Research article

Methylenetetrahydrofolate reductase polymorphism and susceptibility to breast cancer

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Abstract

Background: A growing body of evidence suggests that variations in the levels of folate may contribute to the development of cancer. A functional polymorphic variant (C→T substitution at nucleotide 677) in the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene results in the conversion of an alanine to a valine and may modify the risk of breast and other cancers.

Method: We have investigated the possible influence of this *MTHFR* variant on breast cancer risk in a case-control study of 233 healthy women and 335 women who had breast cancer that occurred under the age of 40 years, bilateral breast cancer or a family history of breast cancer.

Results: A significant excess of the valine genotypes was observed among the cases (odds ratio 1.43, 95% confidence interval 1.02–2.00). The effect was more pronounced among the cases with a breast cancer diagnosis under the age of 40 years, with an odds ratio of 1.66 (95% confidence interval 1.12–2.41). A nonsignificant excess of the valine genotypes was observed among the cases with a family history of breast cancer or bilateral breast cancer.

Conclusions: The low activity C677T (valine) genotype of *MTHFR* may increase the risk of early onset breast cancer.

Keywords: breast cancer, MTHFR, polymorphism

Introduction

The enzyme 5,10-methylenetetrahydrofolate reductase (MTHFR) catalyses the reduction of 5,10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate, which is the carbon donor for *de novo* methionine synthesis and DNA methylation. Approximately 10% of Caucasian populations are homozygous for a common C→T substitution at nucleotide 677 of the *MTHFR* gene. This mutation converts an alanine to a valine at codon 225 and is associated with reduced enzyme activity and increased thermolability [1]. The potential influence of MTHFR activity on DNA methylation and on the availability of uridylates and thymidylates for DNA synthesis and repair makes *MTHFR* an attractive candidate for a cancer predisposing gene [2,3].

Deficiencies in thymidylate have been shown to increase the rate of misincorporation of uridylate into DNA and may in turn lead to an increased rate of DNA strand breaks and other chromosomal damage [4]. Consequently, reduced MTHFR activity, which leads to increased levels of cytosolic 5,10-methylenetetrahydrofolate available for thymidylate synthesis, may protect cells from DNA damage induced by uridylate misincorporation. Consistent with this model, several independent studies have shown that the mutant (valine) allele of *MTHFR* may confer a twofold reduction in risk of colon cancer [2,5] and a fourfold reduction in risk of acute lymphocytic leukemia [6]. Mutant *MTHFR* has also been associated with a 2.8-fold increase in risk of endometrial cancer [7] and a 2.9-fold increase in risk of

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bp = basepairs; CI = confidence interval; MTHFR = 5,10-methylenetetrahydrofolate reductase; OR = odds ratio.

cervical intraepithelial neoplasia [8]. In the latter situation the increased cancer risk associated with mutant *MTHFR* might be explained by impairment of DNA methylation due to a reduction in the availability of 5-methyltetrahydrofolate. This is a plausible model because DNA methylation plays a critical role in regulation of gene expression and maintenance of genomic stability [9,10] and aberrations in normal methylation patterns have been associated with the development of cancer [10,11]. More recently, Stern *et al.* [12] demonstrated that individuals who are homozygous for the mutant C677T *MTHFR* polymorphism show a higher degree of genomic DNA hypomethylation than do homozygotes for the wild-type *MTHFR.*

The role of folate in breast cancer has been investigated in several dietary studies and most have shown folate consumption to be inversely related to breast cancer risk [13–16]. By contrast, few studies have investigated the influence of the *MTHFR* C677T polymorphism on breast cancer risk. In one study the frequency of the low MTHFR activity valine allele was reported to occur significantly more frequently in Jewish women diagnosed with bilateral breast cancer or combined breast and ovarian cancer [17]. Another study of unselected breast cancer cases among a Scottish population did not detect any association with the C677T polymorphism and breast cancer risk [18], although the study involved only 62 cases and 66 controls and lacked statistical power.

Given the plausible role of variations in MTHFR activity in breast cancer susceptibility we undertook an analysis of the C677T polymorphism in a case-control study of 335 women with breast cancer and 233 controls.

