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Original Research Article

The Recessive model of 677C>T Polymorphism in MTHFR gene increase moderatelythe risk of Colorectal Cancer in Moroccan patients

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Abstract

Methylenetetrahydrofolate reductase (MTHFR) is a critical folate-metabolising enzyme and a polymorphism at position 677 (C677T), is associated with reduced enzyme activity. We investigated whether this functional polymorphism modulates the risk of developing Colorectal Cancer (CRC).

We conducted a hospital-based case-control study to assess the association of MTHFR gene polymorphism C677T with risk for colorectal cancer in a Moroccan population. Odds ratios [ORs] with corresponding 95 % confidence intervals (Cis) were used to assess the association.

The analysis had shown the significant elevated risk of cancer was associated with the*MTHFR* C677T polymorphism in recessive model (OR = 2.81, 95 % CI; (1.13-7.06), *P*=0.027) compared the other genetic models. Simultaneously, the T-allele genotype versus C-allele genotype was not associated with CRC risk (OR^a = 1.355; 95% CI = 0.94-1.96and *p* = 0.10).

Thusrecessive model are significantly associated in the risk of colorectal cancer. Further larger-scale studies are necessary to confirm our finding.

Keywords:MTHFR, C677T, polymorphism, colorectal cancer, Moroccan patients.

1.Introduction

Colorectal cancer (CRC) is a worldwide public health problem, which is the third most commonly diagnosed cancer in males and females with over 1.2 million new CRC patients and 608 700 deaths occurred in the world[1,2]. Its incidence varies worldwide and is significantly increased in industrialized countries.The incidence tends to be low in Africa and in Asia and intermediary in the southern parts of South America. These important geographical differences for the colorectal cancer can be explained by different environmental exposures[3]. In Morocco, the colorectal cancer is the third cause of death and situated of the first digestive pathology cancer[4]. Colorectal cancer is a complex disease that involves multiple genetic and nutritional factors[5]. Among the latter, folate was shown to play a preventive role in colorectal carcinogenesis probably because of its involvement in the processes of DNA methylation and synthesis[6]. Other nutrients such as methionine, vitamin B-6, and vitamin B-12, which interact metabolically with folate in this process, may also influence the risk of CRC[7]. In some of those studies the observed inverse association between folate status and CRC risk was further modified by genetic polymorphisms of the enzymes involved in folate metabolism, most notably Methylene Tetrahydrofolate Reductase [MTHFR]. Although several single nucleotide polymorphisms in

the MTHFR gene have been reported, this paper focuses on the common MTHFR C677T polymorphism which is associated with decreased enzyme activity, and thus increases the availability of 5,10-methylenetetrahydrofolate for DNA synthesis, which partially explains the reduced risk of CRC in subjects carrying the TT genotype [8, 9].

2. Materiel and Methods

2.1 Study Population

The cases were 100 patients with a histologic diagnosis of CRC(Adenocarcinoma) attending Mohammed V military Hospital of Rabat city, National Oncology Institute (Sidi Mohammed Ben Abdellah, Rabat) and Avicenne University Hospital of Rabat. Data on all CRC patients were obtained from personal interviews with patients, medical records and pathology reports. The data collected included age, gender, tumor location and smoking status. Informed consent was obtained from all the patients in this study. A total of 198 healthy unrelated were recruited into the control group after being interviewed with regard to whether they had been diagnosed with no previous history of cancer at any site or associated diseases, using age and gender as frequency-matching criteria.

2.2 Genetic Analyses

2.2.1. DNA Isolation

Genomic DNA was isolated from peripheral blood samples using MagMax™Total Nucleic Acid Isolation Kit (Ambion® by Thermo Fischer Scientific, USA)according to manufacturer's instructions.

2.2.2 Real Time PCR

Twenty to 50 ng of DNA from patients and control group were used to amplify a233 pb fragment of the MTHFR gene with specific primers using the LightMix® Kit MTHFR C677T with Roche LightCycler® FastStart DNA Master HybProbe. PCR were carried out in the LightCycler® 2.0 instrument. The resulting PCR fragments are analyzed with hybridization probes labeled with LightCycler®Red 640 [detected in channel 640]. The genotype is identified by running a melting curve with specific melting points [Tm]. The wildtype MTHFR C677 DNA exhibits a Tm of 63.0°C in channel 640. The mutant MTHFR 677T exhibits a Tm of 54.5°C in channel 640.

