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Contribution of *5-Methyltetrahydrofolate-Homocysteine Methyltransferase Reductase* Genotypes to Colorectal Cancer in Taiwan

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Abstract. *Background/Aim: 5-methyltetrahydrofolate-homocysteine methyltransferase reductase (MTRR) is responsible for folate metabolism, and we aimed to investigate its genetic role in colorectal cancer (CRC) among Taiwanese. Materials and Methods: A total of 362 cases and 362 controls were recruited and their MTRR rs1801394 (A66G) and rs1532268 (C524T) genotypes were examined. The behavioral factors and clinical-pathological factors were also analyzed. Results: MTRR rs1801394 genotypes were associated with CRC risk (p for trend=0.0087). In detail, G/G genotype was associated with lower risk (p=0.0049, OR=0.39, 95%CI=0.20-0.76). As for allelic frequency analysis, G allele was also associated with decreased CRC risk (p=0.0026, OR=0.68, 95%CI=0.53-0.88). There was no significant association as for MTRR rs1532268. Among non-smokers and non-alcohol drinkers, those with G/G genotype were at 0.38- and 0.46-fold odds of having CRC. There were no significant protective effects among smokers or alcohol drinkers. Conclusion: MTRR rs1801394 GG genotype can be a protective marker for CRC risk in Taiwan.*

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Key Words: Colorectal cancer, genotype, *MTRR*, polymorphism, Taiwan.

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Colorectal cancer (CRC) is the third most common cancer among men and women worldwide (1-3). The incidence and mortality rates of CRC can change as high as ten folds among countries (1, 2, 4). Many factors may contribute to this variation, for instance, meat consumption, cigarette smoking, and exposure to carcinogens contribute to about 85% of CRC etiology (5, 6). In Taiwan, the problem of CRC is rather noticeable. The incidence rate of CRC is number one among all types of cancer, and the mortality rate of CRC is third, just behind lung and liver cancer. As 15-20% of CRC cases have a familial history of cancer (7, 8), genetic factors have been believed to play a very important part in the etiology of CRC. Although some genetic biomarkers for CRC have been revealed during recent years (9-14), however, the interactions between genomic and other risk factors are still of great interest among translational scientists. The understanding of genetic contribution to CRC can help translational scientists to achieve precise medication and therapy.

In the literature, it is demonstrated that folate metabolism has played a critical part in regulating DNA repair capacity and DNA methylation status (15, 16). There were a few famous genetic polymorphic sites of folate metabolism enzymes, including methionine synthase (MTR), methionine synthase

reductase (MTRR), and 5,10-methylenetetrahydrofolate reductase (MTHFR). There were also studies reporting that the various genotypes could influence the levels of serum folate (17-19). Consequently, it is biologically plausible that these polymorphisms will affect DNA repair capacity or cause abnormal DNA methylation, which may subsequently lead to gene instability and give rise to development of various kinds of cancers. Among them, MTRR is a vital enzyme involved in folate metabolism. Folate, a water-soluble B-vitamin, is essential for the prevention of malignancy initiation (20). This vitamin functions as a coenzyme in the process of nucleotide synthesis as well as in the DNA and protein methylation (21). The enzymatic activity of MTRR can be affected by a missense *MTRR* A66G (rs1801394) polymorphisms. A substitution of A to G at the nucleotide 66 changes isoleucine to methionine at position 22 (Ile22Met) of MTRR (22, 23). The *MTRR* rs1801394 GG genotype is also associated with lower plasma homocysteine concentration compared to the AA genotype (24, 25). Mutation in the gene encoding this enzyme can cause hyper-homo-cysteinemia, which probably contributes to DNA hypomethylation and lowering DNA repair capacity, and leading to CRC initiation (26, 27). *MTRR* C524T (rs1532268) is considered to be a risk factor for the development of ventricular septal defects (VSD) (28, 29) and gastric cancer (30, 31), but not entirely concluded in CRC risk. Based on the above information, we hypothesized that the variant genotypes at *MTRR* rs801394 (A66G) and rs1532268 (C524T) may also be involved in determining the personal susceptibility for CRC among Taiwanese. In addition, we will check the genetic-behavioral and genetic-clinical interactions.

