Methylenetetrahydrofolate Reductase C677T polymorphism and breast cancer risk in Moroccan women

Diakite B[1], Tazzite A[1], Hamzi K[1], Jouhadi H[2], *Nadifi S[1]

1. Genetics and Molecular Pathology Laboratory, Medical school of Casablanca, University Hassan II, Morocco.
2. Department of Oncology, Ibn Rochd University Hospital, Casablanca, Morocco.

Abstract

Background: Methylenetetrahydrofolate (MTHFR) enzyme plays an important role in folate metabolism which is involved in DNA methylation, repair, and synthesis.

Objective: We investigated if the MTHFR C677T polymorphism modulates the risk of developing breast cancer in Moroccan women.

Methods: Genotyping was performed by PCR-RFLP method on a sample of 96 patients with breast cancer and 117 controls.

Results: A positive correlation was found between the MTHFR C677T polymorphism and progesterone receptors expression (p= 0.04). According to menopausal status, the heterozygous CT (OR = 2.29 and P = 0.03) was statistically significant in pre-menopausal women. There was a significant association between C677T polymorphism and breast cancer risk in both additive (OR = 2.2, 95% CI = 1.24-3.86, p = 0.007) and dominant (OR = 2.10, CI 95% = 1.21-3.64, p = 0.008) models. In addition, the T allele were associated with a high breast cancer risk (OR = 1.59, 95% CI = 1.04-2.44, p = 0.03).

Conclusion: In the light of our preliminary study, 677T allele and 677CT MTHFR genotype may represent a genetic determinant increasing breast cancer risk in Moroccan women. A larger study including a larger sample size and more information is needed to confirm our conclusions.

Key words: MTHFR polymorphism, breast neoplasm, Morocco.

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Introduction

Breast cancer is the most common cancer among Moroccan women. According to the Cancer Registry of Casablanca, breast cancer presents 36% of women malignancies with a standardized incidence of 35.04[1]. Several risk factors have been involved in the development of this cancer. Nevertheless, the interaction between genetics and environmental factors play an important role in carcinogenesis[2]. Several epidemiologic studies have shown that folate deficiency may cause DNA damage leading to genetic instability and increasing the risk of several cancers including breast cancer [3,4].

Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism. It catalyzes the irreversible conversion of 5,10-methylenetetrahydrofolate reductase (5,10-methylene THF) to 5-methyl-tetrahydrofolate (5-methyl-THF), an essential substrate in remethylation of homocysteine to methione and subsequently in DNA methylation. The disruption of the DNA methylation control can influence genes stability and expression, leading to the DNA hypomethylation [5] or promoter hypermethylation in some genes which may initiate the development of cancer [6,7]. Moreover, 5,10-methylene THF is also involved in the production of purines and thymidylate for DNA synthesis[8]. The common 677C>T (A222V) polymorphism in exon 4 of MTHFR gene leads to a thermolabile enzyme with decreased activity[9] associated to an increased homocysteine levels and a reduced plasma folate concentration[10,11].

The involvement of the MTHFR C677T polymorphism in breast cancer is controversial. While some studies have not found a significant association between the polymorphism and the risk of breast cancer, several reports have suggested that the MTHFR 677C>T polymorphism might influence breast cancer risk. This variability may be due to the sample size and ethnic origin, especially since MTHFR gene mutations have shown ethnic variations [12]. Therefore the aim of this study is to understand the potential contribution of this commonly occurring

***Correspondence author:**
Sellama Nadifi
Genetics and Molecular Pathology Laboratory
Medical School of Casablanca
19 Rue Tarik Ibnou Ziad, B.P. 9154
Tel: 00212663244338
E-mail: labgenmed@yahoo.fr, amal_tazzite@yahoo.fr

polymorphism to breast cancer risk in a sample of the Moroccan population.

