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Association of the C677T polymorphism in the *MTHFR* gene with breast and/or ovarian cancer risk in Jewish women

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Abstract

The C677T mutation in the methylenetetrahydrofolate reductase (*MTHFR*) gene is associated with reduced enzyme activity, hyperhomocysteinaemia and increased risk for atherosclerosis in homozygotes. We examined the frequency of this mutation and its association with disease pattern in 491 Jewish women with either sporadic ($n=355$; 72%) or hereditary ($n=136$; 28%) breast and/or ovarian cancer and in 69 asymptomatic *BRCA1/2* mutation carriers, genotyped for the three predominant Jewish founder *BRCA1/2* mutations (185delAG, 5382insC and 6174delT). 677T homozygotes were equally distributed among women with sporadic breast and/or ovarian cancer (71/355; 20.0%) and among *BRCA1/2* mutation carriers (43/205; 21.0%) ($P=$ non-significant). 677T homozygotes were equally distributed among women diagnosed with breast cancer prior to (22/122; 18.0%) and after 42 years of age (42/243; 17.3%). Among *BRCA1/2* carriers, the rate of 677T homozygotes in manifesting cancer (32/136; 23.5%) and asymptomatic individuals (11/69; 15.9%) was not significantly different. The rate of 677T homozygotes (24/72; 33.3%) was higher ($P=0.0026$) among women with bilateral breast cancer and those with both breast and ovarian carcinoma than among those with unilateral breast cancer (64/365; 17.5%). Differences in morbidity (one versus multiple breast/ovarian tumours) are mainly attributed to 677T homozygosity and partly to *BRCA1/2* mutations. Confirmation of these data, namely, that the 677T allele is significantly more common in cases of bilateral breast cancer or combined breast and ovarian cancer would have important clinical implications.

Keywords: *MTHFR*; *BRCA1*; *BRCA2*; Bilateral breast cancer; Breast/ovarian cancer

1. Introduction

Methylenetetrahydrofolate reductase (*MTHFR*) catalyses the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the primary circulatory form of folate and carbon donor for the re-methylation of homocysteine to methionine. A common missense mutation (C to T transition at nucleotide 677, substituting alanine for valine at codon 225) in the *MTHFR* gene is associated with increased thermolability and reduced enzyme activity. This specific polymorphism is heralded by elevated plasma levels of homocysteine, and has been linked to an increased risk for atherosclerotic cardiovascular disease and apparently venous thrombosis in homozygotes [1].

The C677T mutation, very common among Caucasians, shows a heterogeneous distribution among different ethnic groups with allele frequencies ranging from 5 to 54% [2–4]. The frequency of the 677T allele in the Israeli Jewish population is 45% (data not shown).

The notion that that 677T allele might be associated with cancer susceptibility was recently examined in colorectal, endometrial and ovarian cancer [5–8]. 677T homozygotes had a marginal decrease in colorectal cancer risk compared with controls and this protective effect was absent in men with folate deficiency [5,6]. Alternatively, a positive association of the C677T polymorphism with endometrial carcinoma was described [7] and allelic loss of 1p36.3, presumably targeting the

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MTHFR gene, was detected in ovarian cancer [8]. Mechanistically, this could be the result of increased levels of 5,10-methylenetetrahydrofolate required for DNA synthesis, or due to depletion of methyltetrahydrofolate which can potentially lead to DNA methylation alterations.

Female carriers of germ line mutations in the *BRCA1/2* genes have a 40–60% lifetime risk of developing breast cancer and a 16–40% risk of ovarian cancer [9,10]. The incomplete penetrance of the *BRCA1/2* genes mutations, suggests that other factors, genetic and non-genetic, determine the phenotypic expression of mutant *BRCA1/2* alleles in these individuals. The *MTHFR* polymorphism is one possible genetic modifier of cancer penetrance.

