Folate Intake, Methylenetetrahydrofolate Reductase Polymorphisms, and Breast Cancer Risk in Women from the Malmö Diet and Cancer Cohort

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Abstract

Background: **Single nucleotide polymorphisms (SNP) of the folate-metabolizing enzyme methylenetetrahydrofolate reductase (MTHFR) may modify associations between folate intake and breast cancer. We examined if the association between tertiles of dietary folate equivalents (DFE) and breast cancer was different in subgroups according to genotypes of the *MTHFR* 677 C>T (rs1801133) and 1298A>C (rs1801131) SNPs and if the polymorphisms per se were associated with breast cancer.**

Methods: **This nested case-control study included 544 incident cases with invasive breast cancer and 1,088 controls matched on age and blood sampling date from the population-based Malmö Diet and Cancer cohort. Genotyping of the *MTHFR* SNPs was done with PCR-based matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. Odds ratios (OR) were obtained by unconditional logistic regression.**

Results: **DFE was positively associated with breast cancer in *MTHFR* 677CT/TT−1298AA women (*P* for trend = 0.01) but inversely associated in compound heterozygous women (*P* for trend = 0.01). Interaction was observed between DFE and the 1298C allele (*P* = 0.03). The 677T allele was associated with increased breast cancer risk in women above 55 years [multivariate adjusted OR, 1.34; 95% confidence interval (95% CI), 1.01-1.76] and an interaction was observed between the T allele and age (*P* = 0.03). Homozygosis for the 1298C allele was associated with increased risk in women between 45 and 55 years (multivariate adjusted OR, 1.89; 95% CI, 1.09-3.29).**

Conclusion: **In conclusion, a positive association between DFE and breast cancer was observed in *MTHFR* 677CT/TT−1298AA women but an inverse association was observed in 677CT−1298AC women. The 677T allele was associated with higher breast cancer risk in women above 55 years of age.** (Cancer Epidemiol Biomarkers Prev 2009;18(4):1101–10)

Introduction

Vegetables, fruits, and cereals are important dietary sources of folate. Especially high concentrations of this B vitamin are detected in liver, green leafy vegetables, and legumes (1). The biological function of folate is to allow enzymes to transmit one-carbon groups to diverse biological reactions, and different biochemical forms of folate transmit one-carbon units to specific pathways (2). In particular, two forms seem to be crucial in carcinogenesis. 5,10-Methylene tetrahydrofolate (5,10-MTHF) may influence cancer development via transmission of one-carbon units for DNA synthesis and repair, and deficiency may lead to misincorporation of uracil instead of thymine to DNA (3). 5-Methyltetrahydrofolate (5-MTHF) can provide one-carbon units for DNA methylation reactions via homocysteine, methionine, and *S*-adenosylmethionine, and the expression of protooncogenes and tumor suppressor genes could be affected by changes in DNA methylation patterns (4). Methylenetetrahydrofolate reductase (MTHFR) is an enzyme that catalyzes the irreversible transformation of 5,10-MTHF into 5-MTHF (5). The minor alleles of two well-known single nucleotide polymorphisms (SNP) of *MTHFR*, 677C>T (rs1801133 ref dbSNP) and 1298A>C (rs1801131 ref dbSNP), have been related to reduced activity of this enzyme (6, 7). Consequently, the distribution of one-carbon units available for DNA synthesis and DNA methylation may be altered in carriers of these alleles, and the importance of folate intake in cancer development might be different in these individuals (8).

High folate intake has been associated with lower breast cancer risk in the Malmö Diet and Cancer (MDC) cohort (9) but most prospective studies have not observed any overall association (10). One study even reported an increased risk at high folate intake (11). The conflicting observations may depend on diverse intake ranges in the study populations and different intakes of other B vitamins involved in folate metabolism (12, 13). It may also reflect the importance of effect modifiers, such as *MTHFR* polymorphisms and alcohol consumption (14). Only a few studies have examined the modifying effects of *MTHFR* polymorphisms on the

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association between folate intake and breast cancer, and the results are inconclusive (8, 15-18).

This study on women from the MDC cohort was conducted to investigate if the total folate intake was associated with invasive breast cancer in subgroups according to the *MTHFR* 677C>T and 1298A>C polymorphisms. We also wanted to find out if these polymorphisms per se were associated with invasive breast cancer in our study population. We intended to perform age-stratified analyses (women below and above 55 years of age) because we have earlier observed that folate intake was more closely related to breast cancer in women above 55 years of age from the MDC cohort (9). Previous studies have also indicated that relations between *MTHFR* polymorphisms and breast cancer may depend on menopausal status (8).

Materials and Methods

Study Design. The MDC study is a prospective cohort study in Malmö, a city in the south of Sweden with ~250,000 inhabitants. In 1991, the MDC source population was defined as all persons living in the city of Malmö and born between 1926 and 1945. However, in May 1995, the cohort was extended to include all women born from 1923 to 1950, and all men born from 1923 to 1945. With this extension, 74,138 persons constituted the source population. The women were between ages 45 and 73 y at baseline. The MDC study was approved by the Ethical Committee at Lund University (LU 51-90). Details of the recruitment procedures and the cohort have been described elsewhere (19). Briefly, participants were invited by personal letters or came spontaneously after invitation by advertisement in local newspapers, in public places, or in primary health care centers. Inadequate Swedish language skills and mental incapacity were the only exclusion criteria. The participants visited the MDC screening center twice. During the first visit, groups of six to eight participants were instructed how to register meals in a menu book and how to fill out the diet questionnaire and the extensive general questionnaire covering socioeconomic and lifestyle factors. Nurses drew blood samples, registered blood pressure, and made anthropometric measurements. All questionnaires were completed at home. During the second visit, ~10 d after the first, the socioeconomic questionnaire was checked and a dietary interview was conducted. In October 1996, when recruitment closed, 28,098 participants had completed all baseline examinations.