Methods

Study population

We conducted a case–control study involving subjects admitted for primary breast cancer between 2009 and 2010 at the Department of Oncology in Ibn Rochd University Hospital in Casablanca, Morocco. Cases were defined as unrelated subjects with clinically and histopathologically confirmed primary breast cancer, without previous history of any cancer. Control subjects were recruited from the DNA bank serving in population studies conducted by the Genetics and Molecular Pathology Laboratory at the Medical School of Casablanca, Morocco. The control group included volunteer healthy women without any clinical signs or medical history of any type of cancer. Both the study groups were drawn from the region of Casablanca.

A total of 96 women with primary breast cancer (average age 41.05 ± 2.13) and 117 age-matched controls (average age 41.05 ± 2.28) were included in the study after giving an informed consent.

Additional data about age at diagnosis, tumor location, histological type, tumor size (T), node involvement (N), metastasis (M), menopausal status and estrogen receptor (ER), and progesterone receptors (PR) status were obtained from medical records and pathology reports.

DNA extraction and MTHFR genotyping

Genomic DNA was extracted from whole blood of cases and controls using the standard salting-out method. The DNA concentration was determined by spectrophotometry.

Genotyping for the MTHFR C677T polymorphism was performed using PCR–RFLP method reported by Frosst et al [10]. The C to T substitution at nucleotide 677 in the coding region of the MTHFR gene creates a restriction site for the HinfI enzyme. The PCR products amplified and digested showed the following fragments: two fragments of 175 bp and 23 bp for homozygous (TT), three fragments of 198, 175 and 23 bp for heterozygous (CT) and one band of 198 bp for homozygous (CC). The digested PCR products were separated on 3% agarose gel electrophoresis, stained by ethidium bromide and visualized by UV transilluminator.

Statistical Analysis

Statistical analyses were performed using MedCalc 11.6 software. The Hardy-Weinberg test was performed separately for cases and controls to measure the distribution of the polymorphism. Odds ratio (OR) with 95% confidence intervals (CI) was used to assess the strength of association between the MTHFR gene polymorphism and breast cancer risk. The MTHFR genotypes were examined with additive, dominant, and recessive models. A p-value of 0.05 was considered statistically significant. The x^2 test or Fisher's exact test were performed to verify the association of clinical and histopathological variables with MTHFR polymorphism.

Results

Cases and controls were in Hardy-Weinberg equilibrium for the distribution of MTHFR polymorphism (x^2= 33.94, $p < 0.0001$; x^2= 49.44, $p < 0.0001$). The average age was 41.05 ± 2.13 for cases and 41.05 ± 2.28 for controls.

Table 1 shows the association between the clinicopathological features and MTHFR C677T polymorphism. No correlation was observed between this common polymorphism and tumor location (unilateral or bilateral), tumor size, histological type, lymph node invasion and metastasis. In contrast, a significant correlation was seen with progesterone receptor status (x^2 = 10.28, p = 0.04).

Table 2 presents the data on genotypes of the MTHFR polymorphism among cases and controls stratified by menopause status. A significantly increased breast cancer risk was observed in pre menopausal women carrying CT genotype with OR = 2.29, CI 95% = 01.07 - 4.88 and p = 0.03). On the contrary, no statistically significant association was observed in post menopausal breast cancer women.

Table I: Frequencies of C677T MTHFR polymorphism in patients with breast cancer according to clinico-pathological features

