AJCN. First published ahead of print September 16, 2009 as doi: 10.3945/ajcn.2009.28064.

Increased breast cancer risk at high plasma folate concentrations among women with the *MTHFR 677T* allele[1–3]

Ulrika C Ericson, Malin IL Ivarsson, Emily Sonestedt, Bo Gullberg, Joyce Carlson, Håkan Olsson, and Elisabet Wirfält

ABSTRACT

Background: Folate is involved in DNA synthesis and methylation and may thereby influence carcinogenesis.

Objectives: We examined plasma folate (P-folate) concentration in relation to genotypes of the folate-metabolizing enzyme methylenetetrahydrofolate reductase [*MTHFR 677C→T* (rs1801133) and *1298A→C* (rs1801131)]. We also explored whether P-folate was associated with risk of postmenopausal breast cancer overall and in subgroups with genetic variants of the *MTHFR* single nucleotide polymorphisms (SNPs).

Design: This nested case-control study included 313 cases (age 55–73 y at baseline) with invasive breast cancer and 626 control subjects, matched on age and blood-sample date, from the population-based Malmö Diet and Cancer cohort. P-folate and *MTHFR* genotypes were determined for 310 cases and 611 controls. P-folate according to genotype was calculated by using analysis of variance. Odds ratios were obtained by using logistic regression. All tests were 2-sided.

Results: The variant *677T* allele was associated with lower P-folate. In women with the *677T* allele, a high P-folate concentration was associated with increased breast cancer risk (P for trend across P-folate tertiles = 0.03). Interaction was seen between the *677C→T* SNP and P-folate (P = 0.002). A positive association, which was seen between P-folate and breast cancer risk in *1298AA* women (P = 0.01), was probably due to linkage between the 2 SNPs. Overall, and in women with other genotypes, no significant associations were observed.

Conclusions: Our results suggest an association of high P-folate concentration with increased risk of postmenopausal breast cancer in carriers of the *677T* allele. The findings underline the importance of genetic variation of *MTHFR* in the complex relation between folate and cancer. *Am J Clin Nutr* doi: 10.3945/ajcn.2009.28064.

INTRODUCTION

Folate is a B vitamin that is found mainly in plant foods. In humans, folate is metabolized into different chemical forms (**Figure 1**), which function as methyl donors for specific biochemical reactions. Two main forms could potentially be important in cancer development: 5,10-methylene tetrahydrofolate (5,10-methylene THF) and 5-methyltetrahydrofolate (5-methyl THF). The 5,10-methylene form may be involved in carcinogenesis via its transmission of methyl groups for DNA synthesis and repair. A possible consequence of deficiency is chromosome breakage due to misincorporation of uracil instead of thymine into DNA (1). An irreversible conversion of 5,10-methylene THF leads to the other crucial form: 5-methyl THF. This form may have a critical role in carcinogenesis, because it transmits methyl groups for reactions that lead to DNA methylation (2). The consequences of altered availability of methyl units for DNA methylation are complex (3).

Global genomic hypomethylation may lead to cancer development because it causes chromosomal instability (3). Methyl deficiency, however, through induced activity of DNA methyltransferases (4), may facilitate methylation of CpG islands in the promoter regions of many genes (5). Hypermethylation in the promoter regions potentially causes gene silencing (6), and silenced tumor suppressor genes enhance cancer development. Methylation patterns may also affect the expression of proto-oncogenes (6).

Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that accelerates the conversion of 5,10-methylene THF to 5-methyl THF. Consequently, this enzyme has a key role balancing the pool of methyl groups between DNA synthesis and DNA methylation. The minor allele of a well-known single nucleotide polymorphism (SNP) of *MTHFR*, *677C→T* (rs1801133) (http://ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1801133), has been linked to a 70% reduction of MTHFR activity (7) and reduced plasma folate (P-folate) concentration (8). In addition, the minor allele of the *MTHFR 1298A→C* (rs1801131) (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=1801131) has been linked to a less-pronounced reduction of MTHFR activity (9).

Moreover, genotypes with the minor T alleles at locus *MTHFR 677C→T* have been related to increased risk of postmenopausal breast cancer in the Malmö Diet and Cancer (MDC) cohort (10). However, the mechanisms that relate *MTHFR* polymorphisms to

[1] From the Department of Clinical Sciences, Malmö, Nutrition Epidemiology (UCE, ES, BG, and EW); the Department of Clinical Chemistry, Malmö (MILI and JC); the Department of Medical Microbiology, Malmö, (MILI), Lund University, Sweden; and the Departments of Cancer Epidemiology and Oncology, Clinical Sciences, Lund, Lund University, Sweden (HO).

[2] Supported by grants from the Swedish Research Council (K2006-27X-20060-01-3), the Swedish Research Council Formas (222-2005-1833), the Swedish Cancer Society (4886-B03-01XAB), The Albert Påhlsson Foundation, The Swedish Nutrition Foundation and the City of Malmö.

[3] Address correspondence to U Ericson, Nutrition Epidemiology, Clinical Research Center, UMAS, Entrance 72, Building 60, Floor 13, SE-205 02 Malmö, Sweden. E-mail address: ulrika.ericson@med.lu.se.

Received May 12, 2009. Accepted for publication August 24, 2009.
doi: 10.3945/ajcn.2009.28064.

Copyright (C) 2009 by the American Society for Nutrition

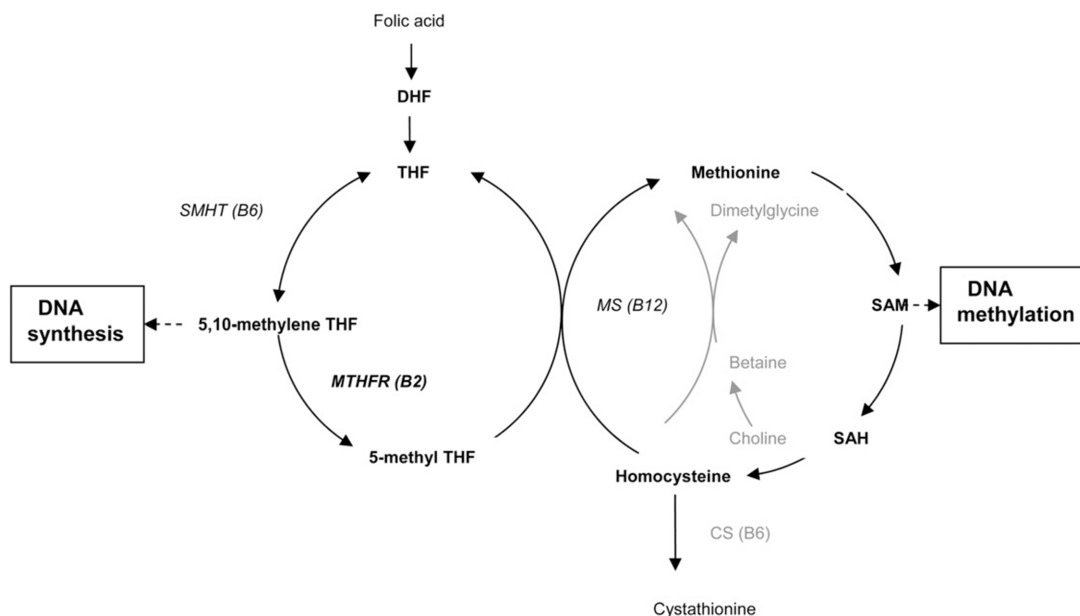

FIGURE 1. A simplified scheme of the folate metabolism of importance for distribution of one-carbon units between DNA synthesis and methylation. DHF, dihydrofolate; THF, tetrahydrofolate; MTHFR, methylenetetrahydrofolate reductase; MS, methionine synthase; CS, cystathionine β-synthase; SHMT, serinehydroxymethyl transferase; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine.