Study Population. Participants with prevalent cancers at baseline, except those with cervix cancer *in situ*, were excluded. Cases are all women with invasive breast cancer diagnosed during follow-up (until December 31, 2004). The study includes 544 cases of breast cancer. Two controls (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 study participants had migrated from Sweden.

Breast Cancer Case Definition and Ascertainment. The Swedish Cancer Registry and the Southern Swedish Regional Tumor Registry provided data on case definition and ascertainment. Invasive cancer was defined as all cancers except *in situ* cancer. As *in situ* cancer does not necessarily progress into invasive cancer (20), inclusion of *in situ* cancer may obscure true associations between diet and serious disease. Information on vital status was obtained from the National Tax Board, which provides up-to-date information on vital status for all Swedish residents.

Dietary Data. The MDC study used an interview-based, modified diet history method that combined (*a*) a 7-d menu book for registration of lunch and dinner meals, cold beverages (including alcohol), drugs, natural remedies, and nutrient supplements; (*b*) a 168-item questionnaire for assessment of meal pattern, consumption frequencies, and portion sizes of regularly eaten foods; and (*c*) a 45-min complementary interview. The consistency of the information provided was carefully checked so that the questionnaire and menu book did not overlap.

The mean daily intake of foods was calculated based on frequency and portion size estimates from the questionnaire and menu book. The food intake was converted to energy and nutrient intakes using the MDC nutrient database in which the majority of the nutrient information comes from PC-KOST2-93 from the National Food Administration in Uppsala, Sweden. The MDC method is described in detail elsewhere (21, 22). Nutrient intakes from supplements were calculated based on registration of supplement consumption in the menu book. The supplement consumption was converted to nutrient intakes using the MDC supplement database. This database contains information about nutrient levels in medical drugs, herbal remedies, and supplements consumed in the MDC study (23).

The relative validity of the MDC method was evaluated in 1984 to 1985 in a sample of Malmö residents (105 women and 101 men, 50-69 y old) using 18 d of weighed records, 3 d every 2nd month during a year, as the reference method (24, 25). The Pearson correlation coefficients, adjusted for total energy, between the reference method and the MDC method were, in women, 0.75 for folate, 0.69 for dietary fiber, 0.70 for carotene, and 0.71 for ascorbic acid.

Dietary Variables. This study examined total intakes, including supplements, of energy and the following nutrients: folate (μg), vitamin B12 (μg), vitamin B6 (mg), riboflavin (mg), dietary fiber (g), carotene (mg), and ascorbic acid (mg). These variables were selected because they are involved in folate metabolism or found in the same foods as folate. Because intake of folate from foods and intake of dietary folate equivalents previously have shown similar associations with breast cancer in the MDC cohort (9), we chose to focus on dietary folate equivalents. Dietary folate equivalents were calculated based on the assumption that the bioavailability of synthetic folic acid consumed in a meal is 1.7 times the bioavailability of food folate (26); that is, dietary folate equivalents = μg food folate + (1.7 × μg folic acid from supplements). Energy-adjusted variables were obtained by regressing intakes of all nutrients on total energy intake (27). Tertiles of nutrient residuals in controls were used as exposure categories.

Other Variables. In September 1994, the processing of dietary data was slightly altered (22). Method version (indicating data collection before or after September 1,

1994) and season of data collection were examined as potential confounders of dietary relations. Information on age was obtained from the personal identification number. Time of blood sampling was referred to the screening week in the MDC study. Age below or above 55 y at baseline was used to examine if observed associations were different in perimenopausal or postmenopausal women. The younger age group (45-55 y) is probably a heterogeneous group with respect to menopausal status, although it is likely that most of them had reached menopause at the date of diagnosis. An exact date of menopause was not available for all women. This is because of missing data on self-reported cessation of menses, imprecise cessation of menses due to menopausal hormone therapy (MHT), or lack of detailed information on history of hysterectomy.

The smoking status of the participants was defined as smokers (including irregular smokers), ex-smokers, and never smokers. Information on total alcohol consumption was converted into a four-category variable. Women reporting zero consumption in the menu book and indicating no consumption of any type of alcohol during the previous year in the general questionnaire were categorized as zero reporters. The other category ranges were <15 g of alcohol/d (low), 15 to 30 g alcohol/d (medium), and >30 g of alcohol/d (high). Leisure-time physical activity was assessed using a questionnaire adapted from the Minnesota Leisure Time Physical Activity Questionnaire (28, 29). 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 hours per week and divided into four groups with cut points every 10 h (0-9, 10-19, 20-29, and 30 or more).

Participants were divided into four categories according to their highest level of education (≤8 y, 9-10 y, 11-13 y, university degree). Classification of socioeconomic index was based on information on job title, tasks, and position at work. The procedure was adapted from that of the 1989 Swedish population census (30). In this study, the information was collapsed into five categories: blue collar workers, white collar workers (low, medium, and high), and self-employed. Retired and unemployed were classified according to their position before retirement/unemployment.