Tumor characteristics		MTHFR Polymorphism			*P* value(*P*<0.05)	Test x^2
		CC	CT	TT		
Localisation	96				0.71	0.67
Unilateral	87	35 (40.2)	46 (52.9)	6 (6.9)		
Bilateral	9	4 (44.4)	5 (55.6)	-		
Histology	96				0.53	5.07
IDC	91	38 (41.8)	47 (51.6)	6 (6.6)		
ILC	3	-	3 (100)	-		
PC	1	1 (100)	-	-		
MC	1	1 (100)	-	-		
Tumor size	96				0.91	2.13
<2 cm	13	6 (46.2)	6 (46.2)	1(7.7)		
2-4 cm	47	19 (40.4)	24 (51.1)	4 (8.5)		
>4 cm	16	7 (43.8)	9 (56.2)	-		
Extension	20	7 (35.0)	12 (60.)	1 (5.0)		
Node involvement	96				0.83	0.36
N+	56	22 (39.3)	31 (55.4)	3 (5.3)		
N-	40	17 (42.5)	20 (50.0)	3 (7.5)		
Metastasis	92				0.53	1.29
M+	15	8 (53.3)	6 (40.0)	1 (6.7)		
M-	81	31 (38.3)	45 (55.5)	5 (6.2)		
PR	96				**0.04**	**10.28**
PR+	54	21 (39.0)	32 (59.0)	1 (2.0)		
PR-	38	18 (47.0)	15 (39.0)	5 (13.0)		
Unknown	4	-	4 (100.0)	-		
RE	96				0.31	4.82
RE+	56	22 (39.3)	31 (55.3)	3 (5.4)		
RE-	36	17 (47.2)	16 (44.4)	3 (8.3)		
Unknown	4	-	4 (100.0)	-		

CC MTHFR wild genotypes, CT MTHFR variant heterozygous, TT MTHFR variant homozygous, - : zero, IDC Invasive Ductal Carcinoma, ILC Invasive Lobular Carcinoma, PC Papillary Carcinoma, MC Medullary Carcinoma, RE Estrogen Receptors, PR Progesterone Receptors.

Table 2: Comparison of the distribution of MTHFR polymorphism according to menopause status

MTHFR	Pre-menopause				Post menopause			
Genotype	F. Cases n=62	F .Controls n= 61	OR (95%CI)	p	F. Cases n= 34	F. Controls n= 56	OR (95%CI)	p
CC	24 (38.7)	34 (55.7)	1 Ref		15 (44.1)	35 (62.5)	1 Ref	
CT	34 (54.8)	21 (34.4)	2.29 (1.07-4.88)	0.03*	17 (50.0)	20 (35.7)	1.98 (0.82-4.81)	0.13
TT	4 (6.45)	6 (9.8)	0.94 (0.24-3.71)	0.93	2 (5.9)	1 (1.8)	4.67 (0.39-55.48)	0.22
Allele C	82 (66.1)	89 (73.0)	1 Ref		47 (69.1)	90 (80.4)	1 Ref	
Allele T	42 (33.9)	33 (27)	1.25 (0.74-2.11)	0.40	21 (30.9)	22 (18.8)	1.83 (0.91-3.66)	0.09

CC wild genotypes, CT MTHFR variant heterozygous, TT MTHFR variant homozygous, F frequency (%), OR odds ratio, CI confidence interval, *significative

The distribution of MTHFR C667T genotypes among cases and controls were respectively 0.41 (CC), 0.53 (CT), 0.06 (TT), and 0.59 (CC), 0.35 (CT), and 0.06 (TT), as shown in table 3. The C677T polymorphism was significantly associated with breast cancer in both additive (CT vs. CC: OR = 2.2, CI 95% = 1.24-3.86, p = 0.007) and dominant (CT + TT vs. CC: OR = 2.10, CI 95% = 1.21-3.64, p = 0.008) models, but no association was seen in the recessive model (TT vs. CC+CT: OR = 1.05, CI 95% = 0.34-3.23, p = 0.94).

Furthermore, C and T allele frequencies were respectively 0.67 and 0.33 in cases, 0.76 and 0.24 in controls (table 3). A statistically significant increase in breast cancer risk was observed in individuals carrying 677T allele with OR = 1.59 (95% CI = 1.04-2.44) and *p* = 0.03.

