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Reduced breast cancer risk with increasing serum folate in a case–control study of the C677T genotype of the methylenetetrahydrofolate reductase gene

J. Beilby[a], D. Ingram[b], R. Hähnel[c], E. Rossi[a,*]

[a]*Clinical Biochemistry, PathCentre Locked Bag 2009, Nedlands, Western Australia 6009, Australia*
[b]*Breast Centre, Sir Charles Gairdner Hospital, QE II Medical Centre, Nedlands, Western Australia 6009, Australia*
[c]*Centre for Molecular Immunology and Instrumentation, University of Western Australia, Australia*

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Abstract

Breast cancer risk may be associated with folate status or the *C677T* genotype of the methylenetetrahydrofolate reductase (*MTHFR*) gene. We compared serum folate concentrations and *C677T* genotype in 141 breast cancer patients and 109 age-matched controls. Serum folate was significantly lower in cases compared to controls (geometric means, 5.7 versus 6.6 μg/l; $P = 0.005$). Breast cancer risk was not associated with *C677T* genotype. After adjusting for age of menarche, parity, alcohol intake and total fat intake we observed reductions in odds ratios for breast cancer risk comparing the highest with the lowest quartiles of serum folate concentrations of 0.23 (95% confidence interval (CI) 0.09, 0.54) for the entire group, 0.27 (CI 0.09, 0.80) for the wild-type and 0.08 (CI 0.01, 0.52) for the heterozygous *C677T* genotype. We conclude that for the whole group, and the wild-type and heterozygous *C677T* genotypes, increased serum concentrations of folate were associated with reduced risks of breast cancer.

Keywords: Breast cancer; Folic acid; Methylenetetrahydrofolate reductase

1. Introduction

Folate plays a key role in cell division by participating in the production of purine and thymidine nucleotides necessary for the synthesis and repair of DNA and regenerating *S*-adenosylmethionine, a universal methyl donor that also methylates DNA. Folate deficiency may be involved in carcinogenesis through impaired synthesis and repair of DNA, or by causing global hypomethylation of DNA, a possible early event in carcinogenesis [1].

Folate status shows a strong interactive effect with the *C677T* polymorphism of the methylenetetrahydrofolate reductase (*MTHFR*) gene. Published data on the relation between *C677T* polymorphism and cancer risk suggest that the T allele protects against cancer in folate-replete individuals but increases the risk under conditions of impaired folate status [2]. The most compelling clinical and epidemiological evidence linking a lower folate with increased cancer risk, including concentrations not necessarily previously classed as deficient, has been obtained for colorectal cancer. Recent data confirm the *MTHFR C677T* polymorphism is a strong genetic modifier of the effect of folate status on the risk of colorectal cancer; in folate-replete individuals, the homozygous T genotype afforded 50% risk reduction compared to an increased risk for the wild-type genotype [3].

Previous case–control and prospective cohort studies have assessed dietary folate intake rather than serum folate concentrations and the risks of breast cancer have obtained inconsistent results. In two population-based case–control studies, a study of Chinese women found that a high dietary intake of folate was associated with reduced breast cancer risk [4], whereas no such association was found in a study of German women [5]. A large prospective cohort study of 3483 breast cancer

* Corresponding author. Tel.: +61-8-9346-2845; fax: +61-8-9346-3882.
E-mail address: ric.rossi@health.wa.gov.au (E. Rossi).

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cases also examined folate intake and found no association with the overall risk of breast cancer [6]. An advantage of the present case–control study is that folate nutrition was assessed by direct measurement of the serum folate and we compared serum folate concentrations and the *C677T* genotype of the *MTHFR* gene in breast cancer patients and age-matched controls to determine if either might play a part in breast cancer risk.

2. Materials and methods

2.1. Study population

Women referred for management of confirmed breast cancer at either a private clinic or the outpatient clinic of Sir Charles Gairdner Hospital, Perth, Western Australia, were recruited for the study between December 1992 and November 1994. Eligible cases were aged between 30 and 84 years, and were residents of the Perth area. Breast cancer cases ineligible for the study were: pregnant women; women with a history of antibiotic use within the previous 6 weeks; women with a previous history of breast cancer; women unable to speak or read sufficient English; women undergoing surgery within 3 days of diagnosis; and cases where a definite diagnosis of breast cancer was not established until the time of surgery. The present data were collected as part of a larger dietary case–control study of breast cancer in which 3-day urine specimens were analysed for lignan and isoflavonoid phyto-oestrogen content [7]. Recent antibiotic use disqualified women from participation because urinary phyto-oestrogen metabolism is affected by gut flora. 341 women were diagnosed with breast cancer during the study period. A large number of women were excluded, as they did not meet the study criteria, which stipulated collection of blood and the onerous task of collecting 3-day urine samples after diagnosis of breast cancer but before any surgery or other treatment. Serum samples were available from 141 cases.