breast cancer seem to be complex. There is inconsistency among published studies (11), and *MTHFR* SNPs seem to interact with menopausal status (10, 12) and folate intake (10, 13). In addition, alcohol is known to interact with folate metabolism (14, 15). The use of biomarkers, as objective measures of dietary intakes, is an asset when estimating the relation between diet and disease. However, results from the few studies that have examined the association of folate in blood with breast cancer are conflicting (16–21). A meta-analysis concluded that there was no evidence for an association (22). However, none of the prospective studies included in the analysis have considered the genetic predisposition caused by *MTHFR* SNPs.

In this study of women from the MDC cohort, we intended to examine whether P-folate concentrations could be related to the *MTHFR 677C→T* and *1298A→C* SNPs and whether P-folate was associated with invasive breast cancer in subgroups according to the *MTHFR* SNPs. In a subsample of 20 women, we examined 3 repeated blood samples to estimate the reliability of using a single blood sample when classifying women according to P-folate concentrations.

SUBJECTS AND METHODS

The Malmö Diet and Cancer Study

The MDC Study is a prospective cohort study performed in Malmö, a city in the south of Sweden. All women who were born during 1923–1950 and all men who were born during 1923–1945 were invited to participate. The source population included 74,138 persons. The MDC Study was approved by the Ethical Committee at Lund University (LU 51–90). Details of the recruitment procedures and the cohort are described elsewhere (23). Inadequate Swedish language skills and mental incapacity were the only exclusion criteria. The participants filled out questionnaires that covered socioeconomic, lifestyle, and dietary factors; registered their meals; and underwent a dietary history interview (24, 25). Nurses collected blood samples, registered blood pressure, and made anthropometric measurements. During the screening period (March 1991–October 1996), 28,098 participants completed all baseline examinations (17,035 women).

Breast cancer study

Participants with prevalent cancers at baseline, except for those with cervix cancer in situ, were excluded. Cases are women >55 y of age at baseline with invasive breast cancer that was diagnosed during follow-up (until 31 December 2004). The study includes 313 cases of breast cancer. Two controls (n = 626) (alive, living in Sweden, and without breast cancer at the time of diagnosis of the corresponding case) were matched on age at baseline (±3 mo) and date of blood sample (±1 mo). During follow-up, 0.5% of the MDC participants had migrated from Sweden. The study was performed after approval by the Regional Ethical Review Board in Lund (567/2005).

Breast cancer case definition and ascertainment

The Swedish Cancer Registry and the Southern Swedish Regional Tumor Registry provided data on case definition and ascertainment until the end of follow-up. Invasive cancer was defined as all cancer except for in situ cancer. Information on vital status was obtained from the National Tax Board, which provides up-to-date information on vital status for all Swedish residents.

P-folate analysis

Nonfasting blood samples (Na-heparin tubes) were drawn at baseline. The plasma was separated within 1 h and then stored at −80°C. P-folate concentration was analyzed by using a 2-step immunoassay with alkaline phosphatase, enzyme marking, and magnetic separation. Blood samples from cases were analyzed

together with their matched controls to avoid problems with variability between assays. The laboratory personnel were unaware of the case-control status of the specimens. P-folate concentrations were determined for 312 cases (99.7%) and 623 controls (99.2%).

DNA analysis

Nonfasting blood samples drawn at baseline and stored in EDTA-coated tubes, were separated within 1 h, producing granulocyte or buffy coat cell suspensions, which were subsequently stored at −80°C (26). DNA was extracted from the cell suspensions with the use of QiaAmp mini-kits (Qiagen, Hilden, Germany). Genotyping of *MTHFR* SNPs *677C→T* (rs1801133) and *1298A→C* (rs1801131) was performed at the Department of Clinical Chemistry at the university hospital in Malmö on a matrix-assisted laser desorption/ionization time-of-flight mass spectrometer (MALDI-TOF MS) (Sequenom MassArray; Sequenom Inc, San Diego, CA) by using iPLEX reagents and protocol (Sequenom Inc) and a 10-ng DNA template. Primer sets were as follows: forward: ACG TTG GAT GAC CTG AAG CAC TTG AAG GAG, reverse: ACG TTG GAT GGA AAA GCT GCG TGA TGA TG, and massextend:GCG TGA TGA TGA AAT CG for the rs1801133 SNP; and forward: ACG TTG GAT GTC TCC CGA GAG GTA AAG AAC, reverse: ACG TTG GAT GAG GAG CTG CTG AAG ATG TGG, and massextend:ATG AGC TGA CCA GTG AAG for the rs1801131 SNP (Metabion). All procedures were performed according to SEQUENOM standard protocols. The MALDI-TOF MS analysis was repeated on 4.2% (n = 68) of the samples for rs1801133 and 4.3% (n = 70) of the samples for rs1801131. There were no discrepancies between repeated analyses. A few samples were not successfully genotyped on the MALDI-TOF MS (32 for *MTHFR 677C→T* and 24 for *1298A→C*). The genotyping of these samples was performed on an ABI PRISM 7900HT Sequence detection system (Applied Biosystems, Foster City, CA) with the use of commercial SNP detection assays: C_1202883_20 for *MTHFR 677C→T* and C_850486_20 for *MTHFR 1298A→C* (Applied Biosystems). In each case, 2 μL DNA at 1 ng/μL was used as template in a 6-μL reaction in 384 formats by using all reagents and instrument settings according to the manufacturers' recommendations.

Genotypes for the *677C→T* SNP were successfully determined for 312 cases (99.7%) and 618 controls (98.4%), and genotypes for *1298A→C* were successfully determined for 312 cases (99.7%) and 615 controls (97.8%). Genotype distributions among controls from the MDC Study did not deviate from Hardy-Weinberg equilibrium (*677C→T*, P = 0.71, and *1298A→C*, P = 0.49) (10). The frequency of the minor allele was 30% at locus *MTHFR 677C→T*, and 32% at *MTHFR 1298A→C* (10). Genotypes were not independently distributed across the 2 loci. The minor *677T* allele was linked to the major *1298A* allele (10). The genotypes were in complete linkage disequilibrium, ie, >2 minor alleles were never present in a single sample (10).