Weight was measured to the nearest 0.1 kg using balance-beam scale with subjects wearing light clothing and no shoes. Standing height was measured with a fixed stadiometer calibrated in centimeters. Body mass index (kg/m^2) was calculated from weight and height. A three-category variable was created (body mass index ≤25, 25-29, ≥30 kg/m^2).

Age at menopause was divided into four categories (<45, 45-50, 50-55, and >55). Duration of contraceptive pills (years) was divided into four categories with zero consumption in the lowest category. Current MHT (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 (31). Parity was the number of participants with no children in the lowest category and with four or more children in the highest. Missing values for the variables were treated as separate categories.

DNA Analysis. Nonfasting blood samples were drawn at baseline. The samples were separated within 1 h, as previously described, producing granulocyte or buffy coat cell suspensions subsequently stored at −80°C (32). DNA was extracted from the cell suspensions using QiaAmp minikits (Qiagen). Genotyping of the *MTHFR* SNPs 677C>T (rs1801133) and 1298A>C (rs1801131) was done 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) using iPLEX reagents and protocol (SEQUENOM) and 10 ng DNA template. Primer sets were forward: ACGTTGGATGACCTGAAGCACTTGAAGGAG, reverse: ACGTTGGATGGAAAAGCTGCGTGATGATG, and massextend: GCGTGATGATGAAATCG for the rs1801133 SNP and forward: ACGTTGGATGTCTCCCGAGAGGTAAAGAAC, reverse: ACGTTGGATGAGGAGCTGCTGAAGATGTGG, and massextend: ATGAGCTGACCAGTGAAG for the rs1801131 SNP (Metabion). All procedures were done 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 of *MTHFR* 677C>T and 24 of 1298A>C). The genotyping of these samples was done on an ABI PRISM 7900HT Sequence detection system (Applied Biosystems) using 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 template at 1 ng/μL were used as template in a total of 6 μL reaction in 384 format using all reagents and instrument settings according to the manufacturer's recommendations.

Genotypes for the 677C>T SNP were determined for 540 cases (99%) and 1,074 controls (99%), whereas genotypes for the 1298A>C were determined for 541 cases (99%) and 1,072 controls (99%).

Statistical Analysis. The SPSS statistical computer package (version 14.0; SPSS Inc.) and Stata (version 10; StataCorp) were used for the statistical analyses. Nutrient variables were log transformed (e-log) to normalize the distribution before analysis.

Differences in baseline status of anthropometric and nutrient variables were examined in cases and controls with ANOVA. Odds ratios (OR) and 95% confidence intervals (95% CI) for breast cancer across categories of reproductive, socioeconomic, and lifestyle characteristics at baseline were computed with unconditional logistic regression with adjustment for matching variables (age and blood sampling date). To detect signs of genotyping error or confounding due to population admixtures, deviation from Hardy-Weinberg equilibrium, among controls, was tested with Pearson's $\times^2$ test. Genotypes of *MTHFR* 677C>T and *MTHFR* 1298A>C were cross-classified. ORs for breast cancer according to *MTHFR* genotypes were computed with unconditional logistic regression with adjustments for matching variables. The analysis was repeated with adjustments for weight, height, household work, smoking, alcohol intake, socioeconomic status, age at menopause, parity, and MHT. The analysis was also stratified on age at baseline above

Table 1. Background characteristics and ORs of breast cancer associated with lifestyle, reproductive, and socioeconomic factors among cases and controls from the MDC cohort

	No. cases/controls	Means in		*P**
		Cases	Controls	
Age	544/1,088	57.1	57.1	0.99
Screening week of blood sample	544/1,088	144	144	0.98
Total crude mean daily intake				
Folate (μg/d)	544/1,088	283	279	0.53 †
B12 (μg/d)	544/1,088	11.1	15.4	0.34 †
B6 (mg/d)	544/1,088	3.1	5.8	0.28 †
Riboflavin (mg/d)	544/1,088	2.6	2.6	0.33 †
Fiber (g/d)	544/1,088	19.4	19.4	0.77 †
β-Carotene (mg/d)	544/1,088	4.0	4.0	0.92 †
Ascorbic acid (mg/d)	544/1,088	181	183	0.80 †
Anthropometry				
Weight	544/1,088	69.2	68.6	0.36
Height	544/1,088	164.5	163.6	0.005
Body mass index	544/1,088	25.6	25.7	0.82

	No. cases/controls	OR ‡ (95% CI)	*P* for trend
MHT			
No	337/796	1.00	
Yes	165/208	1.88 (1.47-2.39)	
Contraceptive pill use, duration category (y)			0.27
0	265/540	1.00	
1-5	95/205	0.95 (0.70-1.28)	
5-15	103/210	1.00 (0.75-1.34)	
>15	73/109	1.37 (0.97-1.94)	
Parity category			0.07
No children	64/96	1.00	
1 child	111/237	0.70 (0.48-1.04)	
2 children	234/469	0.75 (0.52-1.07)	
3 children	94/180	0.78 (0.52-1.17)	
4 or more	26/84	0.46 (0.27-0.80)	
Age at menopause category (y)			0.81
<45	54/116	1.00	
45-50	138/270	1.10 (0.75-1.60)	
50-55	169/354	1.02 (0.70-1.48)	
>55	32/60	1.12 (0.65-1.94)	
Unknown§	151/288	1.17 (0.77-1.78)	
Household work (h/wk)			0.006
0-9	103/166	1.00	
10-19	206/397	0.83 (0.61-1.12)	
20-29	163/327	0.80 (0.58-1.09)	
≥30	62/176	0.56 (0.38-0.82)	
Leisure-time physical activity			0.74
Low	180/360	1.00	
Medium	188/360	1.04 (0.82-1.34)	
High	172/359	0.96 (0.74-1.24)	
Socioeconomic status‖			<0.001
Blue collar workers	179/427	1.00	
White collar workers, low	168/341	1.18 (0.91-1.52)	
White collar workers, medium	104/162	1.55 (1.14-2.10)	
White collar workers, high	47/60	1.89 (1.24-2.88)	
Self-employed§	37/87	1.02 (0.67-1.56)	
Education (y)			0.05
>8	199/438	1.00	
9-10	175/349	1.12 (0.87-1.43)	
11-13	69/140	1.11 (0.79-1.56)	
University degree	98/157	1.41 (1.03-1.93)	
Alcohol category			0.07
Zero consumption (g/d)	37/92	1.00	
<15	412/823	1.25 (0.84-1.86)	
15-30	75/151	1.24 (0.77-2.00)	
>30	20/20	2.50 (1.20-5.20)	
Smoking			0.34
Never	227/492	1.00	
Ex	166/298	1.21 (0.94-1.55)	
Current	151/296	1.11 (0.86-1.43)	

