism −43T>C with red cell folate levels and of MTHFR polymorphism 677C>T with plasma folate levels. Clin Biochem 2008;41:174–176. [PubMed: 18053808]
56. Miyao M, Morita H, Hosoi T, et al. Association of methylenetetrahydrofolate reductase (MTHFR) polymorphism with bone mineral density in postmenopausal Japanese women. Calcif Tissue Int 2000;66:190–194. [PubMed: 10666493]
57. Bi XH, Zhao HL, Zhang ZX, Zhang JW. Association of RFC1 A80G and MTHFR C677T polymorphisms with Alzheimer's disease. Neurobiol Aging. in press.
58. Gambacciani M, Mannella P. Homocysteine, menopause and cardiovascular disease. Menopause Int 2007;13:23–26. [PubMed: 17448264]
59. Marchesoni D, Driul L, Plaino L, Villani MT, Becagli L, Mozzanega B. Menopause rather than estrogen modifies plasma homocysteine levels. Int J Gynaecol Obstet 2003;81:293–297. [PubMed: 12767572]

60. Russo GT, Di BA, Alessi E, et al. Menopause modulates homocysteine levels in diabetic and non-diabetic women. J Endocrinol Invest 2008;31:546–551. [PubMed: 18591889]
61. Ebisch IM, Thomas CM, Peters WH, Braat DD, Steegers-Theunissen RP. The importance of folate, zinc and antioxidants in the pathogenesis and prevention of subfertility. Hum Reprod Update 2007;13:163–174. [PubMed: 17099205]
62. Forges T, Monnier-Barbarino P, Alberto JM, Gueant-Rodriguez RM, Daval JL, Gueant JL. Impact of folate and homocysteine metabolism on human reproductive health. Hum Reprod Update 2007;13:225–238. [PubMed: 17307774]
63. O'Callaghan P, Meleady R, Fitzgerald T, Graham I. Smoking and plasma homocysteine. Eur Heart J 2002;23:1580–1586. [PubMed: 12323157]
64. Sakuta H, Suzuki T. Alcohol consumption and plasma homocysteine. Alcohol 2005;37:73–77. [PubMed: 16584970]
65. Ramlau-Hansen CH, Moller UK, Moller J, Thulstrup AM. Amning - en risikofaktor for forhojet plasma-homocystein? [Lactation – a risk factor for elevated plasma homocysteine?]. Ugeskr Laeger 2003;165:2819–2823. [PubMed: 12891905]
66. Milman N, Byg KE, Hvas AM, Bergholt T, Eriksen L. Erythrocyte folate, plasma folate and plasma homocysteine during normal pregnancy and postpartum: a longitudinal study comprising 404 Danish women. Eur J Haematol 2006;76:200–205. [PubMed: 16412135]
67. Chiuve SE, Giovannucci EL, Hankinson SE, et al. Alcohol intake and methylenetetrahydrofolate reductase polymorphism modify the relation of folate intake to plasma homocysteine. Am J Clin Nutr 2005;82:155–162. [PubMed: 16002814]
68. Eastell R. Role of oestrogen in the regulation of bone turnover at the menarche. J Endocrinol 2005;185:223–234. [PubMed: 15845915]
69. Pan F, Xiao P, Guo Y, et al. Chromosomal regions 22q13 and 3p25 may harbor quantitative trait loci influencing both age at menarche and bone mineral density. Hum Genet 2008;123:419–427. [PubMed: 18379822]
70. Zhang ZX, Lei SF, Deng FY, et al. Bivariate genome-wide linkage analysis for traits BMD and AAM: Effect of menopause on linkage signals. Maturitas 2009;62:16–20. [PubMed: 19019586]
71. He LN, Xiong DH, Liu YJ, Zhang F, Recker RR, Deng HW. Association study of the oestrogen signalling pathway genes in relation to age at natural menopause. J Genet 2007;86:269–276. [PubMed: 18305346]
72. Long JR, Xu H, Zhao LJ, et al. The oestrogen receptor α gene is linked and/or associated with age of menarche in different ethnic groups. J Med Genet 2005;42:796–800. [PubMed: 16199553]
73. Yang F, Xiong DH, Guo Y, et al. The chemokine (C-C-motif) receptor 3 (CCR3) gene is linked and associated with age at menarche in Caucasian females. Hum Genet 2007;121:35–42. [PubMed: 17146638]
74. Zhang F, Xiong DH, Wang W, et al. HDC gene polymorphisms are associated with age at natural menopause in Caucasian women. Biochem Biophys Res Commun 2006;348:1378–1382. [PubMed: 16919600]
75. Abrahamsen B, Madsen JS, Tofteng CL, et al. Are effects of MTHFR (C677T) genotype on BMD confined to women with low folate and riboflavin intake? Analysis of food records from the Danish osteoporosis prevention study. Bone 2005;36:577–583. [PubMed: 15777680]
76. Reynolds RF, Obermeyer CM. Correlates of the age at natural menopause in Morocco. Ann Hum Biol 2003;30:97–108. [PubMed: 12519657]
77. Parazzini F. Determinants of age at menopause in women attending menopause clinics in Italy. Maturitas 2007;56:280–287. [PubMed: 17069999]
78. van Noord PA, Dubas JS, Dorland M, Boersma H, te Velde ER. Age at natural menopause in a population-based screening cohort: the role of menarche, fecundity, and lifestyle factors. Fertil Steril 1997;68:95–102. [PubMed: 9207591]
79. Anderson CA, Zhu G, Falchi M, et al. A genome-wide linkage scan for age at menarche in three populations of European descent. J Clin Endocrinol Metab 2008;93:3965–3970. [PubMed: 18647812]