Cases were individually matched according to 5-year age groups to control for women living in the same postal code area and selected randomly from the Perth electoral roll. Matched controls were invited by letter to participate in a dietary study, with no mention of breast cancer being made. Women who reported a personal history of breast cancer were considered ineligible as controls. All participants were informed of the nature and requirements of the study and provided written consent. In most instances, the same researcher interviewed both the case and matched control and applied a standard questionnaire to obtain demographic, reproductive and life-style information. Of the 441 women randomly chosen as controls for the study, 249 did not wish to participate and 45 could not be contacted. Serum samples were available from 109 control participants.

2.2. Serum and DNA analyses

A single blood sample was drawn by venipuncture and the separated serum stored at -70 °C. Sera from both cases and controls were stored together in the same freezer for a median storage time of 7.6 years. Serum samples were available from 141 cases and 109 controls. Serum folate was analysed by competitive immunoassay using folate-binding protein [8] on an AxSym analyser (Abbott, USA). *MTHFR* genotype was determined on genomic DNA by *Hin*fI digestion of PCR products as described by Frosst and colleagues [9]. The interpretation of the patterns of polymerase chain reaction (PCR) products in electrophoretic gels was checked separately by two scientists.

2.3. Urine analyses

A sample of urine pooled over a 3-day collection period was assayed for urea and ammonia so a measure of total nitrogen excretion could be obtained as an index of total food intake [10]. Urinary urea was measured using an enzymatic rate method on an automatic random-access biochemistry analyser (Hitachi 747, Tokyo, Japan). Urinary ammonia used a glutamate dehydrogenase enzymatic method and was measured on a centrifugal analyser (Cobas Bio, Roche, Switzerland).

2.4. Statistical analysis

The following descriptive statistics were obtained: reproductive variables including age at menarche, age at first full-term birth, parity, months of lactation, and age at menopause were categorised according to classifications in the published literature [11]. An initial analysis of risk factors in participants showed that age at menarche, parity, and dietary fat intake are associated with breast cancer risk and the regression model was therefore adjusted for these variables. Since some studies show that alcohol consumption has an association with breast cancer risk [12], we included alcohol intake as a confounding variable, so the final regression model adjusted for age at menarche, parity, total fat intake and alcohol intake.

The serum folates were skewed, and were logarithmically transformed and presented as geometric means with 95% confidence intervals (CI). Statistical analysis was by multivariate logistic regression with SPSS statistical software version 11.0, with an odds ratio (OR) and its 95% CI used to represent the relative risk for breast cancer. Whether a variable had a significant effect was judged by the *P*-values (two-sided) being $\leqslant 0.05$.

Table 1
Serum folate concentrations in cases and controls related to *MTHFR C677T* genotype

	Folate μg/l, geometric mean (95% confidence interval)		
	Cases	Controls	*P*
Wild-type (CC)	6.1 (5.6, 6.8)	7.3 (6.4, 8.2)	0.03
Heterozygous (CT)	5.6 (5.0, 6.2)	6.6 (5.9, 7.4)	0.03
Homozygous (TT)	4.8 (3.8, 6.0)[a]	5.1 (4.3, 6.2)[b]	0.60

[a] $P=0.04$ for comparison to CC (wild-type genotype) cases.
[b] $P=0.008$ for comparison to CC controls.

3. Results

Descriptive characteristics of cases and controls were as previously published [7]. In brief, statistical analysis showed no significant differences between the case and control groups in age, age at menarche or menopause, parity, age at first full-term birth, duration of lactation, anthropometric variables or the following nutritional variables: intake of alcohol, total energy, total fat or the percentage energy from fat. There was also no significant difference between the cases and controls for the excretion of total nitrogen over a 3-day period.