Variables

This study examined P-folate concentrations. The categories were defined as tertiles of P-folate concentrations among the controls. *MTHFR 677C→T* and *1298A→C* categories were defined as genotypes (ie, *677CC*, *677CT*, *677TT*, *1298AA*, *1298AC*, and *1298CC*) and carriage of the minor allele (yes/no; ie, *677CC* or *677CT+TT*, and *1298AA* or *1298AC+CC*). Combinations of the *677C→T* and *1298A→C* genotypes were also used to define the *MTHFR* categories.

Self-reported food and supplement intakes were converted to folate intakes by using PC-KOST2-93 from the National Food Administration in Uppsala, Sweden, and the MDC supplement database (27). Dietary folate equivalents were calculated on the basis of the assumption that the bioavailability of synthetic folic acid consumed in a meal is 1.7 times the bioavailability of food folate (28) [ie, dietary folate equivalents = μg food folate + (1.7 × μg folic acid from supplement)] (Food fortification has not been taken into consideration in this calculation because very few foods are fortified with folic acid in Sweden.)

Information on age was obtained from the personal identification number. Age was divided into categories, with cutoffs at quarters of a year. Date of blood sample referred to the screening week in the MDC Study. The smoking status of participants was defined as current smokers (including irregular smokers), ex-smokers, and never smokers. Information on total alcohol consumption was converted into a 4-category variable. Women who reported zero consumption of alcohol in a 7-d food record, and who indicated no intake of any type of alcohol during the previous year, were categorized as zero-reporters. The other category ranges were as follows: <15 g alcohol/d (low), 15–30 g alcohol/day (medium), and >30 g alcohol/d (high). Alcohol intake was also dichotomized (≤4.3 or >4.3 g/d). Leisure-time physical activity was assessed by using a questionnaire, adapted from the Minnesota Leisure Time Physical Activity Questionnaire (29, 30). The number of minutes per week of 18 different activities was multiplied with an activity-specific intensity coefficient, and an overall leisure-time physical activity score was created. The score was divided into tertiles and categorized as low, medium, and high. Household activities were estimated in h/wk and divided into 4 groups with cutoffs every 10 h (0–9, 10–19, 20–29, or ≥30). Participants were divided into 4 categories according to their highest level of education (≤8 y, 9–10 y, 11–13 y, or university degree). Classification of socioeconomic index was based on information of job title, tasks, and position at work. The procedure was adapted from that of the 1989 Swedish population census (31). In this study, the information was collapsed into 5 categories: blue-collar workers, white-collar workers (low, medium, and high), and self-employed. Retired and unemployed participants were classified according to their position before retirement/unemployment.

Weight was measured by using a balance-beam scale with subjects wearing light clothing and no shoes. Standing height was measured with a fixed stadiometer, which was calibrated in centimeters. Body mass index (BMI; kg/m^2) was calculated from direct measurement of weight and height and a 3-category variable was created (BMI ≤25, 25–29, or ≥30). Age at menopause was divided into 4 categories plus a separate category for missing values (<45 y, 45–49 y, 50–55 y, >55 y, or unknown). Current menopausal hormone therapy (MHT) status (yes/no) was based on the questionnaire item "Which medications do you use on a regular basis?" in combination with information on drug use from the 7-d menu book (32). Parity was defined as the number of children, with no children in the lowest category and

≥4 children in the highest. Missing values for the variables were treated as separate categories.

Statistical analysis

The SPSS statistical computer package (version 14.0; SPSS Inc, Chicago, IL) and STATA (version 10; StataCorp, College Station, TX) were used for statistical analyses. P-folate was log-transformed (e-log) to normalize the distribution before analysis. The energy-adjusted partial correlation coefficient between intake of dietary folate equivalents and P-folate concentration was computed. Differences in baseline statuses were examined in cases and controls by using analysis of variance (ANOVA) for continuous variables and chi-square analysis for categorical variables. Baseline characteristics according to tertiles of P-folate concentrations in breast cancer controls were calculated by using ANOVA for continuous variables. Adjustments were made for age and date of blood sample. Chi-square analysis was performed for categorical variables. Mean P-folate concentrations according to *MTHFR* genotype were calculated by using ANOVA and adjusted for age and date of blood sample. In subgroup analysis according to *MTHFR* genotype, ORs for breast cancer in tertiles of P-folate were computed by using unconditional logistic regression, after matching variables were controlled for. A second model included adjustments for established risk factors and potential confounders (ie, weight, height, household work, smoking, alcohol intake, socioeconomic status, age at menopause, parity, and MHT). Candidate covariates were identified from the literature. Selected covariates had to be associated with invasive breast cancer in this case-control study, modify the risk estimate for invasive breast cancer according to P-folate concentration by >10%, or by prior knowledge be considered important risk factors. As a last step in the selection procedure, a few covariates that were not associated with breast cancer in the multivariate model were excluded. A test for interaction with regard to breast cancer was performed between tertiles of P-folate (treated as a continuous variable) and *MTHFR* genotypes (of *677C→T* and *1298A→C*, respectively, treated as continuous variables). To avoid confounding between the 2 loci, the analysis was also restricted to women homozygous for the major *1298A* allele when the test for interaction between P-folate and the *677C→T* genotype was performed and to women homozygous for the major *677C* allele when the test for interaction between P-folate and the *1298A→C* genotype was performed. Risk estimate correction for within person P-folate variation was performed using the following formula: $OR_{observed} = (OR_{corrected})^{ICC}$ (33), with the ICC (intraclass correlation coefficient) obtained in the reproducibility study. All statistical tests were 2-sided.

Reproducibility study

Twenty women, between 55 and 65 y of age and without any serious comorbidity, participated in 3 nonfasting blood collections during May and June 2005. They were recruited by mail and telephone from 100 women who were randomly selected from the MDC cohort. The study was performed after approval by the Regional Ethical Review Board in Lund (56/2005).

Statistical analysis

P-folate was ln-transformed to normalize the distribution. For the 20 women with repeated blood samples, median folate concentrations and the number and range of days between blood collections were determined. Pearson correlation coefficients were also calculated. Estimates of between-subject (σ^2_B), within-subject (σ^2_W), total variances, and the ICC (between-subject variance divided by total variance) with 95% CIs were obtained by using reliability analysis with one-factor ANOVA.