*ANOVA.
† Adjusted for energy.
‡ ORs were calculated with unconditional logistic regression. Adjusted for matching variables (age and blood sampling date).
§ Not included in the trend analysis.
‖ The *P* value does not strictly indicate a trend.

or below 55 y. A test for interaction with regard to breast cancer was done using the cross-product term (age below or above 55 y at baseline × occurrence of the variant *MTHFR* allele; 677T and 1298C, respectively). In subgroup analysis according to *MTHFR* genotype, ORs for breast cancer in tertiles of folate intake were computed with unconditional logistic regression, with wild-type women at low folate intake as the reference. Adjustments were made for the matching variables, method version, season, and total energy intake. A second model also included adjustments for established risk factors and potential confounders (i.e., weight, height, household work, smoking, alcohol intake, socioeconomic status, age at menopause, parity, and MHT). An additional model included adjustments for quintile of total vitamin B12 intake, quintile of total vitamin B6 intake, and quintile of total riboflavin intake. 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 total folate intake by more than 10%, or be considered as important risk factors by prior knowledge. As a last step in the selection procedure, a few covariates that were not associated with breast cancer in the multivariate model were excluded. The model was stratified on age at baseline above or below 55 y.

A test for interaction with regard to breast cancer was done (tertile of dietary folate equivalents × occurrence of the variant *MTHFR* allele; 677T and 1298C, respectively).

Results

Baseline anthropometric and nutrient characteristics were similar in cases and controls, but the cases were significantly taller than the controls (Table 1). Averages of the matching variables (age and blood sampling date) were equal in cases and controls. ORs for invasive breast cancer according to reproductive, lifestyle, and socioeconomic characteristics are shown in Table 1. Breast cancer risk was higher among users of MHT. Women with four or more children had lower breast cancer risk than women without children. Women who spent much time on household work (>30 h/wk) were at lower risk than women who spent little time on household work (<10 h/wk). Medium and high white collar workers were at higher breast cancer risk than blue collar workers, and a trend toward increased risk with higher education was observed. There was also a tendency of increased risk with higher alcohol consumption, and women who consumed more than 30 g alcohol/d were at significantly higher risk than zero consumers.

Genotype distributions among controls did not deviate from Hardy-Weinberg equilibrium (677C>T; $P = 0.71$ and 1298A>C; $P = 0.40$). The minor allele frequency was 30% for *MTHFR* 677C>T and 32% for *MTHFR* 1298A>C. The occurrence of combined genotypes of the *MTHFR* 677C>T and 1298A>C polymorphisms are displayed in Table 2. The table shows that the genotypes were not independently distributed across the two loci. No subjects were homozygous for a minor allele at one locus and, simultaneously, carriers of a minor allele at the other locus.

Neither the 677C>T SNP nor the 1298A>C SNP was associated with breast cancer risk in analyses, including all women (ages 45-73 years; Table 3). However, a significant interaction was observed between occurrence of the minor 677T allele and age below or above 55 years at baseline (P for interaction = 0.03). The T allele was related to increased risk of breast cancer in women above 55 years of age. An increased risk was observed for women with the 677CT ($P = 0.05$) genotype or with at least one T allele (CT +TT; $P = 0.04$). Moreover, in analysis of genotype combinations, the 677T allele was associated with a nonsignificant increased risk ($P = 0.06$; 677CT/TT-1298AA compared with 677CC-1298AA). These associations were not observed in women below 55 years of age. In addition, a borderline interaction was observed between occurrence of the minor 1298C allele and age below or above 55 years at baseline ($P = 0.07$). In the younger age group, the 1298CC genotype was associated with an increased risk ($P = 0.02$), and the genotype combination 677CC-1298CC was also at a higher risk compared with 677CC-1298AA ($P = 0.04$). Adjustments for established breast cancer risk factors did not change the associations between *MTHFR* SNPs and breast cancer (data not shown).

Table 4 shows a positive association between dietary folate equivalents and breast cancer risk among all 677 TT women (P for trend = 0.01). A similar tendency is seen among 1298 AA women. Positive associations are also seen for women with the 677TT-1298AA and 677CT/TT-1298AA combinations (P for trend = 0.01). However, an inverse association was observed for compound heterozygous women (677CT-1298AC; P for trend = 0.01). In addition, a significant interaction between tertiles of folate equivalents and occurrence of the variant 1298C allele was detected ($P = 0.03$). The results for both women below and above 55 years of age went in the same directions as in all women. Moreover, in the older age group, the increased risk with higher folate intake among 677CT/TT-1298AA women remained significant.