2.2.3. Statistical Analysis

Hardy-Weinberg equilibrium [HWE] in the cases and control group was tested by the Chi-square test and *p*-value of <0.05 was considered significant. All statistical analyses were performed using STATA software (version 11.0; Stata Corporation, College Station, TX).

3. Results

The characteristics of the study population are presented in Table 1. Hundred cases and 198 controls were included in this analysis.Genotypic distribution of MTHFR 677 did not show any deviation from Hardy-Weinberg equilibrium (χ^2=0.75). The age of the cases is situated from 22 to 82 and the mean age of the cases (51.86 ± 12.34) are statistically similar as that observed in controls (54.11±11.32)(p>0.05). The median age was 53 years in the entire cohort. No statistical differences were observed between cases and controls in the distribution of age and sex, suggesting that frequency matching was adequate. Sex ratio did not significantly differ between the two groups. A statistically significant difference in smoking status was also not found between patients with colorectal cancer and healthy controls(Table 1).

Table 1: General characteristics of Moroccan subjects

	Cases [N=100]		Controls [N=198]		
	N	%	N	%	*p*-value
Age [years]					
≤ 50 years	52	52	74	37,37	0.76
> 50 years	48	48	124	62,63	0.38
Gender					
Female	41	41	98	49,5	0.44
Male	59	59	100	50,5	0.47
Smoking statut					
Ever	33	33	64	32,33	1
Never	67	67	134	67,68	1

In this cases-control study, we examined the relation between the MTHFR genotype and colorectal adenocarcinoma. The main results of this analysis were listed in Table 2. Overall, significantly elevated CRC risk were associated with recessive model (OR^a= 2.81; 95%CI=1.13-7.06 and *p* =0.027 for TT vs CC+CT). In contrast, there were no associations were found in Additive 1 model (OR^a= 1.07; 95%CI=0.64-1.79 and *p*=0.784), Additive 2 model (OR^a= 0.35; 95%CI=0.13-0.91 and *p*=0.03) andin Dominant model (OR^a= 0.87; 95%CI=0.54-1.42 and *p*=0.595). Simultaneously, the T-allele genotype was not associated with an increased CRC risk (OR^a= 1.355; 95%CI=0.94-1.96and *p*=0.10)(Table 3).

Table 2: Genotypes frequencies of MTHFR C677T gene polymorphism in cases and controls and their associations with the risk of CCR

Model	Controls [n=198]	Patients [n=100]	OR [95% CI]	*p*-value
Additive 1				
CC	91	50	1.00	
CT	77	44	1.04; [0.62-1.72]	0.879
			1.07; [0.64-1.79][a]	0.784[a]
Additive 2				
CC	91	50	1.00	
TT	30	6	0.36; [0.14-0.93]	0.04
			0.35;[0.13-0.91][a]	0.03[a]
Dominant				
TT+CT	91	50	1.00	
CC	107	50	0.85; [0.52-1.37]	0.510
			0.87; [0.54-1.42][a]	0.595[a]
Recessive				
TT	30	6	1.00	
CC+CT	168	94	2.79; [1.12-6.96]	0.027
			2.81; [1.13-7.06][a]	0.027[a]

[*] CI, confidence interval; OR, odds ratio; a, adjusted by gender and age

Table 3: Allele frequencies of MTHFR C677T polymorphism in healthy people and patients

SNP	Allele [Major/Minor]	Allele Frequencies Patients [%]	Controls [%]	OR [95% IC]	*p*-value
MTHFR 677					
	C	144 [72]	25 [65]		
	T	56 [28]	137 [35]	1.355 [0.94 - 1.96]	0.10

Furthermore, we examine the combined effect of genotypes of MTHFR at 677 position according to gender. Results of the logistic regression analysis suggested that there is no significant association in all the genetic models between TT genotypes and risk to develop CRC for men (OR^a= 0.98; 95%CI=0.49-1.95 and *p*=0.970 for CC vs CT; ORa= 0.35; 95%CI=0.11-1.15 and *p*=0.084 for CC vs TT; OR^a= 0.79; 95%CI=0.41-1.50 and *p* =0.476 for TT+ CT vs CC; OR^a= 2.81; 95%CI=0.89-8.81 and *p*=0.075 for TT vs CC+CT) and women (OR^a= 1.12; 95%CI=0.52-2.39 and *p*=0.769 for CC vs CT ; OR^a= 0.35; 95%CI=0.07-1.71 and *p*=0.197 for CC vs TT; OR^a= 0.92; 95%CI=0.44-1.92 and *p*=0.843 for TT+ CT vs CC; OR^a= 2.98; 95%CI=0.64-13.85 and *p*=0.163 for TT vs CC+CT)(Table 4).