Table 3: Genotype and allele frequencies of MTHFR C677T polymorphism in patients with breast cancer and healthy controls

MTHFR Genotype	Cases (F) N= 96	Controls (F) N=117	Additive model		Dominant model		Recessive model	
			OR (95%CI)	*P* value (*P*< 0.05)	OR (95%CI)	*P* value (*P*< 0.05)	OR (95%CI)	*P* value (*P*< 0.05)
CC	39 (0.41)	69 (0.59)	2.2 (1.24-3.86)*	0.007	2.10 (1.21-3.64)*	0.008	1.05 (0.34-3.23)	0.94
CT	51 (0.53)	41 (0.35)						
TT	6 (0.06)	7 (0.06)						

MTHFR Genotype	Cases (F) N= 96	Controls (F) N=117	OR (95%CI)	*P* value (*P*< 0.05)
Allele C	129 (0.67)	179 (0.76)	1 Ref	1 Ref
Allele T	63 (0.33)	55 (0.24)	1.59 (1.04-2.44)*	0.03

F Frequency, N Number, OR odds ratio, CI confidence interval, *significative

Discussion

In the current study, there was no statistically significant positive association between the MTHFR C677T polymorphism and tumor histology, tumor size and location, lymph node invasion, metastasis and expression of estrogen receptors. These results are consistent with Langsenlehner U et al. [13] and Batschauer AP et al. [14] findings. Furthermore, in contrast to these published reports, our data support a positive correlation between the MTHFR C677T polymorphism and progesterone receptors status with p = 0.04.

According to menopausal status, premenopausal women with CT genotype had a higher statistically significant risk of developing breast cancer whereas in a meta-analysis of Qi X et al. [15], including 16 480 cases and 22388 controls, no significant association was shown in menopausal status-based population. On the other side, Macis D et al. [16] showed an increased risk of breast cancer in premenopausal women with 677TT genotype with an odds ratio of 1.42 (95% CI, 1.02–1.98). Moreover, Lin et al. [17] reported a significantly elevated risk of breast cancer among subjects with MTHFR 677TT and CT genotypes who had a longer duration (>10 years) of estrogen exposure prior to first full-term pregnancy (adjusted OR = 4.98, 95% CI=2.00-12.43).

Interestingly, our study revealed a positive correlation between the T allele of MTHFR C677T variant and risk of breast cancer among Moroccan women with an odds ratio of 1.59 (95% CI = 1.04-2.44, p = 0.03). We also found a significant difference between patients with breast cancer and controls regarding the frequency of C677T genotypes in the additive and dominant models. These results suggest that the T allele may influence the risk of breast cancer. Similarly, Qi X et al. [15] showed that T allele vs. C was associated with breast cancer in women of East Asia with an odds ratio of 1.041 (95% CI = 1.009-1.073) compared to the Caucasian population. Maruti SS et al. [18] concluded also that women with a higher number of variant T alleles had higher risk of breast cancer with p = 0.04. Another recent meta-analysis [19] suggested that women with at least one T allele had lower risk of developing breast cancer. The distribution of MTHFR polymorphism may vary

by group or patient characteristics especially since the MTHFR gene has a stronger influence on homocysteine plasma level in Asian populations who have different habits of tea consumption, smoking and drinking and Africa where malnutrition may lead to an imbalance in folate metabolism.

These contradictions about the relationship between MTHFR 677T polymorphism and breast cancer risk could be related to several factors in particular sample size, population study ethnicity, genetic predisposition, exogenous hormones and environmental factors such as folate intake.

In this study, we have to emphasize some limitations. The most important limitation was the reduced statistical power due to the relatively small sample size. The second limitation was the lack of supplement information on dietary folate intake and other measures of overall plasma folate levels. Therefore, the role of folate in the association between MTHFR and breast cancer could not be addressed in this study. For these reasons, results must be interpreted with caution and cannot be generalized especially for a genetic association study. A larger study including a larger sample size and more precise data and variables is needed to confirm our conclusions.

Conclusion

The present study found positive correlation between MTHFR C677T polymorphism and expression status of progesterone receptor with $p = 0.04$. Furthermore, a significant association between MTHFR 677T allele and the risk of breast cancer in Moroccan women with odds ratio = 1.59 suggests that women with T allele have an increased risk of breast cancer. Additional studies with a large sample and more information about folate intake and plasma folate levels are needed to better understand the relationship between this common polymorphism and breast cancer risk in our population.

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