RESULTS

Participant characteristics at baseline

Comparison of baseline characteristics between breast cancer cases and controls is presented in **Table 1**. The cases were significantly taller than the controls ($P = 0.02$). The use of MHT, as well as high alcohol consumption (>30 g/d), was more frequent among cases compared with controls ($P < 0.001$ and $P = 0.001$, respectively). The percentage of medium- or high-white-collar workers was also higher among the cases ($P = 0.003$). In addition, occurrence of the variant *MTHFR 677T* allele was higher among the cases ($P = 0.04$). The means or the percentage distributions of baseline characteristics in control women across tertiles of P-folate concentrations are shown in **Table 2**. Folate intake increased across tertiles of P-folate. (The partial correlation coefficient between energy-adjusted dietary folate equivalents and P-folate was 0.52 in breast cancer controls from the MDC cohort). Mean age at menopause was higher in the mid-tertile of P-folate than in the lowest tertile. The lowest tertile of P-folate included fewer women who spent >20 h/wk on household work, fewer women with high socioeconomic status, and more current smokers (compared with fractions among all control women).

Plasma folate according to *MTHFR* genotypes

The *MTHFR 677TT* genotype was associated with lower P-folate concentrations compared with the *677CC* genotype ($P < 0.001$) (**Table 3**), and a significant trend for decreased P-folate across *677C→T* genotypes was observed. When the analysis was limited to *1298AA* women (**Table 4**), the same associations between the *677T* allele and P-folate were observed. We did not observe any significant association between the *MTHFR 1298A→C* polymorphism and P-folate concentrations. However, in analysis limited to *677CC* women, the variant C allele at locus *1298A→C* tended to be associated with lower P-folate concentrations (P for trend = 0.07).

Plasma folate and breast cancer

In the adjusted model, no overall association between P-folate concentrations and breast cancer risk were observed (**Table 5**). However, there was a statistical interaction between the *MTHFR 677C→T* polymorphism and tertiles of P-folate concentration in relation to breast cancer risk ($P = 0.002$). P-folate was positively associated with invasive breast cancer among women with the *677T* allele (CT/TT women) (P for trend = 0.03). In contrast, no trend across tertiles of P-folate was observed among *677CC* women. The interaction remained significant when the analysis

TABLE 1
Background characteristics of cases and controls >55 y of age from the Malmö Diet and Cancer cohort

	Cases/controls	Values[1]		P value[2]
		Cases	Controls	
	n			
Plasma folate (nmol/L)	313/626	13.2	12.4	0.29[3]
Folate intake (μg/d)[4]	313/626	310	316	0.45[5]
Age (y)	313/626	62.1	62.1	0.99
Height (cm)	313/626	163.5	162.5	0.02
Weight (kg)	313/626	70.1	69.4	0.39
BMI (kg/m^2)	313/626	26.3	26.3	0.90
Age at menopause (y)	296/611	50.2	50.0	0.54
Parity (no. of children)	304/618	1.8	2.0	0.08
Menopausal hormone therapy; current use (%)	288/584	32	18%	<0.001
Household work >20 h/wk (%)	305/613	46	52	0.07
Leisure time physical activity, highest tertile (%)	311/622	34	34	0.96
Socioeconomic status, high (%)[6]	308/620	26	17	0.003
Education, high level (%)[7]	311/625	19	20	0.67
Alcohol intake >30 g/d (%)	313/626	4	1	0.001
Smoking, current (%)	313/626	20	22	0.40
MTHFR 677C→T genotype (%)				0.04[8]
CC	141/324	45	52	
CT	143/246	46	40	
TT	28/48	9	8	
MTHFR 1298A→C genotype (%)				0.33[9]
AA	147/269	47	44	
AC	136/275	44	45	
CC	29/71	9	11	
Genotype combinations (%)				
677CC-1298AA	39/96	13	16	
677CC-1298AC	73/154	24	25	
677CC-1298CC	29/71	9	11	
677CT-1298AA	79/125	25	20	
677CT-1298AC	63/121	20	20	
677CT-1298CC	0/0	0	0	
677TT-1298AA	28/48	9	8	
677TT-1298AC	0/0	0	0	
677TT-1298CC	0/0	0	0	

[1] Values are means or percentages, where indicated.
[2] Calculated by using ANOVA for continuous variables and chi-square test for categorical variables.
[3] Test of differences between geometric means
[4] Dietary folate equivalents
[5] Test of differences between energy-adjusted geometric means
[6] Medium- or high-white-collar workers compared with blue-collar workers and low-white-collar workers (self-employed participants were excluded in the analysis).
[7] ≥11 y.
[8] For distribution of carriers with or without the minor *677T* allele. The odds ratio for breast cancer among carriers of the *677T* allele compared with *677CC* women was 1.34 (95% CI: 1.01–1.76) (10).
[9] For distribution of carriers with or without the minor *1298C* allele.

was restricted to *1298AA* women (*677 C→T* genotype × tertile of P-folate; *P* = 0.006). P-folate was positively associated with invasive breast cancer among women with the T allele (*677CT/TT-1298AA* women) (*P* for trend = 0.002) but not among *677CC-1298AA* women.

P-folate was also positively associated with invasive breast cancer among *1298AA* women (*P* for trend = 0.01). However, no such tendencies were observed among *677CC-1298AA* women (*P* for trend = 0.71). In addition, statistically significant interaction between the *MTHFR 1298A→C* polymorphism and P-folate was not observed in all women (*P* = 0.22), even when the analysis was restricted to *677CC* women (*1298A→C* genotype × tertile of P-folate; *P* = 0.32).

No significant interaction was observed between alcohol intake above or below the median intake and P-folate concentration (*P* = 0.13). However, among women who reported an alcohol intake above the median (>4.3 g/d), an increase of P-folate from one tertile to the next tended to be associated with increased breast cancer risk (OR: 1.24; 95% CI: 0.95–1.60; *P* for trend = 0.11), but similar tendencies were not observed among women

TABLE 2
Distribution of baseline characteristics according to tertiles of plasma folate concentrations in breast cancer controls >55 y of age from the Malmö Diet and Cancer cohort

		Tertiles of plasma folate			
	n	1	2	3	*P* values[1]
Plasma folate (nmol/L)	619	6.2 (5.6, 6.9)[2]	9.9 (9.2, 10.6)	20.4 (19.7, 21.1)	<0.001
Folate intake (μg/d)[3]	619	231 (173, 289)	268 (211, 329)	436 (379, 493)	<0.001[4]
Height (m)	619	1.63 (1.62, 1.64)	1.63 (1.62, 1.64)	1.62 (1.61, 1.63)	0.44
Weight (kg)	619	70.5 (68.7, 72.2)	68.4 (66.7, 70.2)	69.3 (67.5, 71.0)	0.28
BMI (kg/m^2)	619	26.6 (25.9, 27.2)	25.9 (25.2, 26.5)	26.4 (25.8, 27.0)	0.31
Age at menopause (y)	602	49.5 (48.9, 50.1)	50.7 (50.1, 51.3)	49.8 (49.3, 50.5)	0.01
Parity (no. of children)	609	2.1 (1.9, 2.3)	1.9 (1.7, 2.1)	2.0 (1.8, 2.2)	0.37
Menopausal hormone therapy, current use (%)	575	18	16	20	0.52
Leisure time physical activity, highest tertile (%)	604	34	33	36	0.75
Household work >20 h/wk (%)	604	48	51	60	0.04
Socioeconomic status, high (%)[5]	571	12	21	19	0.04
Education, high level (%)[6]	616	16	20	23	0.24
Alcohol intake >30 g/d (%)	618	1.0	1.0	0.5	0.81
Smoking, current (%)	617	30	20	18	0.01

[1] Calculated by using ANOVA and adjusted for age and date of blood sample for continuous variables and by using chi-square tests for categorical variables.
[2] Mean; 95% CI in parentheses (all such values).
[3] Dietary folate equivalents.
[4] Energy adjusted.
[5] Medium- or high-white-collar workers compared with blue-collar workers and low-white-collar workers (self-employed participants were excluded in the analysis).
[6] ≥11 y.

who reported an alcohol intake below the median (OR: 0.98; 95% CI: 0.76–1.26; *P* for trend = 0.86).

