RESEARCH COMMUNICATION

Association between the 1793G>A MTHFR Polymorphism and Sporadic Colorectal Cancer in Iran

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Abstract

Background: It has been proposed that folate and polymorphisms of the enzyme methylenetetrahydrofolate reductase (MTHFR), which regulates influx of folate for methylation reactions for DNA synthesis and repair, are involved in colorectal cancer. This study was designed to determine the influence of a genetic variant (MTHFR G1793A) and folate on colon cancer in Iran. Materials and Methods: We analyzed 227 cases and 239 normal unmatched controls using pyrosequencing. Odds ratios and 95% confidence intervals (95% CI) were calculated to evaluate associations of the MTHFR gene polymorphism with colorectal cancer risk. Results: A significantly reduced risk of recurrence was observed in patients heterozygous for the MTHFR G1793A polymorphism (OR: 0.17; 95% CI, 0.05- 0.52). The frequency of GG, GA and AA genotypes of MTHFR among the colorectal cancer patients were 98%, 2% and 0% respectively, while the frequencies among controls were 90%, 10% and 0%, respectively. Furthermore, a significant reduction in recurrence risk was seen in MTHFR G1793A heterozygotes limited to those who received folate supplements. Conclusion: Our study is compatible with previous findings concerning a reverse association between the MTHFR 1793G>A genotype with cancers in different populations.

Key Words: MTHFR - G1793A - polymorphism - folate - pyrosequencing

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Introduction

Colorectal carcinoma (CRC) is one of the most frequent causes of cancer death in industrialized countries, with a yearly incidence of about 50 new cases for every 100,000 people in the population (Boyle et al., 2005). The prevalence of CRC has been steadily increasing over the last century, while mortality rates have declined as a result of improved treatment and efficient screening and surveillance (Siegel et al., 2006). Folate, the major source of dietary methyl groups, plays an important role in DNA methylation, synthesis, and repair and 5,10-methylenetetrahydrofolate reductase (MTHFR) is the key enzyme in folate metabolism, carrying out the irreversible conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which in turn directs the folic acid pool toward remethylation of homocysteine to methionine (Kim et al.,1999). Methionine, the methyl donor for DNA methylation, is also involved in this pathway and is an alternate dietary source of methyl groups.

Folate deficiency may cause uracil misincorporation and subsequent DNA instability (Wainfan et al., 1993), retarded DNA repair capacity for oxidative or alkylating damage (Sanjoaquin et al., 2005), and global and proto-oncogenic DNA hypomethylation (Shrubsole et al., 2001). All of these effects have been reported to be involved in carcinogenesis, and higher intake of folate has been associated with a decreased risk of some types of cancer, including colorectal cancer (McCann et al., 2000). The relationship between folate and colorectal cancer, however, is inconsistent based on previous reports. Adequate folate intake has been associated with a substantially decreased risk of colorectal cancer (Negri et al., 2004), but unrelated to endometrial cancer risk (Jain et al., 2000).

Polymorphisms in different DNA repair genes that are mainly represented by single-nucleotide polymorphisms (SNPs) can potentially modulate the individual DNA repair capacity (Hoffbrand et al., 1996) (Rampersaud et al., 2000) and therefore exert an impact on individual genetic susceptibility to a wide range of cancers. The main findings of the published studies on the effect of DNA repair genetic polymorphisms on the risk of cancer have been summarized in several reviews. Only in a few instances, consistent evidence for association of genetic polymorphisms with specific cancers has emerged (Cravo et al., 1998; Herman et al., 199; McCann et al., 1998, 2001). In recent years, an increasing number of studies have investigated the role of polymorphisms in DNA repair genes on individual susceptibility to CRC with inconclusive outcomes (Friso et al., 2005)

The aim of the present study was to investigate the

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associations, if any, between polymorphisms in a gene involved in DNA repair and the risk of colorectal cancer in a population from Iran. A novel polymorphic site in MTHFR (G1793A) could influence the homocysteine levels and was first described in 2002. Therefore we decided to examine any association between this polymorphism and colorectal cancer.

Materials and Methods

Patients and Controls

Blood samples were collected from 227 unrelated Iranian patients with no familial colon cancer and 239 healthy individuals as controls which their characteristics is indicated in Table1. All subjects were genetically-unrelated ethnic Iranian patients with histopathologically confirmed incident sporadic colorectal cancers recruited between September 2003 and December 2007 at the Research Center for Gastroenterology and Liver Diseases (RCGLD) in Taleghani hospital.

