Association of the DNMT3A -448A>G polymorphism with genetic susceptibility to colorectal cancer

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Abstract. The DNA methyltransferase 3A (DNMT3A) -448A>G polymorphism is a novel functional single nucleotide polymorphism (SNP) that contributes to the genetic susceptibility to gastric cancer. In this study, we aimed to assess the genotype frequencies of DNMT3A -448A>G in colorectal cancer (CRC) patients and healthy control subjects, and to explore the association of the DNMT3A functional SNP, -448A>G, with genetic susceptibility to CRC. Genomic DNA was extracted from samples of 258 patients with CRC and 280 healthy controls. Polymerase chain reaction-restriction fragment length polymorphism analysis was employed to assess the genotype frequencies of DNMT3A -448A>G in all of the subjects. Stratification analyses were used to study subgroups of subjects by age and gender, and to evaluate the association between the DNMT3A -448A>G polymorphism and the genetic susceptibility to CRC. The allele frequency of -448A among CRC patients and the controls was 26.4 versus 19.8%, respectively. Overall, we found that compared with GG carriers, the DNMT3A -448AA homozygotes had a 3.692-fold increased risk of CRC. Stratification analysis showed a significant difference in this SNP between the CRC patients and the control subjects of different genders. AA homozygotes carried an increased risk in the subgroup of individuals aged ≥50 years in male CRC. Compared with GG homozygotes in females aged ≥50 years, the AG and AA genotypes carried a 0.355-fold decreased risk in this subgroup. These data imply that the DNMT3A SNP -448A>G contributes to genetic susceptibility to CRC. -448A>G may be used as a stratification marker to predict the susceptibility of certain individuals to CRC, particularly in male individuals aged ≥50 years.

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Introduction

Colorectal cancer (CRC) is the leading cause of cancer-related mortality for males and females worldwide (1). Although significant progress has been made to understand the genetic changes associated with cancer, there are few useful biomarkers for identifying individuals at high risk in the general population. DNA methyltransferase 3A (DNMT3A) plays a significant part in the development of embryogenesis and in the generation of aberrant methylation in carcinogenesis (2). The expression of DNMT3A is increased in parallel to the degree of dysplasia, with significant overexpression in the malignant lesion when compared with normal mucosa (3). Elevated Dnmt3a activity promotes polyposis in Apc(Min) mice by relaxing the extracellular restraints on Wnt signaling (4). Our previous study found a novel DNMT3A functional single nucleotide polymorphism (SNP), -448A>G, and showed that a DNMT3A promoter genetic variant increased its transcriptional activity and contributed to the genetic susceptibility to gastric cancer (GC) in the Chinese population (5). However, an association between the DNMT3A -448A>G polymorphism and CRC has not been reported as yet. In this case-control study, we genotyped this DNMT3A polymorphism and investigated the association between the -448A>G functional SNP and CRC.

Materials and methods

Study subjects. CRC patients were selected consecutively from the Zhongda Hospital of Southeast University and Jiangsu Tumor Hospital, China. The 258 case subjects were diagnosed and CRC was histopathologically confirmed. The 280 healthy controls were selected from cancer-free individuals at the same hospital and were frequency-matched to the case patients based on age, gender and ethnicity. All of the subjects were ethnically Chinese and resided in the Jiangsu Province of China or the surrounding area. All samples were obtained with informed consent from the subjects and the approval of the Institutional Review Board at the Zhongda Hospital of Southeast University and Jiangsu Tumor Hospital in Jiangsu province, from September 2006 to February 2011.

The controls were cancer-free individuals who visited the same hospital for routine physical examinations or who had volunteered to participate in the epidemiology survey during the same period.

DNMT3A genotyping. Total genomic DNA was isolated from 258 patients with CRC and 280 healthy controls by proteinase K digestion (6). As the transition of A>G of the DNMT3A SNP creates a *Taa*I restriction site, polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis was used to detect this A-G transition in the promoter of DNMT3A at -448A>G (GenBank accession no. NT_022184.15:g.4387794AG). The DNMT3A -448A>G polymorphism was determined using a PCR-RFLP assay. The PCR reaction was performed in a total volume of 25 μl, containing 100 ng genomic DNA, 0.1 mM dNTPs, 2.0 mM $MgCl_2$, 10 μM primers 5'-ACACACCGCCCTCACCCCTT-3' (forward), and 5'-TCCAGCAATCCCTGCCCACA-3' (reverse), and 1.25 U Taq polymerase (Biocolor BioScience and Technology Company, Shanghai, China). PCR cycle conditions included an initial melting step of 95˚C for 5 min, followed by 32 cycles of 95˚C for 20 sec, 68˚C for 20 sec and 72˚C for 30 sec, and a final extension step of 72˚C for 5 min. The 358 bp fragment was then digested with *Taa*I (Fermentas Co., Glen Burnie, MD, USA) for 5 min at 65˚C, the digested products were then separated on a 3.0% agarose gel, and the RFLP bands were visualized under UV light with ethidium bromide (EB) staining. The wild-type G allele had a *Taa*I restriction site that resulted in three bands (153, 94 and 87 bp), while the variant A allele resulted in four bands (247, 153, 94 and 87 bp).