The observed associations were also seen in the basic model, with adjustments for only matching variables, as well as in a model with additional adjustments for folate intake (dietary folate equivalents) (data not shown). In sensitivity analysis, excluding women diagnosed during the first year of baseline examinations (minimizing effects of preclinical cancer on P-folate concentrations), the results remained unchanged (data not shown). For women with high P-folate concentrations, the breast cancer OR changed from 1.20 to 1.27 when using the ICC from the reproducibility study to correct for within-person variation of P-folate. For women with the *677T* allele and high P-folate concentrations, the OR changed from 1.51 to 1.71.

Reproducibility study

The median interval between the first and the last blood collection was 34 d (range: 19–46 d). In the 20 women, the Pearson correlation coefficients between P-folate concentrations from different blood collections were 0.72, 0.92, and 0.63 (for collection 1 compared with 2, 2 compared with 3, and 1 compared with 3, respectively). The within-person CV was lower (CV = 15%) than the between-person CV (CV = 38%). The ICC was 0.77 (95% CI: 0.59, 0.89).

DISCUSSION

We did not observe any overall association between P-folate and invasive breast cancer in this sample of postmenopausal women from the MDC cohort. The minor *MTHFR 677T* allele was associated with lower P-folate concentrations. Among women with this minor allele, high P-folate concentrations were associated with increased risk of invasive breast cancer. Statistical interaction was observed between the *677C→T* SNP and P-folate.

The prospective design is an advantage of this nested case-control study because it minimizes selection bias and reverse causation. Other advantages are the nearly complete Swedish

TABLE 3
Plasma folate concentrations according to *MTHFR 677C→T* and *1298A→C* genotypes among control women >55 y of age from the Malmö Diet and Cancer cohort[1]

	n	Plasma folate[2]	*P* for trend
		nmol/L	
MTHFR 677C→T			
677CC	324	11.5[a]	<0.001
677CT	246	9.9[a]	
677TT	48	8.1[b]	
MTHFR 1298A→ C			
1298AA	269	10.2[a]	0.91
1298AC	275	11.0[a]	
1298CC	71	10.4[a]	

[1] Homogeneous subsets within each single nucleotide polymorphism are indicated by different superscript letters. *P* values were obtained by using ANOVA. Multiple comparisons were performed by using Tukey's test (α = 0.05). Adjustments were made for age of participant and date of blood sample.
[2] Values are geometric means.

TABLE 4
Plasma folate concentrations according to genotype combinations of the *MTHFR 677C→T* and *1298A→C* single nucleotide polymorphisms among control women >55 y of age from the Malmö Diet and Cancer cohort[1]

	1298AA		*1298AC*		*1298CC*		
Genotype	*n*	Plasma folate[2]	*n*	Plasma folate[2]	*n*	Plasma folate[2]	*P* for trend
		nmol/L		*nmol/L*		*nmol/L*	
677CC	96	12.0[a]	154	11.8[a]	71	10.4[a]	0.07
677CT	125	9.8[a,b]	121	10.0[a,b]			
677TT	48	8.1[b]					
P for trend		<0.01					

[1] Homogeneous subsets are indicated by different superscript letters. *P* values were obtained by using ANOVA. Multiple comparison was performed with Tukey's test (α = 0.05). Adjustments were made for age and date of blood sample.
[2] Values are geometric means.

National Cancer Registry and the extensive information on confounding variables. Examination of genotype combinations of the *677C→T* SNP and *1298A→C* is also a strength because of linkage between these SNPs (10, 34–36). We are aware of only 2 epidemiologic breast cancer studies that have previously examined interactions between folate in blood and the *677C→T* SNP, and none were prospective (16, 17). In addition, study-specific data on the reliability of using a single sample when ranking individuals according to concentrations of a biomarker is valuable, because the ICC may differ between cohorts. Our calculated ICC lay well above the level that often results in severe attenuation of examined associations (37). Nevertheless, corrections by the ICC resulted in a notable increase of the calculated breast cancer risk in *677T* carriers with high P-folate. P-folate is considered to be a good biomarker of folate status in large epidemiologic studies (38). However, erythrocyte folate is less influenced by recent intake and would have been more informative about different folate vitamers (39). Folate in plasma almost exclusively appears as 5-methyl THF (40) and may therefore above all reflect the amount of folate available for DNA methylation. Further conversion of methyl groups from 5-methyl THF is, however, also influenced by the vitamin B-12–dependent enzyme methionine synthase. A limitation of this study might be that blood concentrations of vitamin B-12 were not analyzed. Another limitation is the small number of individuals in subgroups of genotype combinations, which may prevent us from detecting associations in some of the subgroups. In addition, we cannot completely exclude that some of the

TABLE 5
Odds ratios and 95% CIs of invasive breast cancer across tertiles of plasma folate concentrations in strata of *MTHFR* polymorphisms among cases and controls >55 y of age from the Malmö Diet and Cancer cohort[1]