In Ashkenazi Jews at high risk for developing breast and ovarian cancer, three predominant founder mutations have been described in *BRCA1* (185delAG and 5382insC) and *BRCA2* (6174delT) [9,11–13].

To further delineate the putative role that the C677T polymorphism plays in breast and ovarian tumorigenesis, we determined the frequency of this polymorphism and its association with disease pattern in Jewish women with breast and ovarian cancer genotyped in respect to their being carriers for the three predominant Jewish *BRCA1/2* founder mutations.

2. Patients and methods

2.1. Patients

The study cohort includes 491 women with breast and/or ovarian cancer referred to our Oncogenetics counselling services at the Sheba and Rambam Medical centres during 1997–1998. Of these, 136 carried *BRCA1/2* germ line mutations. 69 asymptomatic *BRCA1/2* carriers (healthy individuals at risk, similarly recruited) were included in the study (Table 1).

Data including demographics, histopathological information, treatment and outcome variables were collected and entered into a computerised database. All participants signed an informed consent form approved by the Institutional Review Board (IRB) and were genotyped for the three predominant founder mutations in *BRCA1* (185delAG and 5382insC) and *BRCA2* (6174delT) and for the C677T *MTHFR* polymorphism.

2.2. Genetic testing

Genotyping was performed on DNA extracted from lymphocytes by standard procedures. The 185delAG, 5382insC (*BRCA1*), the 6174delT (*BRCA2*) and the C677T polymorphism (*MTHFR*) were detected by polymerase chain reaction (PCR) amplification with specific primers that produce a modified restriction enzyme digest made to distinguish the wild type allele from the mutant allele, as previously described [1,14,15].

2.3. Statistical analysis

The χ^2 test was used for comparisons between groups. Proportion and 95% confidence intervals were calculated. Logistic regression analysis was used to determine the independent effect of a *BRCA1/2* mutation and the homozygous 677T genotype on type of morbidity (one or two primary tumours). Because the major effect of the *MTHFR* C677T polymorphism is mediated by the recessive TT genotype, the primary tests were applied to this genotype only.

Table 1
Prevalence of 677T homozygotes among breast/ovarian patients with/without *BRCA1/2* mutations and asymptomatic *BRCA1/2* carriers

Groups	Sub-groups	677T homozygotes *n* (%)	Total *n* (%)	%	95% CI
Total		114 (100)	560 (100)	20.4	17.2–24.0
Sporadic cases					
	Unilateral breast cancer	53 (46)	285 (51)	18.6	14.4–23.7
	Bilateral breast cancer	12 (11)	42 (8)	28.6	16.2–44.8
	Ovarian cancer	5 (4)	22 (4)	22.7	8.7–45.8
	Breast and ovarian cancer	1 (1)	6 (1)	16.7	0.9–63.5
	All	71 (62)	355 (63)	20.0	16.0–24.6
BRCA1/2 carriers					
	Unilateral breast cancer	11 (10)	80 (14)	13.8	7.4–23.7
	Bilateral breast cancer	6 (5)	15 (3)	40.0	17.5–67.1
	Ovarian cancer	10 (9)	32 (6)	31.3	16.8–50.1
	Breast and ovarian cancer[a]	5 (4)	9 (2)	55.6	22.7–84.7
	Asymptomatic carriers	11 (10)	69 (12)	15.9	8.6–27.2
	All[b]	43 (38)	205 (37)	21.0	15.8–27.3

95% CI, 95% confidence interval.

[a] Including 2 cases with ovarian cancer and bilateral breast cancer.

[b] Including 69 cases of asymptomatic *BRCA1/2* carriers.

3. Results

3.1. Characteristics

3.1.1. Patients

491 women (mean age 50.9±12.27 years): 422 with breast cancer (of whom 138 were diagnosed prior to age 42 years and 284 after that age), 54 with ovarian cancer and 15 with both breast and ovarian cancer (Table 1). Of these 136 were *BRCA1/2* mutation carriers; 95 with breast cancer, 32 with ovarian cancer and 9 with both breast and ovarian cancer.