Statistical analysis. SPSS 13.0 software (SPSS Inc., Chicago, IL, USA) was used to perform all statistical comparisons. All comparisons were two-tailed, and P<0.05 was considered to be statistically significant. DNMT3A genotype and allele distributions among the study groups were calculated with odds ratios (ORs) and 95% confidence intervals (CIs) by means of two-sided contingency tables using the χ^2 test. Stratification analysis was used to study subgroups of subjects by age and gender.

Table I. Characteristics of the study population.

Variables	CRC cases (n=258)	Controls (n=280)	P-value[b]
Age (years)			0.076
<50	58 (22.5)[a]	46 (16.4)	
≥50	200 (77.5)	234 (83.6)	
Gender			0.153
Male	158 (61.2)	188 (67.1)	
Female	100 (38.8)	92 (32.9)	

[a]Numbers in parentheses, percentage; [b]P-values for Chi-square (χ^2) test. CRC, colorectal cancer.

Results

The DNMT3A genotypes, AA, AG and GG, were detected in the CRC patients and the controls. There were no significant differences in the distribution of the mean age and gender between the cases and the controls, suggesting that the matching based on these two variables was satisfactory (Table I). The genotyping by PCR-RFLP analysis was confirmed by DNA sequencing analysis. All of the cases and controls were successfully genotyped for the DNMT3A polymorphism. The distributions of -448A>G genotypes in the 280 control subjects were GG 63.6%, GA 33.2% and AA 3.2%, and the A allele frequency was 19.8%, while the -448A allele frequency was 26.4% in CRC (Table II). There are significantly different frequencies of -448A>G between the male and female patients in CRC (Table III). The -448A>G polymorphism of the DNMT3A promoter evaluated the risk related to CRC groups in the case-control study. The CRC risk related to the DNMT3A -448A>G genotype is shown in Table IV. The distributions of -448A>G genotypes in the CRC group (GG 58.1%, AG 31.0% and AA 10.9%) were significantly different from those among the controls (GG 63.6%, GA 33.2% and AA 3.2%). As compared with the reference group -448GG genotypes, AA homozygotes carried a 3.692-fold increased risk of CRC (P=0.001).

Table II. DNMT3A -448A>G genotype and allele frequencies of colorectal cancer cases and control.

Genotype	Colorectal cancer (n=258)		Control subjects (n=280)		Crude OR (95% CI)	P-value[a]
	No.	(%)	No.	(%)		
-448A>G						
GG (ref.)	150.0	58.1	178	63.6	1	
AG	80.0	31.0	93	33.2	1.021 (0.705-1.477)	0.913
AA	28.0	10.9	9	3.2	3.692 (1.689-8.068)	0.001
AG+AA	108.0	41.9	102	36.4	1.256 (0.888-1.778)	0.197
A allele	26.4	19.8				

[a]Chi-square (χ^2) test, colorectal cancer cases versus control. OR, odds ratio; CI, confidence interval.

Table III. DNMT3A -448A>G genotype and allele frequencies in colorectal cancer cases.

Groups	Genotype			Allele	
	GG (%)	AG (%)	AA (%)	A (%)	P-value[a]
Total	150 (58.1)	80 (31.0)	28 (10.9)	26.4	
Age (years)					0.710[b]
<50	33 (12.8)	17 (6.6)	8 (3.1)	28.4	
≥50	117 (45.3)	63 (24.4)	20 (7.8)	25.8	
Gender					0.015[c]
Male	103 (39.9)	40 (15.5)	15 (5.8)	22.2	
Female	47 (18.2)	40 (15.5)	13 (5.1)	33.0	

[a]Chi-square (χ^2) test; [b]the frequency of the A allele in individuals aged <50 years versus ≥50 years; [c]the frequency of the A allele in males versus females.

Table IV. Distribution of -448A>G DNMT3A genotypes and the associated odds ratios (ORs) in relation to age and gender in CRC cases.