		Tertiles of plasma folate concentrations[2]			
	No. of cases/controls	1 (6 nmol/L)	2 (10 nmol/L)	3 (17 nmol/L)	*P* for trend (*P* for interaction)[3]
All women	312/617	1.00	1.00 (0.70, 1.43)	1.20 (0.84, 1.70)	0.31
Strata of *MTHFR 677C→T*					(0.002)
CC	141/322	1.00	0.79 (0.46, 1.35)	0.85 (0.51, 1.44)	0.87
CT	142/245	1.08 (0.63, 1.86)	0.99 (0.56, 1.75)	1.29 (0.74, 2.23)	0.53
CT+TT[4]	170/292	0.87 (0.52, 1.47)	1.10 (0.64, 1.89)	1.51 (0.89, 2.55)	0.03
Strata of *MTHFR 1298A→C*					(0.22)
AA	146/268	1.00	1.87 (1.08, 3.25)	1.97 (1.16, 3.34)	0.01
AC	136/273	1.92 (1.11, 3.33)	1.24 (0.71, 2.16)	1.30 (0.75, 2.26)	0.34
AC+CC[4]	165/343	1.76 (1.04, 2.98)	1.09 (0.64, 1.86)	1.48 (0.88, 2.50)	0.64
Strata of *MTHFR* combined genotypes					
677 CC-1298AA	39/96	1.00	1.82 (0.62, 5.35)	1.07 (0.37, 3.11)	0.71
677CC-1298AC	73/153	2.21 (0.78, 6.20)	1.27 (0.46, 3.44)	1.22 (0.44, 3.35)	0.19
677 CC-1298AC/CC[4]	102/223	1.86 (0.69, 4.97)	1.06 (0.40, 2.82)	1.55 (0.59, 4.08)	0.91
677 CT-1298AA	78/125	1.48 (0.53, 4.11)	1.71 (0.61, 4.85)	2.40 (0.88, 6.57)	0.20
677CT-1298AC	63/120	1.86 (0.68, 5.14)	1.36 (0.46, 3.97)	1.60 (0.56, 4.57)	0.73
677 CT/TT-1298AA[4]	106/172	1.08 (0.40, 2.91)	2.04 (0.75, 5.54)	3.00 (1.13, 7.93)	0.002

[1] Models were adjusted for age, blood-sampling date, weight, height, menopausal hormone therapy, age at menopause category, parity, household work category, socioeconomic status, smoking, total energy intake, and alcohol intake category. Odds ratios were calculated by using unconditional logistic regression.
[2] Tertiles of ln plasma folate in controls; tertile median plasma concentration in parentheses.
[3] *P* values for trend for tertiles of plasma folate (treated as a continuous variable). *P* values for interaction between tertiles of plasma folate (treated as a continuous variable) and *MTHFR* genotype (treated as a continuous variable).
[4] Separate analysis of genotypes that are homozygous for variant alleles was not conducted because the strata included too few subjects.

subgroup associations could have occurred by chance as a consequence of multiple tests. However, the fact that the main finding of increased breast cancer risk at high P-folate concentrations among *677T* carriers became stronger when the analysis was restricted to *1298AA* women reduces the risk that these observations occurred due to chance. Information on family history of breast cancer was missing. However, this issue may be more crucial in studies in premenopausal women (41).

There are inconsistencies among studies that have investigated the relation between breast cancer and blood concentrations of folate. An Australian case-control study found that breast cancer risk was lower at higher concentrations of serum folate (16), and similar, although not statistically significant, observations were made in a case-control study on P-folate from Taiwan (17). Reverse causation, however, cannot be excluded in case-control studies. Four prospective studies investigated blood folate status in relation to breast cancer. In an Australian study, low erythrocyte folate, but not serum folate, was associated with increased risk (18). Three studies were conducted in the United States. The Nurses' Health Study indicated a nonsignificant inverse association between P-folate and breast cancer (20). The association was statistically significant only among women with relatively high alcohol intake. In the Washington County Study, no associations were observed between serum folate and breast cancer (19). In the Women's Health Study, plasma folate concentrations were not associated with breast cancer risk in postmenopausal women, but high concentrations were associated with increased risk in premenopausal women (21). In addition, tendencies of an increased risk of death from breast cancer have been observed among women who received 5 mg folic acid/d during pregnancy (thereby increasing their serum folate) in a clinical trial (42).

The *677T* allele has in most studies shown positive, or no, associations with postmenopausal breast cancer (11, 12, 43, 44). However, in a case-control study from Taiwan, the minor alleles of the *677C→T* and *1298A→C* SNPs were associated with decreased risk of breast cancer among women with P-folate concentrations below the median, but no associations were observed among women with P-folate concentrations above the median. In the Australian case-control study, no interaction was found between serum folate and the *677C→T* polymorphism. A case-control study from Shanghai reported an interaction between the *MTHFR 677C→T* polymorphism and dietary folate intake on breast cancer risk ($P = 0.05$) (13). In that study, high folate intake was associated with an overall decreased breast cancer risk. The highest risk was observed among *677TT* women with low folate intake. However, our observations of an increased risk for women with the *677T* allele and high P-folate concentrations are in line with the high risk observed among *677TT* women with high folate intakes in the same study cohort (10). Our analysis of genotype combinations indicates that the positive association between plasma folate concentrations and breast cancer risk in all *1298AA* women was a consequence of linkage between the major *1298A* allele and the minor *677T* allele.

Conflicting results from studies on folate, *MTHFR*, and cancer at different sites suggest complicated mechanisms may be involved (45). The *677T* allele has in most studies been associated with moderately decreased risks of colorectal cancer (46). Possibly because of prevention of impaired DNA synthesis, this might be more harmful to the highly proliferating epithelial cells of the colon than to breast cells. In some studies, low folate intake (47, 48), or low plasma folate concentrations (49), seem to reduce the protective effect of the *677T* allele on colon cancer. In contrast, other studies indicate protective effects only with low folate intake (50, 51). Differences in intakes of folate or alcohol may explain the contradictory results (52). The *1298A→C* SNP has been less studied. However, in one study, the *1298C* allele seemed to be more protective in relation to colon cancer than the *677T* allele (51). Although circulating folate has not shown any important overall associations with prostate cancer (53, 54), it is interesting to note that, similar to our observations for breast cancer, another Swedish study indicated that the *677T* allele in combination with high plasma folate concentrations may increase the risk of prostate cancer (55). In addition, a clinical trial has recently shown a positive association between folic acid supplementation and risk of prostate cancer (56).

The *677T* allele (as a marker of reduced MTHFR activity and low P-folate) indicates that the balance of folate isomers is pushed toward 5,10-methylene THF (needed for DNA synthesis) rather than 5-methyl THF (needed for DNA methylation). In contrast to our findings, it is reasonable to expect that high P-folate (mainly 5-methyl THF) would promote DNA methylation and counteract the high breast cancer risk among *677T* carriers in the MDC cohort. On the other hand, high P-folate concentrations indicate high total folate status and thereby also further increase intracellular 5,10-methylene THF. Effective DNA synthesis is especially important in rapidly dividing cells, such as neoplasms (52). It is possible that accumulation of 5,10-methylene THF promotes progression of neoplastic cells that would otherwise not necessarily progress into cancer cells. Folate has shown dual influence on carcinogenesis; it seems to suppress tumor development in normal tissue but stimulate the progression of neoplasms (57). Supraphysiologic doses may even stimulate tumor development in normal tissue (57). In addition, the effects of very high total folate status are not fully understood, but this may inhibit the activity of enzymes in the folate cycles (58), which may influence DNA synthesis and repair and DNA methylation. Reduction of synthetic folic acid into bioactive tetrahydrofolate may also be affected, possibly leading to accumulation of unmetabolized folic acid in the blood. It is unclear, however, whether circulating folic acid is connected with any health risks. In one study, folic acid was related to lower natural killer cell cytotoxicity (59). Unmetabolized folic acid binds to carrier proteins and may compete with the bioactive forms for transport into the cells (60), with unknown consequences. Although high plasma folate concentration is a marker of high total folate status, unchanged results after adjustment for intake of dietary folate equivalents may indicate that plasma folate per se is of importance in breast cancer development among carriers of the *677T* allele.