Adjustments for other B vitamins did not change the observed patterns. However, only the inverse association between dietary folate equivalents and breast cancer among compound heterozygous women (677CT-1298AC; $P = 0.03$), and the interaction between tertiles of folate equivalents and occurrence of the 1298C allele ($P = 0.03$), remained significant. In sensitivity analysis, excluding women diagnosed during the 1st year after baseline examinations, the results remained unchanged (data not shown). Examinations of dietary folate intakes gave results in the same directions as those from dietary folate equivalents (data not shown). However, the inverse association with breast cancer among 677CT/TT-1298AA women was somewhat weaker for dietary folate intake (P for trend = 0.03), and the highest risk estimate was found in the mid-tertile. 677CT/TT-1298AA women consuming folate supplements had higher breast cancer risk than compound wild-type women who did not consume supplements (OR, adjusted for dietary intake: 2.00; 95% CI, 1.14-3.50).

Discussion

In women above 55 years of age, the minor *MTHFR* 677T allele was associated with higher breast cancer risk, and

Table 2. Number of persons with genotype combinations of MTHFR 677C>T and 1298A>C among cases and controls from the MDC study

	677C>T		
	CC	CT	TT
1298A>C			
AA	234	351	141
AC	387	335	0
CC	162	0	0

an interaction was observed between the T allele and age. In women between 45 and 55 years of age, homozygosis for the minor *MTHFR* 1298C allele was associated with higher risk. When examining associations between folate intake and breast cancer in strata of *MTHFR* SNPs, similar associations were seen in both age groups. In analysis of both age groups together, a significantly increased breast cancer risk with higher folate intake was observed in *MTHFR* 677CT/TT-1298AA women; however, in compound heterozygous (677CT-1298AC) women, the risk decreased with higher intakes. A significant interaction was observed between folate intake and the *MTHFR* 1298 A>C SNP.

This is one of few epidemiologic studies that have examined the modifying effect of *MTHFR* SNPs on the association between folate intake and breast cancer (16). The prospective design of this population-based nested case-control study minimizes selection bias, recall bias, and reverse causation. Other advantages are the nearly complete Swedish national cancer registry and the extensive information on confounding variables. The high validity and reliability of estimated nutrient intakes from foods are also valuable (21, 24, 33, 34), as well as the detailed estimation of folic acid from supplements (23). In addition, intakes of other B vitamins of importance in one-carbon metabolism were included in the statistical models. However, we present the analysis without other B vitamins because assessment of dietary exposures is always connected with various degrees of misreporting (35) and should be interpreted with caution. Adjustments for other B vitamins might lead to unstable results, especially in the subgroup analyses. A limitation is that information on family history of breast cancer was missing. On the other hand, this issue may have stronger impact in studies on premenopausal women (36). Moreover, it has been estimated that only 13% of all breast cancer is explained by family history (37). Another limitation is that the number of individuals in genotype subgroups was rather small. This might be especially critical when examining *MTHFR*1298A>C because this SNP seems to influence the MTHFR activity to a lesser extent than *MTHFR* 677C>T (6, 7). Linkage between the examined SNPs has also been observed in other populations (38-40), and occurrence of compound homozygous variant genotypes has only been observed in spontaneous abortion fetal tissues (41). The lack of independence between the two SNPs also makes potential effects connected to one of them difficult to derive. Because most other studies have only examined the *MTHFR* 677 C>T SNP (16, 42-44), examinations of genotype combinations are especially valuable.

Concordant with our study, the 677T allele was associated with increased breast cancer risk only among postmenopausal women in the Japanese Hospital-Based Epidemiologic Research Program at Aichi Cancer Center

Table 3. ORs of breast cancer according to genotypes of MTHFR 677C>T and 1298A>C in cases and controls from the MDC cohort