Cases consisted of patients with positive colonoscopic results for malignancy, histologically confirmed as carcinomas of the colon or rectum. Controls were randomly selected subjects undergoing colonoscopy for various gastrointestinal complaints and sampled at the same time as the cases. They all had negative colonoscopic results for malignancy or idiopathic bowel diseases and were frequency-matched to the cases by age within five years and sex. At recruitment, informed consent was obtained from all participants and the Ethical Review Boards of the centr approved the study protocol.

Red Cell and Plasma Folate

Briefly, a fasting blood sample was drawn in the morning from 227 cases and 239 controls. RBCs, plasma, and whole blood folate were determined for the cases and controls. We calculated red cell folate values from whole blood folate concentrations and corrected for hematocrit and plasma folate levels, according to an established formula. (Hoffbrand et al., 1996).

Genotyping by Pyrosequencing

Pyrosequencing is based on the detection of released pyrophosphate (PPi) during DNA synthesis. In a cascade of enzymatic reactions, visible light proportional to the number of incorporated nucleotides is generated.

We designed three primers (forward, reverse and sequencing) which either forward or reverse was biotinylated. Forward and reverse primers were used for doing PCR and sequencing primer for running pyrosequencing (Assay Design software v1.0.6). PCR primers used in the study are given in Table 2 and Fig 1, and the reaction conditions were as follows: 1µl of genomic DNA solution (10ng/µl), 20µM of each primer, 0.2mmol/l of each dNTP, and 2.0mmol/l $MgCl_2$ and 0.02U/µl AmpliTaq Gold DNA polymerase in 50µl total valume. Thermal cycling was performed as following: 94˚C for 5 min followed by 35 cycles of 95˚C for 30s, annealing temperature for 45s and 72˚C for 40s, followed by 72˚C for 10min. The biotinylated products of the single PCR were immobilized on streptavidin-coated paramagnetic beads (Magnetic Biosolutions), and the strands were separated using 0.10mol/l NaOH. This ssDNA were genotyped for the polymorphic locus by sequencing primer and pyrosequencer (PSQ 96MA) Direct sequencing was also conducted for 8 randomly selected subjects to confirm our data. Sequencing results were analyzed with DNASIS MAX software v2.6 (Hitachi Software Engineering Co.).

Table 1. Characteristics of the Cases and Controls

Factor		Cases (n = 227)	Controls (n = 239)
Gender	Male	125	134
	Female	102	105
Average Age		68.3 ± 7.9	59.1 ± 6.6
Folates	Total (mg/day)	443.1 ± 251.2	446.5 ± 259.2
	RBC (ng/ml)	257.2 ± 165.8	263.5 ± 141.7
	Plasma (ng/ml)	11.3 ± 7.8	15.1 ± 9.7

Data are Means plus SDs

Table 2. PCR and Pyrosequencing primers Used for Genotyping MTHFR 1793G>A

PCR and Pyrosequencing primers (5'- 3') Id	Sequence	Score: 98 Bp	Tm°C	GC*
Forward	GTGGGCCTTGTTCTATTCCG	20	70.6	55.0
Reverse	ACGGGGACTCCTCCTCAT	18	68.4	61.1
Sequencing	TTGCCCTGTGGATTG	15	54.6	53.3

*(%)

Statistical Analysis

The distribution of individual characteristics was evaluated by simple descriptive statistic. Standard techniques for unmatched case-control studies were used. Odds ratios and 95% confidence intervals were calculated as a measure of association between MTHFR genotypes or environmental exposure and colon cancer. Exposure was defined as homozygosity for the glycin substitution (AA). The association between MTHFR genotype and colorectal cancer was estimated in the entire studied population.

Results

During the accrual period, we identified 244 cases and 251 controls who were potentially eligible of these, 17 cases and 12 controls refused interview, thus we analyzed 227 cases and 239 controls including 125 male and 102 female in the cases and 134 men and 105 women as a control group (Table 1) .The genotypes were analyzed by pyrosequencing (Fig. 2). Allele frequencies in cases were G=99% and A=1% while among the controls were G= 94.5% and C= 4.95%.