This prospective Swedish study suggests that high P-folate concentrations may be associated with increased risk of postmenopausal breast cancer in women with the *MTHFR 677T* allele. Our results need to be confirmed in other studies. However, in light of the debate concerning the need and amounts of folic acid fortification (58, 61, 62), these findings underline the importance of more research on folate in different population subgroups and in relation to different health outcomes.

We thank the staff and participants of the Malmö Diet and Cancer Study.
The authors' responsibilities were as follows—UCE: responsible for writing the manuscript, performed the data analysis, and contributed to the study design; MILI: responsible for the blood analyses and gave advice concerning genetic matters in the revision of the manuscript; ES: contributed to the revision of the manuscript; BG: gave statistical advice and contributed to the study design and revision of the manuscript; HO: gave advice on matters related to oncology and contributed to the interpretation of results and revision of the manuscript; JC: gave significant advice concerning biochemical and genetic matters in the study design and in the revision of the manuscript; and EW: contributed to the study design, the interpretation of results, and revision of the manuscript. No conflicts of interest were reported.

REFERENCES

1. Blount BC, Mack MM, Wehr CM, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci USA 1997;94:3290–5.
2. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr 2000;130:129–32.
3. Kim YI. Nutritional epigenetics: impact of folate deficiency on DNA methylation and colon cancer susceptibility. J Nutr 2005;135:2703–9.
4. Zhu K, Davidson NE, Hunter S, et al. Methyl-group dietary intake and risk of breast cancer among African-American women: a case-control study by methylation status of the estrogen receptor alpha genes. Cancer Causes Control 2003;14:827–36.
5. Baylin SB, Herman JG, Graff JR, Vertino PM, Issa JP. Alterations in DNA methylation: a fundamental aspect of neoplasia. Adv Cancer Res 1998;72:141–96.
6. Wajed SA, Laird PW, DeMeester TR. DNA methylation: an alternative pathway to cancer. Ann Surg 2001;234:10–20.
7. 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;10:111–3.
8. Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in US physicians. Circulation 1996;94:2410–6.
9. van der Put NM, Gabreels F, Stevens EM, et al. A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects? Am J Hum Genet 1998;62:1044–51.
10. Ericson U, Sonestedt E, Ivarsson MI, et al. Folate intake, methylenetetrahydrofolate reductase polymorphisms, and breast cancer risk in women from the Malmo Diet and Cancer cohort. Cancer Epidemiol Biomarkers Prev 2009;18:1101–10.
11. Lissowska J, Gaudet MM, Brinton LA, et al. Genetic polymorphisms in the one-carbon metabolism pathway and breast cancer risk: a population-based case-control study and meta-analyses. Int J Cancer 2007;120:2696–703.
12. Chen J, Gammon MD, Chan W, et al. One-carbon metabolism, MTHFR polymorphisms, and risk of breast cancer. Cancer Res 2005;65:1606–14.
13. Shrubsole MJ, Gao YT, Cai Q, et al. MTHFR polymorphisms, dietary folate intake, and breast cancer risk: results from the Shanghai Breast Cancer Study. Cancer Epidemiol Biomarkers Prev 2004;13:190–6.
14. Giovannucci E. Alcohol, one-carbon metabolism, and colorectal cancer: recent insights from molecular studies. J Nutr 2004;134:2475S–81S.
15. Halsted CH, Villanueva JA, Devlin AM, Chandler CJ. Metabolic interactions of alcohol and folate. J Nutr 2002;132:2367S–72S.
16. Beilby J, Ingram D, Hahnel R, Rossi E. Reduced breast cancer risk with increasing serum folate in a case-control study of the C677T genotype of the methylenetetrahydrofolate reductase gene. Eur J Cancer 2004;40: 1250–4.
17. Chou YC, Wu MH, Yu JC, et al. Genetic polymorphisms of the methylenetetrahydrofolate reductase gene, plasma folate levels and breast cancer susceptibility: a case-control study in Taiwan. Carcinogenesis 2006;27:2295–300.
18. Rossi E, Hung J, Beilby JP, Knuiman MW, Divitini ML, Bartholomew H. Folate levels and cancer morbidity and mortality: prospective cohort study from Busselton, Western Australia. Ann Epidemiol 2006;16: 206–12.
19. Wu K, Helzlsouer KJ, Comstock GW, Hoffman SC, Nadeau MR, Selhub J. A prospective study on folate, B12, and pyridoxal 5′-phosphate (B6) and breast cancer. Cancer Epidemiol Biomarkers Prev 1999;8:209–17.
20. Zhang SM, Willett WC, Selhub J, et al. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer: a prospective study of folate intake and the risk of breast cancer. J Natl Cancer Inst 2003;95: 373–80.
21. Lin J, Lee IM, Cook NR, et al. Plasma folate, vitamin B-6, vitamin B-12, and risk of breast cancer in women. Am J Clin Nutr 2008;87:734–43.
22. Larsson SC, Giovannucci E, Wolk A. Folate and risk of breast cancer: a meta-analysis. J Natl Cancer Inst 2007;99:64–76.
23. Manjer J, Carlsson S, Elmstahl S, et al. The Malmo Diet and Cancer Study: representativity, cancer incidence and mortality in participants and non-participants. Eur J Cancer Prev 2001;10:489–99.
24. Callmer E, Riboli E, Saracci R, Akesson B, Lindgarde F. Dietary assessment methods evaluated in the Malmo food study. J Intern Med 1993;233:53–7.
25. Wirfalt E, Mattisson I, Johansson U, Gullberg B, Wallstrom P, Berglund G. A methodological report from the Malmo Diet and Cancer study: development and evaluation of altered routines in dietary data processing. Nutr J 2002;1:3.
26. Pero RW, Olsson A, Berglund G, Janzon L, Larsson SA, Elmstahl S. The Malmo biological bank. J Intern Med 1993;233:63–7.
27. Elmståhl S, Wallström P, Berglund G, et al. The use of dietary supplements in relation to dietary habits in a Swedish middle-aged population. Scand J Nutr 1994;38:94–7.
28. Willett W, Stampfer MJ. Total energy intake: implications for epidemiologic analyses. Am J Epidemiol 1986;124:17–27.
29. Taylor HL, Jacobs DR Jr, Schucker B, Knudsen J, Leon AS, Debacker G. A questionnaire for the assessment of leisure time physical activities. J Chronic Dis 1978;31:741–55.
30. Richardson MT, Leon AS, Jacobs DR Jr, Ainsworth BE, Serfass R. Comprehensive evaluation of the Minnesota Leisure Time Physical Activity Questionnaire. J Clin Epidemiol 1994;47:271–81.
31. National Bureau of Statistics. Occupations in population and housing census 1985 (FoB 85) according to Nordic standard occupational classification (Nordisk yrkesklassificering NYK) and Swedish socioeconomic classification (Socioekonomisk indelning SEI), 1989:5. Stockholm, Sweden: Statistics Sweden, 1989 (in Swedish).
32. Merlo J, Berglund G, Wirfalt E, et al. Self-administered questionnaire compared with a personal diary for assessment of current use of hormone therapy: an analysis of 16,060 women. Am J Epidemiol 2000;152: 788–92.
33. White E. Effects of biomarker measurement error on epidemiological studies. IARC Sci Publ 1997;142:73–93.
34. Chen J, Ma J, Stampfer MJ, Palomeque C, Selhub J, Hunter DJ. Linkage disequilibrium between the 677C>T and 1298A>C polymorphisms in human methylenetetrahydrofolate reductase gene and their contributions to risk of colorectal cancer. Pharmacogenetics 2002;12:339–42.
35. Mao R, Fan Y, Chen F, Sun D, Bai J, Fu S. Methylenetetrahydrofolate reductase gene polymorphisms in 13 Chinese ethnic populations. Cell Biochem Funct 2008;26:352–8.
36. Zetterberg H, Regland B, Palmer M, et al. Increased frequency of combined methylenetetrahydrofolate reductase C677T and A1298C mutated alleles in spontaneously aborted embryos. Eur J Hum Genet 2002;10:113–8.
37. Vineis P. Sources of variation in biomarkers. IARC Sci Publ 1997;142: 59–71.
38. Drogan D, Klipstein-Grobusch K, Wans S, Luley C, Boeing H, Dierkes J. Plasma folate as marker of folate status in epidemiological studies: the European Investigation into Cancer and Nutrition (EPIC)-Potsdam study. Br J Nutr 2004;92:489–96.
39. Smulders YM, Smith DE, Kok RM, et al. Red blood cell folate vitamer distribution in healthy subjects is determined by the methylenetetrahydrofolate reductase C677T polymorphism and by the total folate status. J Nutr Biochem 2007;18:693–9.
40. Fazili Z, Pfeiffer CM. Measurement of folates in serum and conventionally prepared whole blood lysates: application of an automated 96-well plate isotope-dilution tandem mass spectrometry method. Clin Chem 2004;50:2378–81.
41. Loman N, Johannsson O, Kristoffersson U, Olsson H, Borg A. Family history of breast and ovarian cancers and BRCA1 and BRCA2 mutations in a population-based series of early-onset breast cancer. J Natl Cancer Inst 2001;93:1215–23.
42. Charles D, Ness AR, Campbell D, Davey Smith G, Hall MH. Taking folate in pregnancy and risk of maternal breast cancer. BMJ 2004;329: 1375–6.