3.1.2. Asymptomatic carriers

69 women (mean age 42.3±12.1 years)

3.2. Distribution of 677T homozygotes

Overall, 114 *MTHFR* 677T homozygotes were detected in several subsets of patients (Table 1). The prevalence of 677T homozygotes among *BRCA1/2* carriers (43/205; 21.0%) was not significantly different from that observed among non-carriers (71/355; 20.0%) (P = non-significant (n.s.)). 677T homozygotes were equally distributed among *BRCA1/2* patients (32/136; 23.5%) and *BRCA1/2* asymptomatic carriers (11/69; 15.9%) (P = n.s.).

Notably, in a subset of patients with bilateral breast cancer and women with both breast and ovarian carcinoma a high rate of 677T homozygotes was observed. The combined frequency of 677T homozygotes among women with bilateral breast cancer and those with both breast and ovarian carcinoma (24/72; 33.3%), compared with that observed in women with unilateral breast cancer (64/365; 17.5%), was significantly higher, irrespective of the *BRCA1/2* carrier status (P = 0.0026). Logistic regression analysis showed that the combined contribution of the 677T homozygous genotype and a *BRCA1/2* mutation to the development of a second primary tumour equals 13%, of which 29% (P = 0.0037) is attributed to 677T homozygosity and 14% to the *BRCA1/2* mutation (P = 0.06) (Table 2).

Table 2
Logistic regression analysis: independent effect of *BRCA1/2* mutations and 677T homozygosity on type of morbidity[a]

	Morbidity	
Variable	β	P value
BRCA1/2	0.1462	0.0601
MTHFR	0.2920	0.0037

[a] Logistic regression analysis showed that the combined contribution of the 677T homozygous genotype and a *BRCA1/2* mutation to the development of a second primary tumour equals 13% (R^2), of which 29% (P = 0.0037) is attributed to 677T homozygosity and 14% to the *BRCA1/2* mutation (P = 0.06). R^2 = The total contribution of both variables (677T homozygous genotype and a *BRCA1/2* mutation). β = The relative contribution of each variable.

677T homozygotes were equally distributed among women with unilateral breast cancer with either early (< 42 years) or late (> 42 years) age at disease diagnosis (22/122; 18.0% and 42/243; 17.3%, respectively).

4. Discussion

Low MTHFR activity disrupts the remethylation of homocysteine to methionine [1]. It is debated whether reduced MTHFR activity is a risk factor in ovarian or endometrial tumorigenesis [7,8]. In this study, we show that 677T homozygotes are equally distributed among women with breast or ovarian cancer, either carriers or not of *BRCA1/2* mutations and does not seem to differ from that observed in the general Israeli or Ashkenazi population (21%; data not shown). However, patients with bilateral breast cancer and those with both breast and ovarian cancer have a significantly increased frequency of homozygous 677T genotypes compared with patients with breast or ovarian cancer only. *BRCA1/2* carriers homozygous for the 677T allele were significantly more prevalent in the subset of patients who developed more than one tumour. This effect although partly attributed to the high frequency of *BRCA1/2* carriers among such patients is mainly related to 677T homozygosity.

It has been appreciated that altered folate metabolism might be implicated in the oncogenic process both in colorectal and ovarian cancer [5,6,8]. Our data, taken together with that reported in the literature favours the opinion that disruptions in folate metabolism may enhance breast/ovarian tumorigenesis. Our results may indicate that breast/ovarian cancer patients homozygous for the 677T mutation could be at greater risk of acquiring a second primary tumour. This applies both to *BRCA1/2* carriers and non-carriers. These findings originate from a relatively small number of patients and should be confirmed by further study, especially, if one considers that MTHFR deficiency can be counteracted by specific intervention schemes such as diet and folic acid supplementation and as such has important clinical implications.

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