Table 3 presents the inverse association between GA genotype and colorectal cancer in this population. The frequencies of 1793AA (Gln/Gln), GA1793 (Arg/Gln), and GG1793 (Arg/Arg) genotypes among the cases were 0, 1.8 and 98.2%, respectively while in controls the frequencies were 0% , 9.7% and 90.3% .(Table 3). The frequency of G1793A genotype among the cases was lower compared with the controls. Thus, one A allele at 1793G>A, was related to the lowest risk. The genotype frequencies for the rs2274976 (1793G>A) polymorphisms

Table 3. MTHFR 1793G>A Genotype Prevalence and Main Effects in Iranian Patients with Colorectal Cancer

Genotype	Cases (%)	Controls (%)	OR	95% CI
GG	223 (98.2)	216 (90.3)	1.00	Reference
GA	4 (1.8)	23 (9.7)	0.17	0.05-0.52
AA	0 (0.0)	0 (0.0)	--	----

deviated significantly from Hardy-Weinberg equilibrium among cases or controls (data not shown in the Table). Therefore, we found a reverse association with colorectal cancer for heterozygotes, GA, among our population. OR=0.17 (95% CI=0.05-0.52)

Table 4 shows data for the joint effects of the MTHFR genotype and folate on CRC risk. Subjects with the GA genotype had approximately the adenoma risk of those with at least one wild-type allele. At the highest folate levels, CRC risk was <1.0 for both GA heterozygotes and those with a wild-type allele.

Discussion

Polymorphisms in critical genes can potentially alter the susceptibility to different cancers including CRC. In this study, we evaluated this hypothesis, whether the SNP in MTHFR gene encoding a key enzyme in folate metabolism, influence the risk of this cancer. The present investigation demonstrated an inverse association between codon G1793A and colon cancer in both sexes, in line with earlier findings of an impact on susceptibility to several cancers (Mao et al., 2008).

Folate has been suggested to play an important role in DNA methylation, synthesis, and repair, and thus may be involved in carcinogenesis. Folate deficiency may lead to reduced 5-methyltetrahydrofolate availability, which is used for the remethylation of homocysteine to methionine. This, in turn, may result in decreased levels of methionine, S-adenosyl-L-methionine, and global hypomethylation of DNA (McCann et al., 2000; Rampersaud et al., 2000; Shrubsole et al., 2001) leading to increased mutation rates via genomic instability (Cravo et al., 1998) . Folate deficiency may also increase DNA replication errors through misincorporation of uracil into DNA, causing higher levels of chromosomal breaks (Herman et al., 1998; McCann et al., 2000; Rampersaud et al., 2000). Folate seems to be associated with hypermethylation of CpG islands in the promoter regions of several tumor suppressor genes and DNA repair genes, reducing their expression (Herman et al., 1998; McCann et al., 2001).

This is the first report in association between colorectal cancer and novel MTHFR genotype in the world. However, a previous study was performed in China regarding association between endometrial cancer and Chinese women.They did not find any association between MTHFR genotype and cancer risk although dietary folate intake was inversely associated with risk of endometrial cancer (Xu et al., 2007).

There were several limitations to our study. First, we were unable to distinguish between intake of vitamin B - 6, B-12 and folate in the form of supplements, thus we did not estimate intake of B vitamin or methionine from food. Second, alcohol consumption is associated with folate malabsorption, which may lead to folic acid deficiency. However, alcohol consumption is not recommended in our culture and drinking of alcohol was not common in our studied population. Therefore, our results are unlikely to be confounded by the above-mentioned factors. Thereby we had limited power to evaluate interaction with alcohol consumption in our study.

In conclusion, our results are different from some previous reports related to association between endometrial cancer risk and the polymorphism since they did not find any association (Bagley et al.,1998; Paynter et al., 2004; Friso et al., 2005). While ou results showed an inverse association between the MTHFR1793 genotype with colorectal cancer, especially at high levels of folate, larger studies are now needed for confirrmation. Also it is suggested that this effect may be particularly true for advanced colon cancers.

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Table 4. ORs and 95% CIs for Colon Cancer in Relation to MTHFR 1793G>A Genotypes, Total Folate Intake, and Supplement Use in an Iranian Population

Genotype	Intake[a]	Food folate		Total folate	
		Cases/controls	OR (95% CI)	Cases/controls	OR (95% CI)
GG	≤Median	112/117	1.00	125/113	1.00
GG	>Median	111/99	1.17 (0.79-1.75)	98/103	0.86 (0.58-1.27)
GA	≤Median	2/13	0.16 (0.02-0.77)	3/11	0.25 (0.05-0.99)
GA	>Median	2/10	0.21 (0.03-1.05)	1/12	0.08 (0.0-0.57)

[a] The median intake was 320μg/d for folate from foods, 450μg/d for total folate

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