43. Lewis SJ, Harbord RM, Harris R, Smith GD. Meta-analyses of observational and genetic association studies of folate intakes or levels and breast cancer risk. J Natl Cancer Inst 2006;98:1607–22.
44. Suzuki T, Matsuo K, Hirose K, et al. One-carbon metabolism-related gene polymorphisms and risk of breast cancer. Carcinogenesis 2008;29:356–62.
45. Kim YI. Folate and colorectal cancer: an evidence-based critical review. Mol Nutr Food Res 2007;51:267–92.
46. Sharp L, Little J. Polymorphisms in genes involved in folate metabolism and colorectal neoplasia: a HuGE review. Am J Epidemiol 2004;159: 423–43.
47. Le Marchand L, Donlon T, Hankin JH, Kolonel LN, Wilkens LR, Seifried A. B-vitamin intake, metabolic genes, and colorectal cancer risk (United States). Cancer Causes Control 2002;13:239–48.
48. Chen J, Giovannucci E, Kelsey K, et al. A methylenetetrahydrofolate reductase polymorphism and the risk of colorectal cancer. Cancer Res 1996;56:4862–4.
49. Ma J, Stampfer MJ, Giovannucci E, et al. Methylenetetrahydrofolate reductase polymorphism, dietary interactions, and risk of colorectal cancer. Cancer Res 1997;57:1098–102.
50. Sharp L, Little J, Brockton NT, et al. Polymorphisms in the methylenetetrahydrofolate reductase (MTHFR) gene, intakes of folate and related B vitamins and colorectal cancer: a case-control study in a population with relatively low folate intake. Br J Nutr 2008;99:379–89.
51. Keku T, Millikan R, Worley K, et al. 5,10-Methylenetetrahydrofolate reductase codon 677 and 1298 polymorphisms and colon cancer in African Americans and whites. Cancer Epidemiol Biomarkers Prev 2002;11:1611–21.
52. Kim YI. Role of folate in colon cancer development and progression. J Nutr 2003;133:3731S–9S.
53. Johansson M, Appleby PN, Allen NE, et al. Circulating concentrations of folate and vitamin B12 in relation to prostate cancer risk: results from the European Prospective Investigation into Cancer and Nutrition study. Cancer Epidemiol Biomarkers Prev 2008;17:279–85.
54. Weinstein SJ, Hartman TJ, Stolzenberg-Solomon R, et al. Null association between prostate cancer and serum folate, vitamin B(6), vitamin B (12), and homocysteine. Cancer Epidemiol Biomarkers Prev 2003;12: 1271–2.
55. Van Guelpen BR, Wiren SM, Bergh AR, Hallmans G, Stattin PE, Hultdin J. Polymorphisms of methylenetetrahydrofolate reductase and the risk of prostate cancer: a nested case-control study. Eur J Cancer Prev 2006;15:46–50.
56. Figueiredo JC, Grau MV, Haile RW, et al. Folic acid and risk of prostate cancer: results from a randomized clinical trial. J Natl Cancer Inst 2009; 101:432–5.
57. Kim YI. Does a high folate intake increase the risk of breast cancer? Nutr Rev 2006;64:468–75.
58. Smith AD, Kim YI, Refsum H. Is folic acid good for everyone? Am J Clin Nutr 2008;87:517–33.
59. Troen AM, Mitchell B, Sorensen B, et al. Unmetabolized folic acid in plasma is associated with reduced natural killer cell cytotoxicity among postmenopausal women. J Nutr 2006;136:189–94.
60. Qiu A, Jansen M, Sakaris A, et al. Identification of an intestinal folate transporter and the molecular basis for hereditary folate malabsorption. Cell 2006;127:917–28.
61. Kim YI. Will mandatory folic acid fortification prevent or promote cancer? Am J Clin Nutr 2004;80:1123–8.
62. Ulrich CM, Potter JD. Folate supplementation: too much of a good thing? Cancer Epidemiol Biomarkers Prev 2006;15:189–93.