Materials and Methods

Collection of 362 CRC cases and 362 control subjects. The CRC cases were recruited as described in our previous studies (13, 14). Briefly and concisely, the CRC cases have been collected, and the pathological data were defined, graded and well recorded. Then, each of the case was well match by age, sex and behaviors including smoking and alcohol drinking. The collection protocols were approved by the Institutional Review Board of the China Medical University Hospital (coding number: DMR99-IRB-108). Specific characters which are analyzed in the study are presented in Table I.

MTRR genotyping methodology. The genomic DNA from peripheral blood leukocytes of all participants were extracted and stored at –80˚C as previously published (32, 33). The polymerase chain reaction (PCR) conditions set for *MTRR* genotyping were one cycle at 94˚C for 5 min; 35 cycles at 94˚C for 30 s, one cycle at annealing 55˚C for 30 s and one cycle at elongating 72˚C for 30 s and a final extension at 72˚C for 10 min. The sequences of forward and reverse primers are designed by Terry Fox Cancer Research Lab and provided in Table II. In addition, the PCR products, corresponding restriction enzymes, and cutting adducts for *MTRR* rs1801394 and rs1532268 are also presented in Table II.

Statistical analysis. To compare the distribution of *MTRR* genotypic and allelic distributions between stratified sub-groups, the Pearson's Chi-square test without Yates' correction was applied. To check the associations between *MTRR* genotypes and CRC risk, odds ratios (ORs) together with 95% confidence intervals (CIs) was applied. Adaptation of confounding factors as indicated was applied when examining the interactions of behavioral factors with *MTRR* genotypes.

Table I. *Summary of selected data from 362 patients with colorectal cancer and 362 matched non-cancer healthy controls.*

Characteristic	Controls (n=362)		Cases (n=362)		*p*-Value[a]
	n	%	n	%	
Age (years)					
≤60	95	26.2%	95	26.2%	1.0000
>60	267	73.8%	267	73.8%	
Sex					
Male	203	56.1%	203	56.1%	1.0000
Female	159	43.6%	159	43.9%	
Smoking					
Yes	84	23.2%	91	25.1%	0.5434
No	278	76.8%	271	74.9%	
Alcohol drinking					
Yes	51	14.1%	44	12.2%	0.4410
No	311	85.9%	318	87.8%	
BMI					
<24	175	48.3%	193	53.3%	0.1809
≥24	187	51.7%	169	46.7%	
Tumor size (cm)					
<5			195	53.9%	
≥5			167	46.1%	
Location					
Colon			257	71.0%	
Rectum			105	29.0%	
Lymph node involvement					
Negative			210	58.0%	
Positive			152	42.0%	

SD, Standard deviation; BMI, body mass index; [a]based on Chi-square test without Yates' correction.

Results

Basic indexes between the CRC patient and control groups. The distribution of age, sex and other indexes for the 362 CRC patients and 362 non-cancer healthy controls are shown in Table I. There were 203 (56.1%) males and 159 (43.6%) females both in the CRC case and control groups. Ninety-one (25.1%) of the CRC group had the smoking habits, while 44 (12.2%) had alcohol drinking habits, and the percentages were not significant different from those of the control group (both $p>0.05$, Table I). The BMI was not differentially distributed between case and control groups (Table I).

Association of MTRR genotypes with CRC risk. The genotypes of *MTRR* rs1801394 (A66G) and rs1532268 (C524T) among the 362 CRC patients and 362 controls are

Table II. *Information about primers, PCR products, restriction enzymes, and cutting adducts for MTRR rs1801394 and rs1532268.*

Polymorphic sites	Primers	PCR product	Restriction enzyme	Cutting adducts
rs1801394 (A66G)	F: 5'-CAA AGG CCA TCG CAG AAG ACA T-3' R: 5'-CAC TTC CCA ACC AAA ATT CTT CA A-3'	149 bp	*Nde* I	G: 149 A: 128+21
rs1532268 (C524T)	F: 5'-GTC AAG CAG AGG ACA AGA G-3' R: 5'-AGA GAC TCC TGC AGA TGT AC-3'	309 bp	*Xho* I	T: 309 C: 251+58