The three genotypes of the *MTHFR C677T* polymorphism were denoted as follows: wild-type genotype as CC; heterozygous genotype as CT; and homozygotes for the polymorphism as TT. The frequencies of the CC, CT and TT genotypes for the cases were not significantly different to the same genotypes in the controls ($P=0.93$, 0.18 and 0.61, respectively), indicating that breast cancer risk was not significantly associated with the *C677T* polymorphism. The *C677T* genotype frequencies in our total group (cases and controls combined) were as follows: CC in 53% ($n=133$), CT in 33% ($n=82$) and TT in 14% ($n=35$). Although these frequencies indicate our group is not in Hardy–Weinberg equilibrium, the group size of 250 is a relatively small one in which to assess population-equilibrium statistics.

Serum folate concentrations were significantly lower in breast cancer cases compared to controls (geometric means, 5.7 versus 6.6 μg/l; $P=0.01$). When comparing cases and controls within each genotype, breast cancer cases with a CC or CT genotype had significantly lower serum folate concentrations than controls of the respective genotype (Table 1). When TT genotype cases were compared to CC genotype cases and TT cases to TT controls, both TT groups had significantly lower folates (Table 1). A subgroup statistical analysis was performed to test for an interaction between serum folate concentrations, *C677T* genotypes and breast cancer risk and none was detected ($P=0.72$). A further analysis tested for interactions between serum folate concentrations, breast cancer risk and different inheritance modes for the *C677T* genotypes; the dominant mode, CC versus CT and TT genotypes, and the recessive mode, TT versus CC and CT genotypes, and none was found.

For the multivariate logistic regression analysis, serum folate concentrations in the controls were categorised into quartiles and Table 2 shows the OR for breast cancer risk, adjusted for age at menarche, parity, alcohol intake and total fat intake for the group as a whole and the three *C677T* genotypes. Increasing serum folate concentrations were associated with reductions in breast cancer risk, with significant trends through the quartiles for the entire group and the CC and CT genotypes. The adjusted OR for breast cancer risk comparing serum folate concentrations in the highest with the lowest quartiles was 0.23 (CI 0.09, 0.54) for the entire group, 0.27 (CI 0.09, 0.80) for the CC genotype and 0.08 (CI 0.01, 0.52) for the CT genotype. The TT genotype showed no trend.

4. Discussion

We observed significant reductions of breast cancer risk with increasing serum folate concentrations, for the group as a whole and both the CC and CT genotypes. The reduction in breast cancer risk is similar to those

Table 2
Adjusted[a] odds ratios (OR) and 95% confidence intervals (95% CI) for risk of breast cancer associated with serum folate concentrations in the entire group and within *MTHFR C677T* genotypes

	Entire group			Wild-type (CC)	Heterozygous (CT)	Homozygous (TT)
Folate quartiles (μg/l)	Cases (*n*)	Controls (*n*)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
<5.0	62	28	1.00	1.00	1.00	1.00
5.1–6.4	28	27	0.51 (0.23, 1.14)	0.66 (0.19, 2.24)	0.45 (0.17, 4.16)	0.39 (0.06, 2.72)
6.5–8.9	35	27	0.48 (0.23, 1.03)	0.48 (0.20, 1.64)	0.29 (0.07, 1.63)	1.37 (0.10, 18.75)
>9.0	16	27	0.23 (0.09, 0.54)**	0.27 (0.09, 0.80)*	0.08 (0.01, 0.52)*	Nil#
Trend (*P*)			0.001	0.02	0.007	0.93

#There were no cases with a TT genotype. *$P<0.05$; **$P<0.01$.
[a] Adjusted for *C677T* genotype, age of menarche, parity, alcohol intake, total fat intake.

observed for the urine excretion of the phyto-oestrogens equol and enterolactone (OR 0.41 and 0.36, respectively) in the original study [7].

Previous case–control studies assessing dietary folate intake rather than serum folate concentrations and the risks of breast cancer have obtained inconsistent results. As mentioned in the Introduction, in two population-based case–control studies, the highest quartile of dietary folate intake was associated with a reduced risk of breast cancer (OR 0.71 (95% CI, 0.56, 0.92)) in a study of Chinese women [4], whereas no association was found for a study of German women [5]. A well-designed, large, prospective cohort study with 3483 breast cancer cases also examined total folate intake and found no association with the overall risk of breast cancer [6]. An advantage of the present study is that folate nutrition was assessed by direct measurement of serum folate rather than by dietary recall or records.