80. Rothenbuhler A, Fradin D, Heath S, et al. Weight-adjusted genome scan analysis for mapping quantitative trait loci for menarchal age. J Clin Endocrinol Metab 2006;91:3534–3537. [PubMed: 16804047]

81. van Asselt KM, Kok HS, Putter H, et al. Linkage analysis of extremely discordant and concordant sibling pairs identifies quantitative trait loci influencing variation in human menopausal age. Am J Hum Genet 2004;74:444–453. [PubMed: 14872408]

82. Mitchell ES, Farin FM, Stapleton PL, et al. Association of estrogen-related polymorphisms with age at menarche, age at final menstrual period, and stages of the menopausal transition. Menopause 2008;15:105–111. [PubMed: 17589376]

83. Stavrou I, Zois C, Ioannidis JP, Tsatsoulis A. Association of polymorphisms of the oestrogen receptor α gene with the age of menarche. Hum Reprod 2002;17:1101–1105. [PubMed: 11925413]

84. Weel AE, Uitterlinden AG, Westendorp IC, et al. Estrogen receptor polymorphism predicts the onset of natural and surgical menopause. J Clin Endocrinol Metab 1999;84:3146–3150. [PubMed: 10487678]

## 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 |
| Months of breastfeeding | | |
| None | 43.6 | 44.8 |
| 1–6 | 23.5 | 23.3 |
| 7–12 | 11.4 | 11.4 |
| 13–24 | 11.7 | 9.0 |
| 25 and more | 9.7 | 12.4 |
| No. of pregnancies | 4.0 ± 0.1 | 4.2 ± 0.2 |
| Pregnancies (% of sample) | | |
| None | 3.0 | 1.9 |
| 1 or 2 | 25.9 | 25.9 |
| 3 or 4 | 40.0 | 38.4 |
| 5 and more | 31.1 | 33.8 |

**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 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 4**

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

| Haplotype | SNP1 | SNP2 | SNP3 | SNP4 | SNP5 | SNP6 | Haplotype frequency | $R^2$ | β | P |
|---|---|---|---|---|---|---|---|---|---|---|
| A | | | T | G | | | 0.540 | 0.017 | −0.86 | 0.09 |
| B | | | | G | G | T | 0.049 | 0.024 | −2.67 | 0.04 |
| C | | A | T | G | | | 0.268 | 0.018 | −0.95 | 0.08 |
| D | | | T | G | G | T | 0.046 | 0.024 | −2.65 | 0.04 |
| E | | | T | G | A | G | 0.150 | 0.023 | − 1.17 | 0.05 |
| F | | A | T | G | A | G | 0.147 | 0.020 | − 1.09 | 0.06 |
| G | C | A | T | G | A | G | 0.147 | 0.020 | −1.09 | 0.06 |

## Table 5

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

| SNP | Factor | | | |
|---|---|---|---|---|
| | **Smoking** | **Alcohol consumption** | **Length of breastfeeding** | **AM** |
| SNP1 | 0.04 | *0.06* | 0.04 | 0.02 |
| SNP2 | 0.04 | 0.01 | 0.04 | 0.02 |
| SNP3 | *0.07* | | 0.05 | |
| SNP5 | | | 0.02 | |

[a]Only significant ($P < 0.05$) and nearly significant values (italics) are shown.