Materials and methods
Subjects
Women with breast cancer were selected on the basis of an age at onset of less than 40 years, or a family history of breast cancer (defined as two or more cases of breast cancer in a first- or second-degree female relative) irrespective of age at onset, or bilateral breast cancer irrespective of family history or age at onset. All breast cancer cohort cases were systematically ascertained through breast clinics in the Wessex region of southern England as described previously [19]. Briefly, women were invited to take part in a research study, the primary goal of which was to ascertain and verify family histories for segregation analysis. The breast cancer cases diagnosed before 40 years of age were consecutively ascertained without regard to family history. The group of women with bilateral breast cancer were ascertained in the same clinics but the selection criterion was the presence of bilateral breast cancer diagnosed after 40 years of age. The familial breast cancer cases consisted of women presenting to the same clinics with a strong family history of breast or ovarian cancer or both. Family histories were verified as far as

possible using medical records and death certificates. Blood was taken from all recruits who consented to molecular analysis for breast cancer predisposition genes. The age range of the women included in the breast cancer cohort was 19–79 with a mean age of 38 years. The controls represent the population from which the cases arose and consisted of 233 Caucasian female volunteers who were either staff at the Princess Anne Hospital, Southampton, UK or patients attending for non-neoplastic disease conditions. The age of the controls ranged from 18 to 84 with a mean age of 39 years. For all groups normal genomic DNA was prepared from blood lymphocytes. Epidemiological data such as reproductive factors, oral contraceptive use, smoking habits and obesity were not obtained for either the cases or controls. Both control and cancer groups were drawn from the same geographical area, which has predominantly an Anglo-Saxon population.

MTHFR genotyping
Genomic DNA from cases and controls was assessed for the presence of the C677T mutation by PCR amplification using an exonic primer (5′-tgaaggagaaggtgtctgcggga-3′) and an intronic primer (5′-aggacggtgcggtgagagtg-3′) as described previously [1]. PCRs were performed in a reaction volume of 10 μl containing 50 ng of genomic DNA, primers (25 ng of each), 1 × reaction buffer (Genepak, New Milton, UK), 200 nM of dATP, dTTP, dGTP, dCTP (Promega, Annandale, Australia) and 0.2 units of Taq DNA polymerase (Taq Express, Genepak, UK). PCR conditions consisted of an initial denaturation at 94°C for 5 minutes followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 60 s followed by one cycle of 72°C for 5 min. The PCR products were digested with *Hin*fI in a volume of 20 μl and the fragments were separated on 2.0% agarose gels and visualized by ethidium bromide staining. The C→T (valine) variant creates a *Hin*fI restriction enzyme site and produces fragments of 178 basepairs (bp) and 23 bp.

Statistical analysis
Frequency comparisons were analysed using Fisher's exact test. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using the relevant 2 × 2 contingency tables. All statistical calculations were two-sided and performed using InStat version 3.01 (GraphPad Software Inc., San Diego, CA, USA). All statistical tests were based on two-tailed probability.

Results
Table 1 shows the distribution of the C677T polymorphism among the 335 women in the breast cancer cohort and 233 controls. The distribution of the alleles among the controls is consistent with that for previously reported control populations [6,20]. We did not observe a departure from Hardy–Weinberg equilibrium in the breast cancer or the control groups ($P = 0.62$ and $P = 0.37$, respectively).

Table 1

MTHFR C677T genotype distribution among breast cancers and controls

Group	Number of cases	Alanine/Alanine		Alanine/Valine		Valine/Valine		Alanine/Valine or Valine/Valine		Valine allele frequency
		No. (%)	P value[a] (OR, 95% CI)	No. (%)	P value[a] (OR, 95% CI)	No. (%)	P value[a] (OR, 95% CI)	No. (%)	P value[a] (OR, 95% CI)	
Controls	233	118 (50.6)		92 (39.5)		23 (9.9)				0.30
All breast cancer	335	140 (41.8)	1.00	162 (48.4)	0.03 (1.5, 1.0–2.1)	33 (9.8)	0.55 (1.2, 0.7–2.2)	195 (58.2)	0.04 (1.43, 1.02–2.00)	0.34
Under 40 breast cancer	203	78 (38.4)	1.00	104 (51.2)	0.01 (1.7, 1.2–2.6)	21 (10.4)	0.39 (1.4, 0.7–2.7)	125 (61.6)	0.01 (1.64, 1.12–2.41)	0.36
Familial and/or bilateral breast cancer	132	62 (47.0)	1.00	58 (43.9)	0.49 (1.2, 0.8–1.9)	12 (9.1)	1.00 (1.0, 0.5–2.1)	70 (53.0)	0.51 (1.16, 0.75–1.76)	0.31

[a]Fisher's exact test (two-sided) for the relevant genotype using the Alanine/Alanine homozygotes as reference. The odds ratio (OR) and 95% confidence intervals (CI) are shown in parentheses.