Genotype	Controls (%)	CRC cases (%)	OR	P-value[a]
Male				
GG	151 (54.0)	103 (39.9)	1	
AG	33 (11.8)	40 (15.5)	1.777 (1.052-3.003)	0.031
AA	4 (1.4)	15 (5.8)	5.498 (1.774-17.036)	0.001
AA+AG	37 (13.2)	55 (21.3)	2.179 (1.340-3.544)	0.002
Male <50 yrs				
GG	14 (5.0)	19 (7.3)	1	
AG	14 (5.0)	9 (3.5)	0.474 (0.160-1.402)	0.174
AA	1 (0.3)	3 (1.1)	2.211 (0.207-23.555)	0.503
AA+AG	15 (5.3)	12 (4.6)	0.589 (0.211-1.645)	0.311
Male ≥50 yrs				
GG	137 (49.0)	84 (32.6)	1	
AG	19 (6.8)	31 (12.0)	2.661 (1.414-5.008)	0.002
AA	3 (1.1)	12 (4.7)	6.524 (1.789-23.794)	0.001
AA+AG	22 (7.9)	43 (16.7)	3.188 (1.783-5.700)	0.000
Female				
GG	27 (9.6)	47 (18.2)	1	
AG	60 (21.4)	40 (15.5)	0.383 (0.206-0.712)	0.002
AA	5 (1.8)	13 (5.1)	1.494 (0.480-4.646)	0.487
AA+AG	65 (23.2)	53 (20.6)	0.468 (0.258-0.850)	0.012
Female <50 yrs				
GG	10 (3.6)	14 (5.4)	1	
AG	7 (2.5)	8 (3.1)	0.816 (0.223-2.992)	0.759
AA	0 (0.0)	5 (2.0)	-[b]	0.134
AA+AG	7 (2.5)	13 (5.1)	1.327 (0.389-4.520)	0.651
Female ≥50 yrs				
GG	17 (6.0)	33 (12.8)	1	
AG	53 (18.9)	32 (12.4)	0.311 (0.150-0.646)	0.001
AA	5 (1.8)	8 (3.1)	0.824 (0.233-2.910)	0.755
AA+AG	58 (20.7)	40 (15.5)	0.355 (0.175-0.723)	0.004

[a]Chi-square (χ^2) test, CRC cases versus control. [b]No significance as the frequency of the AA genotype in this subgroup of CRC is zero. CRC, colorectal cancer.

When the analyses were stratified by the age and gender of the patients, the -448AA genotype was associated with the genetic susceptibility to CRC in males, particularly in individuals older than 50 years of age (OR, 6.524; 95% CI, 1.789-23.794; P=0.001). Individuals who carried at least one A allele (AG or AA) had a 2.179-fold increased OR of developing CRC compared to those who carried the GG wild-type genotype (95% CI, 1.340-3.544; P=0.002). The risk associated with the combined AA+AG genotypes was more pronounced in older subjects, and in males rather than females. There was a significant difference in the frequency of AG heterozygotes (P=0.002) between the cases and controls among females. Individuals who carried at least one A allele (AG or AA) had a 0.468-fold decreased OR of developing CRC compared to those who carried the GG wild-type genotype (95% CI, 0.258-0.850; P=0.012). In particular, AG heterozygotes had a greater reduced risk of developing CRC in females older than 50 years (OR, 0.311; 95% CI, 0.150-0.646; P=0.001).

Discussion

CRC is one of the main causes of cancer mortality worldwide. A series of studies have suggested that there is aberrant DNA methylation in colorectal carcinoma cells, including inactivation and CpG island hypermethylation of certain tumor-suppressor genes, including CD133 (7), MLHI, MGMT (8), APC, MGMT, RASSF2A and Wif-1 (1). Given that the HIF-1α SNP C1772T (9), the DNA repair gene XRCC3 (10) SNP and the E-cadherin gene SNP -347 G→GA (11) and -160 C→A (12) are associated with the genetic susceptibility to CRC, DNMT3A is responsible for DNA methylation as a *de novo* methyltransferase in the tumorigenesis of numerous types of cancer, including CRC. Previous studies have suggested that ectopic DNMT3A expression plays a significant role in CRC progression (13,14). The restriction of Dnmt3a overexpression showed hypermethylation-mediated transcriptional silencing of the Wnt antagonist Sfrp5, and to a lesser extent, Sfrp1 (4). Our previous study showed a novel functional polymorphism, -448A>G, in the promoter of the DNMT3A gene, and this polymorphism was associated with the risk of cancer, particularly with GC. To the best of our knowledge, the correlation between the DNMT3A polymorphism and the risk of CRC has not been reported as yet. We hypothesized that this functional DNMT3A -448A>G polymorphism is associated with genetic susceptibility to CRC. Stratification analyses showed that there was a significant difference in the frequency of AG heterozygotes and AA homozygotes in CRC.

According to our analysis of the DNMT3A gene promoter -448A>G polymorphism among a Chinese CRC and control group, we found that the A variant genotype was associated with a significantly increased risk of CRC. The AA genotype was associated with a significantly increased risk of CRC in males aged ≥50 years. The data provide crucial evidence that -448A carries a significant likelihood of carcinogenesis for older male CRC patients, at least in this Chinese population. This functional polymorphism modifies the susceptibility to CRC, and may be a risk predictor for male CRC, particularly in the group aged ≥50 years. In addition to this, another significant finding is that the females carrying the -448AA genotype had a decreased risk of CRC, suggesting that this polymorphism may be a protective factor for CRC in females of Chinese origin. Research has shown that the risk of CRC varies in different genders, ethnicities and ages (11,15,16). The mechanism for this should be investigated in further studies, as there is not enough evidence to explain it at the present. Moreover, further studies are required to confirm our findings with larger sample sizes and in more populations. In the present study, we can only highlight this significant result and make the hypothesis that the DNMT3A -448A>G polymorphism is associated with the risk of CRC and relates to gender and age. Given that the size of the samples in the subgroups was not large enough to draw any conclusion, further studies are required.

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