	No. cases/controls	OR* (95% CI)
All women 45-73 y (n = 1,614)		
MTHFR 677C>T		
CC (wild-type)	255/531	1.00
CT	235/452	1.08 (0.87-1.35)
TT	50/91	1.14 (0.78-1.67)
CT+TT	285/543	1.09 (0.89-1.34)
MTHFR 1298A>C		
AA (wild-type)	242/487	1.00
AC	242/480	1.02 (0.82-1.26)
CC	57/105	1.09 (0.76-1.56)
AC+CC	299/585	1.03 (0.84-1.27)
Genotype combinations; 677C>T/1298A>C		
677 CC, 1298 AA	68/166	1.00
677 CC, 1298 AC	130/257	1.23 (0.87-1.76)
677 CC, 1298 CC	57/105	1.32 (0.86-2.03)
677 CT, 1298 AA	122/229	1.30 (0.91-1.86)
677 CT, 1298 AC	112/223	1.23 (0.85-1.76)
677 TT, 1298 AA	50/91	1.34 (0.86-2.10)
677 CC, 1298 AC + CC	187/362	1.26 (0.90-1.76)
677 CT + TT, 1298AA	172/320	1.31 (0.94-1.84)
Women below 55 y of age (n = 684)		
MTHFR 677C>T		
CC (wild-type)	114/207	1.00
CT	92/206	0.81 (0.58-1.13)
TT	22/43	0.93 (0.53-1.63)
CT+TT	114/249	0.83 (0.60-1.15)
MTHFR 1298A>C		
AA (wild-type)	95/218	1.00
AC	106/205	1.19 (0.85-1.66)
CC	28/34	1.89 (1.09-3.29)
AC+CC	134/239	1.29 (0.94-1.78)
Genotype combinations; 677C>T/1298A>C		
677 CC, 1298 AA	29/70	1.00
677 CC, 1298 AC	57/103	1.34 (0.78-2.30)
677 CC, 1298 CC	28/34	2.00 (1.02-3.87)
677 CT, 1298 AA	43/103	1.00 (0.57-1.75)
677 CT, 1298 AC	49/102	1.17 (0.67-2.03)
677 TT, 1298 AA	22/43	1.24 (0.63-2.44)
677 CC, 1298 AC + CC	85/137	1.50 (0.90-2.51)
677 CT + TT, 1298AA	65/147	1.07 (0.64-1.81)
Women above 55 y of age (n = 930)		
MTHFR 677C>T		
CC (wild-type)	141/324	1.00
CT	143/246	1.34 (1.00-1.78)
TT	28/48	1.34 (0.81-2.23)
CT+TT	171/294	1.34 (1.01-1.76)
MTHFR 1298A>C		
AA (wild-type)	147/269	1.00
AC	136/275	0.90 (0.68-1.21)
CC	29/71	0.75 (0.46-1.20)
AC+CC	165/346	0.87 (0.66-1.15)
Genotype combinations; 677C>T/1298A>C		
677 CC, 1298 AA	39/96	1.00
677 CC, 1298 AC	73/154	1.17 (0.73-1.86)
677 CC, 1298 CC	29/71	1.00 (0.57-1.78)
677 CT, 1298 AA	79/125	1.56 (0.98-2.48)
677 CT, 1298 AC	63/121	1.28 (0.79-2.08)
677 TT, 1298 AA	28/48	1.44 (0.79-2.61)
677 CC, 1298 AC + CC	102/225	1.12 (0.72-1.73)
677 CT + TT, 1298AA	107/173	1.52 (0.98-2.37)

*ORs were calculated with unconditional logistic regression. Adjusted for matching variables (age and blood sampling date).

Table 4. ORs and 95% CIs of invasive breast cancer across tertiles of dietary folate equivalents in strata of MTHFR polymorphisms among cases and controls from the MDC cohort