F: Forward; R: reverse.

shown in Table III. First, the various genotypic frequencies of *MTRR* rs1801394 were differentially distributed between CRC and control groups (p for trend=0.0087). In detail, the *MTRR* rs1801394 heterozygous A/G and homozygous G/G genotypes were associated with lower risk for CRC than the wild-type A/A genotype (p=0.0825 and 0.0049, OR=0.76 and 0.39, 95%CI=0.55-1.03 and 0.20-0.76). In the recessive model, the G/G genotype conferred a decreased risk for CRC compared to combination of A/A+A/G genotypes (p=0.0110, OR=0.43, 95%CI=0.22-0.84). In the dominant model, those who carry A/G+G/G conferred a decreased susceptibility of CRC compared to the A/A genotype carriers (p=0.0151, OR=0.69, 95%CI=0.51-0.93). On the contrary, as for rs1532268, there was no difference in genotype distribution in any models analyzed (Table III, lower panel). To sum up, the *MTRR* rs1801394 genotypes play a critical role in determining personal susceptibility to CRC in Taiwan.

The allelic frequency analysis of MTRR with CRC risk. Further analysis of allelic frequency was performed and is presented in Table IV. There is an obvious difference in the distribution of allelic frequencies among the CRC patients and healthy controls regarding *MTRR* rs1801394 (OR=0.68, 95%CI=0.53-0.88, p=0.0026). On the contrary, there is no difference found as for rs1532268 of *MTRR* (OR=0.94, 95%CI=0.70-1.27, p=0.7027). This is consistent with the finding in Table III.

Influence of smoking habit and MTRR rs1801394 genotype on CRC risk. Cigarette smoking is a risk factor for Taiwan CRC, we intended to examine the influence of cigarette smoking and *MTRR* rs1801394 genotypes on CRC risk. As for non-smokers, those with *MTRR* rs1801394 G/G genotype were at 0.38-fold odds of CRC risk (95%CI=0.18-0.83, p=0.0123). There was no significant association between *MTRR* rs1801394 A/G genotypes and CRC risk (OR=0.74, 95%CI=0.52-1.07, p=0.1150). After adjusting for age, sex, alcohol drinking and BMI status, the trends are similar for both *MTRR* rs1801394 G/G and A/G (OR=0.36 and 0.70, 95%CI=0.15-0.80 and 0.48-1.12, respectively). There was no any significant association found among the smokers (Table V).

Influence of alcohol drinking habit and MTRR rs1801394 genotype on CRC risk. Then we aimed to investigate the influence of alcohol drinking and *MTRR* rs1801394 genotypes on CRC risk, another risk factor for Taiwan CRC. As for those non-drinkers, those with *MTRR* rs1801394 G/G genotype were at 0.46-fold odds of CRC risk (95%CI=0.22-0.95, p=0.0336). There was no significant association between *MTRR* rs1801394 A/G genotypes and CRC risk (OR=0.74, 95%CI=0.53-1.03, p=0.0702). After adjusting for age, sex, smoking and BMI status, the trends are similar for both *MTRR* rs1801394 G/G and A/G (OR=0.42 and 0.71, 95%CI=0.18-0.83 and 0.47-1.02, respectively). There was no any significant association found among the alcohol drinkers (Table VI).

Correlations among genotypes of MTRR rs1801394 and clinical indexes. The correlations among genotypes of *MTRR* rs1801394 and clinical features were analyzed (Table VII). No statistically significant correlation was observed between *MTRR* rs1801394 genotypic distributions and age, sex, BMI, tumor size or location, or lymph node metastasis status (all p>0.05) (Table VII).

Discussion

CRC has been the third most common cause of cancer-related mortality worldwide (1-3), and in Taiwan (35). During the last years, the role of folate and its related genetic variations on CRC have attracted great interest of translational scientists. However, several studies have been conducted, with conflicting findings from various populations with different genetic backgrounds (36-43). Taiwan is genetically and geographically conserved and our collection of CRC cases are representative for Eastern Asia. Our results provided evidence for positive association of *MTRR* rs1801394 genotypes with CRC risk (Table III and Table IV). In a meta-analysis consisting of 17 studies investigating 8,371 cases and 12,574 control subjects, the overall results indicated that the genotypes of *MTRR* rs1801394 may be ethnically, associated with CRC risk, which means that Asian populations are more likely to be influenced by *MTRR* rs1801394 while Caucasian populations are not (44).