The frequency of the valine allele among the breast cancer cohort was 0.34, which was higher than that of the controls (0.30), but this was not statistically significant ($P = 0.30$). The combined number of valine/valine homozygotes and alanine/valine heterozygotes was significantly higher among the breast cancer cases as a whole than among the controls (OR 1.43, 95% CI 1.02–2.00). The significant excess of valine genotypes was only observed among the breast cancer cases diagnosed under the age of 40 years (OR 1.64, 95% CI 1.12–2.41). Interestingly, the valine heterozygotes had a significantly increased OR (OR 1.7, 95% CI 1.2–2.6) while the homozygotes did not (OR 1.4, 95% CI 0.7–2.7). This may be due to the effect of the small number of valine homozygotes.

Discussion
The potential influence of MTHFR activity on DNA methylation and on the availability of uridylates and thymidylates for DNA synthesis and repair makes *MTHFR* an attractive candidate cancer-predisposing gene. Reduced MTHFR activity leads to increased levels of cytosolic 5,10-methyl-enetetrahydrofolate, which may protect cells from DNA damage induced by uridylate misincorporation. Indeed, several independent studies have shown that the mutant valine allele of *MTHFR* may reduce the risk of colon cancer [2,5,20] and acute lymphocytic leukemia [6]. Conversely, mutant *MTHFR* has also been associated with an increase in risk of endometrial cancer [7] and cervical intraepithelial neoplasia [8]. An increased cancer risk conferred by the valine allele may be explained by impairment of DNA methylation due to a reduction in the availability of 5-methyltetrahydrofolate.

Only two previous studies have examined the relationship between the MTHFR C677T polymorphism and breast cancer risk. Gershoni-Baruch *et al.* [17] reported that the 677T allele occurred significantly more frequently in Jewish women diagnosed with bilateral breast cancer or combined breast and ovarian cancer. Sharp *et al.* [18] did not observe any significant difference in the genotype distributions within a case-control study of unselected breast cancers from a Scottish population. Comparison of the number of cases that were compound homozygotes of the low-activity 677TT genotype and/or another low-activity *MTHFR* variant (1298CC) revealed a significant reduction in risk (OR 0.26), suggesting that low MTHFR activity may protect against breast cancer. While this association is intriguing, the reliability of the conclusion is tempered by the fact that the study included only 62 cases and 66 controls.

Given the plausible role of variations in MTHFR activity in breast cancer susceptibility and the conflicting conclusions of previous studies we undertook an analysis of the C677T polymorphism in a more substantial number of cancers and controls. Our study revealed a significant excess of valine genotypes among the early onset breast cancers (OR 1.43). In contrast to the study by Gershoni-Baruch *et al.* [17], we did not observe a significant increase in the valine allele frequency among the women diagnosed with bilateral breast cancer or with a family history of breast cancer (although it was higher than in the controls). The data presented by Sharp *et al.* [18] suggest that low MTHFR activity is associated with a decrease rather than an increase in breast cancer risk. The reliability of their conclusions, however, is limited by the lack of

statistical power. The data from our study of 335 cases suggests that, in fact, low MTHFR activity increases the risk of breast cancer. We recognise that the conflicting conclusions may be attributable to the different characteristics of the breast cancer populations studied. Our study included both familial and early onset breast cancers while those of Sharp *et al.* [18] were unselected cases and those of Gershoni-Baruch *et al.* [17] were unselected and familial cases occurring among women of Jewish ancestry.

In conclusion, we suggest that low MTHFR activity is associated with an increased risk of early onset breast cancer. It is possible, however, that chance or confounding factors such as ethnicity and the influence of known risk factors such as oral contraceptive use may have generated a false positive result. Consequently it will be important to replicate our findings in larger population-based case-control studies. In addition, it will be important for future studies to incorporate detailed dietary information, because deficiencies in MTHFR activity may be compensated for by increased folate intake and therefore may confound interpretation of the genetic data.

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