All women (45-73 y of age, n = 1,614)	Cases/controls	Tertiles of dietary folate equivalents*			P for trend [†] (P for interaction [‡])
		1 (179)	2 (240)	3 (378)	
Strata of MTHFR 677C>T[§]					
CC	255/530	1.00	1.01 (0.69-1.49)	0.90 (0.61-1.31)	0.81
CT	235/450	1.01 (0.69-1.49)	1.06 (0.72-1.57)	0.96 (0.63-1.44)	0.66
TT	50/91	0.68 (0.32-1.44)	1.00 (0.50-2.00)	1.86 (0.99-3.50)	0.01
CT+TT	285/541	0.96 (0.66-1.39)	1.05 (0.72-1.53)	1.10 (0.75-1.62)	0.63 (0.35)
Strata of MTHFR 1298A>C[§]					
AA	242/484	1.00	1.34 (0.89 2.00)	1.42 (0.96-2.12.)	0.07
AC	242/480	1.41 (0.95-2.10)	1.39 (0.92-2.09)	1.12 (0.74-1.69)	0.19
CC	57/105	1.95 (1.01-3.75)	1.14 (0.60-2.19)	1.32 (0.69-2.53)	0.83
AC+CC	299/585	1.49 (1.02-2.17)	1.34 (0.90-1.97)	1.15 (0.78-1.71)	0.15 (0.03)
Strata of MTHFR combined genotypes[§]					
677 CC, 1298 AA	68/165	1.00	1.07 (0.51-2.27)	0.84 (0.40-1.75)	0.95
677CC, 1298 AC	130/257	1.08 (0.55-2.12)	1.36 (0.68-2.70)	1.14 (0.58-2.25)	0.78
677CC, 1298CC	57/105	1.78 (0.79-4.00)	1.02 (0.46-2.30)	1.20 (0.53-2.68)	0.83
677 CC, 1298 AC/CC	187/362	1.24 (0.65-2.36)	1.24(0.65-2.38)	1.16 (0.61-2.21)	0.93
677CT, 1298AA		0.90 (0.45-1.80)	1.28 (0.65-2.55)	1.42 (0.70-2.84)	0.13
677CT, 1298AC	112/223	1.54 (0.78-3.06)	1.16 (0.58-2.31)	0.86 (0.41-1.77)	0.01
677TT, 1298AA	50/91	0.79 (0.32-1.95)	1.17 (0.50-2.74)	2.17 (0.97-4.84)	0.01
677 CT/TT, 1298 AA	172/318	0.88 (0.45-1.70)	1.25 (0.65-2.41)	1.63 (0.85-3.14)	0.01
Women below 55 y of age (n = 684)	Cases/controls	Tertiles of dietary folate equivalents*			P for trend [†] (P for interaction [‡])
		1 (179)	2 (240)	3 (378)	
Strata of MTHFR 677C>T[§]					
CC	114/207	1.00	1.12 (0.62-2.02)	0.90 (0.49-1.64)	0.90
CT	92/206	0.85 (0.46-1.57)	0.84 (0.46-1.52)	0.76 (0.40-1.46)	0.73
TT	22/43	0.27 (0.06-1.28)	1.57 (0.59-4.2)	1.13 (0.45-2.82)	1.00
CT+TT	114/249	0.75 (0.41-1.36)	0.93 (0.53-1.64)	0.84 (0.46-1.53)	0.86 (0.58)
Strata of MTHFR 1298A>C[§]					
AA	95/218	1.00	1.93 (1.00-3.71)	1.69 (0.87-3.28)	0.25
AC	106/205	2.08 (1.07-4.01)	1.92 (0.98-3.75)	1.64 (0.82-3.28)	0.44
CC	28/34	4.08 (1.45-11.49)	2.76 (1.04-7.29)	2.59 (0.79-8.52)	0.55
AC+CC	134/239	2.34 (1.25-4.41)	2.07 (1.09-3.91)	1.78 (0.91-2.65)	0.34 (0.08)
Strata of MTHFR combined genotypes[§]					
677 CC, 1298 AA	29/70	1.00	1.60 (0.48-5.45)	1.00 (0.30-3.27)	0. 79
677CC, 1298 AC	57/103	1.47 (0.48-4.50)	1.76 (0.56-5.53)	1.73 (0.56-5.29)	0.74
677CC, 1298 CC	28/34	3.39 (0.92-12.50)	2.24 (0.63-7.93)	2.12 (0.50-8.90)	0.55
677 CC, 1298 AC/CC	85/137	1.88 (0.65-5.45)	1.93 (0.66-5.69)	1.84 (0.62-5.44)	0.64
677 CT, 1298AA	43/103	0.89 (0.28-2.87)	1.29 (0.42-3.98)	1.62 (0.50-5.25)	0.50
677CT, 1298AC	49/102	2.06 (0.66-6.39)	1.47 (0.48-4.46)	0.95 (0.28-3.19)	0.26
677 TT, 1298 AA	22/43	0.44 (0.07-2.63)	2.61 (0.71-9.67)	1.83 (0.53-6.38)	1.00
677 CT/TT, 1298 AA	65/147	0.78 (0.25-2.44)	1.60 (0.54-4.69)	1.71 (0.58-5.05)	0.25
Women above 55 y of age (n = 930)	Cases/controls	Tertiles of dietary folate equivalents*			P for trend [†] (P for interaction [‡])
		1 (179)	2 (240)	3 (378)	
Strata of MTHFR 677C>T[§]					
CC	141/324	1.00	0.97 (0.58-1.64)	0.90 (0.54-1.49)	0.61
CT	143/246	1.20 (0.72-2.00)	1.34 (0.79-2.29)	1.12 (0.65-1.93)	0.83
CT+TT[‖]	171/294	1.17 (0.72-1.92)	1.21 (0.72-2.01)	1.31 (0.78-2.19)	0.61 (0.56)
Strata of MTHFR 1298A>C[§]					
AA	147/269	1.00	1.10 (0.65-1.88)	1.31 (0.78-2.20)	0.16
AC	136/275	1.17 (0.69-1.96)	1.21 (0.71-2.07)	0.88 (0.51-1.52)	0.32
CC	29/71	1.22 (0.50-2.96)	0.62 (0.24-1.60)	1.02 (0.46-2.29)	0.67
AC+CC	165/346	1.18 (0.72-1.93)	1.07 (0.64-1.79)	0.91 (0.55-1.52)	0.20 (0.14)
Strata of MTHFR combined genotypes[§]					
677 CC, 1298 AA	39/95	1.00	0.82 (0.30-2.22)	0.77 (0.29-2.04)	0.86
677CC, 1298 AC	73/154	0.92 (0.38-2.22)	1.28 (0.53-3.07)	0.89 (0.37-2.16)	0.98
677CC, 1298 CC	29/71	1.22 (0.42-3.55)	0.59 (0.19-1.84)	1.00 (0.37-2.73)	0.67

(*Continued on the following page*)

Table 4. ORs and 95% CIs of invasive breast cancer across tertiles of dietary folate equivalents in strata of MTHFR polymorphisms among cases and controls from the MDC cohort (Cont'd)

Women above 55 y of age (n = 930)	Cases/controls	Tertiles of dietary folate equivalents*			P for trend [†]
		1 (179)	2 (240)	3 (378)	(P for interaction [‡])
Strata of MTHFR 677C>T[§]					
677 CC, 1298 AC/CC	102/225	1.00 (0.43-2.29)	1.02 (0.44-2.37)	0.93 (0.41-2.13)	0.53
677 CT, 1298 AA	79/124	0.96 (0.40-2.30)	1.47 (0.61-3.59)	1.42 (0.59-3.44)	0.16
677CT, 1298AC	63/121	1.46 (0.61-3.59)	1.11 (0.45-2.76)	0.81 (0.32-2.08)	0.12
677 CT/TT[‖], 1298 AA	107/172	0.98 (0.43-2.26)	1.20 (0.51-2.80)	1.66 (0.72-3.84)	0.04

NOTE: ORs were calculated with unconditional logistic regression and were adjusted for energy with the residual model.
*Tertiles of natural log of folate intake in controls, with tertile median intake (μg) in parentheses.
† P for trend of tertiles of dietary folate equivalents (treated as a continuous variable).
‡ P for interaction between tertile of folate intake (treated as a continuous variable) and occurrence of the variant allele.
§ Model with adjustment for age, blood sampling date, method version, season, weight, height, MHT, age at menopause category, parity, household work category, socioeconomic status, smoking, total energy intake, and alcohol intake category.
‖ Separate analysis of genotypes homozygous for variant alleles was not conducted because the strata included too few subjects.