Two large prospective studies have reported significantly lower breast cancer risk with higher folate intakes only among women who regularly consumed at least one drink of alcohol per day. These were the recent update of the Nurses Health Study of 712 breast cancer cases [13] and a Canadian study of 1469 cases [14], showing that either higher plasma folates or dietary intakes, respectively, were associated with a significantly lower risk in women who regularly consumed at least 15 g/day of alcohol. These studies suggest that the inverse relation between dietary folate intake and breast cancer is reliably demonstrated in women who regularly consume alcohol. In our total group of 250 participants (141 cases and 109 controls), the mean alcohol intake was 9 g/day, 32% were totally abstinent and only 16% consumed more than 15 g/day. This suggests that the present study is not suitable for detecting the effect of alcohol on folate concentrations. Nevertheless we tested for any effects of alcohol consumption on folate concentrations and did not detect any significant changes. We also adjusted the multivariate statistical analysis (Table 2) for alcohol consumption.

This observation of the association of significant reductions of breast cancer risk with increasing serum folate concentrations does not necessarily imply causality. We cannot exclude the possibility that the development of breast cancer over many years could in itself be a cause a lower serum folate. A previous prospective, nested, case–control study also measured serum folate concentrations obtained before breast cancer diagnosis in 195 incident cases and 195 controls and found no association between serum folate and breast cancer [15]. However, studies with colorectal cancer confirm that the *MTHFR C677T* polymorphism is a strong genetic modifier of the effect of folate status on the risk of colorectal cancer and in folate replete subjects, the TT genotype afforded 50% risk reduction compared to an increased risk for the wild-type CC genotype [3].

We confirm the report of strong interactive effects of the *C677T* genotype on folate status [2]. Cases with the TT genotype had significantly lower serum folates than CC cases, and TT controls also had significantly lower serum folates than CC controls. Cases who were either CC or CT had significantly lower serum folates than controls of the respective genotypes. It has been suggested that TT individuals are at increased risk for colorectal cancer when serum folate concentrations are low [2,3], but we were unable to confirm this for breast cancer as only 35 (14%] of our 250 participants bore the TT genotype.

Breast cancer risk was not associated with *C677T* genotype in our study and findings have been variable in the three other studies thus far reported. The T allele conferred significantly increased risk only in premenopausal breast cancer in one study of 105 cases [16] while another study of 335 cases reported increased risk for both pre- and postmenopausal cancer, although the effect was stronger in premenopausal women [17]. The third study examined the *C677T MTHFR* genotypes in only 62 cases and reported reduced breast cancer risk in the TT genotype, a finding at odds with the other two studies [18].

Our findings may also have been influenced by the timing of collection of blood samples immediately following diagnosis of breast cancer at a very stressful time, which may have caused the women affected to have generally eaten less. However, their total urine nitrogen excretion was not significantly different from that of the controls, suggesting that differences in serum folate reflect differences in the types of foods consumed, not just a general stress-related reduction in food intake following breast cancer diagnosis.

A low participation rate is also a source of potential bias. Of the women diagnosed with breast cancer during the study period, a large number were excluded, mainly on the basis of a personal decision by some of them to request immediate surgery, which did not allow sufficient time for the study protocol. The other common reasons for declining participation were that the final diagnosis would not be confirmed until the time of their operation or the women could not arrange for collection of three consecutive 24-h urine specimens required for the other arm of this study. Both reasons would have excluded breast cancer patients on a random rather than systematic basis and we do not believe either affected ascertainment.

Controls were recruited from the community with a less than 50% participation rate. For controls, the main reason for non-participation was the onerous task of collecting three 24-h urine specimens required for another arm of this study while maintaining their usual activities. It is possible that women with an interest in their health and diet would be more likely to volunteer as controls. We were not permitted to record demo-

graphic details of prospective case or control women who finally elected not to participate in the study, and are therefore unable to comment on the possible effects of this self-selection process. However, we acknowledge that our study may have been biased by recruitment of control women with a special interest in their diet and whose serum folate concentrations may therefore have been higher than the average for the community as a whole. This bias might have contributed to the significantly lower serum folate we observed in cases compared to controls.

With this reservation, we conclude that after adjusting for age of menarche, parity, alcohol intake and total fat intake, increased concentrations of serum folate were associated with reduced risks of breast cancer for the group as a whole and for both the CC and CT genotypes. We also find that breast cancer risk was not associated with *C677T* genotype.

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