Table 4: Genotypic distribution of C677T MTHFR polymorphism and statistic comparison between men and women of CRC subjects and controls

	Men				**Women**			
Model	Controls	Patients	OR [95% CI]	*p*-value	Controls	Patients	OR [95% CI]	*p*-value
Additive 1								
CC	45	30	1.00		46	20	1.00	
CT	38	25	0.98 [0.49-1.95]	0.970	39	19	1.12 [0.52-2.39]	0.769
Additive 2								
CC	45	30	1.00		46	13	1.00	
TT	17	4	0.35 [0.11-1.15]	0.084	20	2	0.35 [0.07-1.71]	0.197
Dominant								
TT+CT	45	30	1.00		46	20	1.00	
CC	55	29	0.79 [0.41-1.50]	0.476	52	21	0.92 [0.44-1.92]	0.843
Recessive								
TT	17	4	1.00		13	85	1.00	
CC+CT	83	55	2.81 [0.89-8.81]	0.075	2	39	2.98 [0.64-13.85]	0.163

CI, confidence interval; OR, odds ratio; a, adjusted by gender and age

4. Discussions

The etiology of colorectal cancer is not well understood, but linkage studies will hopefully result in the identification of new high or moderate risk predisposing genes[10]. MTHFR is one such gene, but this association requires consideration of environmental factors such as geographical region, dietary intake, and homocysteine level and folate status. In the present Moroccan case-control study focused on the MTHFR TT genotype and the risk of CRC. Earlier study published by Diakete *et al*[11] was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). Foremost, this is the first study report analysis of the MTHFR gene polymorphism C677Tfor large case-control samples using the real time PCR. In the case-control study revealed that, the TT genotype was actually found to be associated with an increased risk of CRC (p= 0.027; OR= 2.81; 95% CI (1.13 - 7.06)). These results were similar to previously reported studies [11-13]. Additionally, the results also indicate that the MTHFR TT genotype, which is associated with lower functionality, does not play a real protective in the cell and also affects the methylation status of the cell by limiting the availability of 5, 10-methylenetetrahydropholate, which in turn, also affects thymidine synthesis[14]. Moreover, comparing our present work for the two genetic models (*Dominant*: CC vs TT + CT and *recessive*: TT vs CC + CT) to the meta-analysis in the association between 677 C>T MTHFR polymorphism and CRC susceptibility in the study published by Zan *et al*[15], we found that our Moroccan population for the recessive model (p = 0.027; OR= 2.81; 95% CI (1.13 - 7.06))seems to be very close to the Caucasian population based on 38 studies (p = 0.009; OR= 1.08; 95% CI (1.02 - 1.15)). Whereas, no significant associations were found with four African populations included at the same meta-analysis for all genetic models included the recessive model (p = 0.469; OR= 1.12; 95% CI (1.07 - 1.17)). Furthermore, other studies have found a protective effect of TT genotype against CRC using the recessive model[16].For the C allele genotype compared with the T allele genotype we did not found any significant association with increased colorectal cancer risk in our Moroccan population (p = 0.10; OR= 1.35; 95% CI (0.94 - 1.96))these results are similar to 77 case-control studies for different ethnic groups (p = 0.508; OR= 1.04; 95% CI (0.97 - 1.05))[15].

Many of the studies incorporated both men and women into the case control groups. However in this present study we stratified our results based on gender. The results showed any significant difference between genders of our case-control study. Our study show a similar results of the meta-analysis for 11 studies representing over 7,000 case-control study participants published by Deborah *et al*[17]. In contrast only one reported a significant OR based on gender and genotype Lightfoot and al. found that the men with 677CT genotype had a reduced risk of CRC, and women with 677TT genotype had increased risk[18].

In conclusion, in this study we observed a significant correlation between the recessive model of MTHFR 677 and risk to developing colorectal cancer in the Moroccan population. However, this correlation need to be authenticated by the further studies related with different polymorphisms of homocysteine and folate cycle against status of methylation concerning the same cohort study.

Conflict of interest

None of the authors has any financial interest related to this study to disclose.

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