(HERPACC) study (18). In a report from the Long Island breast cancer study (8), the 677T allele was associated with an increased risk independent of menopausal status. However, meta-analyses do not support an association between the *MTHFR* 677C>T polymorphism and breast cancer in postmenopausal women (16, 42-44), and in contrast to this study, they imply that the T allele might be associated with increased risk in premenopausal women (45, 46). The 1298A>C polymorphism has been less examined. Most studies did not find any associations between the 1298 A>C SNP and breast cancer (15, 17, 47) but one indicated a higher risk for the minor 1298C allele among premenopausal women (45). This is in line with our observations in the perimenopausal group (ages 45-55 years; Table 3), especially at low folate intakes (Table 4). In the Cancer Prevention Nutrition cohort (United States of America), women with at least one minor allele from both the 677C>T and 1298A>C SNPs were at increased risk of postmenopausal breast cancer (48). In general, cancers at other sites have also shown strongest associations with the *MTHFR* 677C>T SNP (49-51). However, about colon cancer, several studies have found that variant genotypes of the 1298A>C SNP is more strongly related to reduced colon cancer risk than variants of the 677C>T (52-54). The 1298C allele has also shown more protective associations with some types of leukemia (55, 56). This implies that both SNPs may be of importance in cancer development but the potential effects may depend on cancer site and population characteristics (e.g., folate intake levels).

In a recent large, case-control study from Poland, no interaction between folate intake and the *MTHFR* polymorphisms 677C>T or 1298A>C was observed (16). Likewise, no significant interactions could be detected in the Multiethnic Cohort Study, in the Long Island Breast Cancer Study, or in the recent Japanese HERPACC study (8, 15, 18). However, in contrast to the high risk observed among 677TT women at high folate intakes in our study, the highest risks were observed among 677TT women at low folate intakes in the Long Island and Shanghai Breast Cancer studies, as well as in the postmenopausal part of the HERPACC study (8, 17, 18). On the other hand, only the Shanghai Breast cancer study could show that the observations remained in analysis of 677C>T and 1298A>C genotype combinations. They observed inverse associations between folate intake and breast cancer in all *MTHFR* 677C>T and 1298A>C genotype groups but the association was stronger in women with the 677TT genotype, and a significant interaction was observed between that polymorphism and folate intake (17).

In epidemiologic studies, polymorphisms known to influence metabolic processes, such as nutrient metabolism, may sometimes be used as a proxy of exposure. This is an advantage because it minimizes problems related to measurement errors of nutrient intakes. In addition, it minimizes confounding by other environmental exposures because the distributions of genetic variants in populations are generally independent of the environment (57). In studies on cancer, *MTHFR* SNPs would rather be used as a proxy for the distribution of total folate available for synthesis and methylation of DNA. Both mechanisms are relevant in cancer development. Owing to the relation of *MTHFR* 677C>T and 1298A>C SNPs to breast cancer (in women above and below 55 years of age, respectively), this study suggests that folate is of importance in the etiology of the disease. Furthermore, the divergent associations with breast cancer in strata of *MTHFR* genotypes indicate that we are capturing folate intakes rather than intakes of other nutrients originating from the same foods. It also highlights the importance of stratification according to the *MTHFR* polymorphisms and folate intake, respectively, when examining these variables in relation to breast cancer.

The positive association between the 677T allele and breast cancer in women above 55 years of age indicates that DNA methylation is of particular importance in the development of postmenopausal breast cancer. This is in agreement with the inverse association between folate intake and breast cancer among compound heterozygous women (677CT-1298AC) because a satisfying folate intake is of importance for both DNA synthesis and methylation (2). The opposite folate–breast cancer associations among 677CT/TT-1298AA and compound heterozygous (677CT-1298AC) women, as well as conflicting results from published studies, may reflect the complexity of folate metabolism. Among 677CC/CT women with high total folate status, an increased proportion of folate vitamers other than 5-MTHF has been observed in RBC (58). This indicates that high folate intake pushes the distribution of folate toward DNA

synthesis on behalf of DNA methylation. Although not shown, it is possible that high folate intake may have similar influence on the distribution among 677TT women. In combination with the low MTHFR activity among the 677TT women, high folate intake may lead to an accumulation of other isomers of folate than the one needed for DNA methylation (5-MTHF). The consequences of this possible imbalance are not known. Because synthetic folic acid must be reduced to tetrahydrofolate to become bioactive, it is possible that the unmetabolized inactive form compete with the bioactive forms in the blood by binding with enzymes and carrier proteins (59). In this study, the high breast cancer risk among 677TT women at high folate intakes was especially pronounced in the models including supplemental folate. An explanation to this observation might be that women homozygous for the 677T allele respond differently to folate exposures due to a disturbed folate metabolism and that they may be especially vulnerable to synthetic folic acid.

In conclusion, this study suggests that age modifies the association between the *MTHFR* 677C>T SNP and breast cancer, and the minor T allele was associated with increased breast cancer risk in women above 55 years of age. In addition, and as far as we are aware, this is the first study to suggest that high folate intake increases the risk of breast cancer among 677CT/TT-1298AA women, whereas it tend to decrease the risk in compound heterozygous (677CT-1298AC) women. These findings imply that folate is involved in the development of breast cancer but the mechanisms seem complicated. Therefore, and because of scarce and inconclusive evidence from other studies, interactions between the *MTHFR* SNPs and folate intake with regard to breast cancer risk need to be further investigated in large epidemiologic studies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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