MTRR is one of the major players in folate metabolism, and plays essential roles in nucleotide neo-synthesis and

Table III. *Distributions of MTRR rs1801394 and rs1532268 genotypic frequencies among colorectal cancer patients and healthy controls.*

	Cases, n (%)	Controls, n (%)	OR (95%CI)	*p*-Value[a]
rs1801394				
A/A	234 (64.6)	202 (55.8)	1.00 (Reference)	
A/G	115 (31.8)	131 (36.2)	0.76 (0.55-1.03)	0.0825
G/G	13 (3.6)	29 (8.0)	**0.39 (0.20-0.76)**	**0.0049***
p_{trend}				**0.0087***
p_{HWE}				0.2380
Carrier comparison				
A/A+A/G	349 (96.4)	333 (92.0)	1.00 (Reference)	
G/G	13 (3.6)	29 (8.0)	**0.43 (0.22-0.84)**	**0.0110***
A/A	234 (64.6)	202 (55.8)	1.00 (Reference)	
A/G +G/G	128 (35.4)	160 (44.2)	**0.69 (0.51-0.93)**	**0.0151***
rs1532268				
C/C	274 (75.7)	270 (74.6)	1.00 (Reference)	
C/T	79 (21.8)	82 (22.6)	0.95 (0.67-1.35)	0.7721
T/T	9 (2.5)	10 (2.8)	0.89 (0.35-2.22)	0.7972
p_{trend}				0.9334
p_{HWE}				0.2216
Carrier comparison				
C/C+C/T	353 (97.5)	352 (97.2)	1.00 (Reference)	
T/T	9 (2.5)	10 (2.8)	0.90 (0.36-2.24)	0.8162
C/C	274 (75.7)	270 (74.6)	1.00 (Reference)	
C/T +T/T	88 (24.3)	92 (25.4)	0.94 (0.67-1.32)	0.7309

OR: Odds ratio; CI: confidence interval; p_{trend}: *p* for trend; p_{HWE}: *p* for Hardy-Weinberg Equilibrium; [a]Based on Chi-square test without Yates' correction; *Bold values indicate statistical significance.

Table IV. *Allelic frequencies for MTRR rs1801394 and rs1532268 polymorphisms among the colorectal cancer patients and healthy controls.*

Allelic type	Cases, n (%) n=724	Controls, n (%) n=724	OR (95%CI)	*p*-Value[a]
rs1801394				
Allele A	583 (80.5)	535 (73.9)	1.00 (Reference)	
Allele G	141 (19.5)	189 (26.1)	**0.68 (0.53-0.88)**	**0.0026***
rs1532268				
Allele C	627 (86.6)	622 (85.9)	1.00 (Reference)	
Allele T	97 (13.4)	102 (14.1)	0.94 (0.70-1.27)	0.7027

OR: Odds ratio; CI: confidence interval. [a]Based on Chi-square test without Yates' correction; *Bold values indicate statistical significance.

methylation status of DNA, histones and proteins. The *MTRR* rs1801394 G/G genotype has been reported to have lower affinity with MTR (45). High folate intake may be a protective diet for CRC risk (46-48). However, all of these studies neither linked with personal *MTRR* genotype, nor assessed the dietary folate intake with the real serum folate status to reveal how folate influences CRC risk. The regulation of folate in serum is complicated, and lots of enzymes may be involved, for instance, MTHFR, MTRR and MTR. In 2018, we provided evidence for the significant association of *MTHFR* rs1801133 T allele serves as a predictive marker for CRC risk (49). It seems that those *MTHFR* rs1801394 G allele carriers can have a lower level of serum homo-cysteine, which by some unknown reason causes a higher efficiency in DNA repair activity, and keeps the human genome from instability and CRC carcinogenesis. The detailed mechanisms of how MTRR interact with other molecules leading to CRC needs further investigation in the future.

There have been various clinical or behavioral indexes reported to play a role in determining CRC risk, such as age, sex, familial cancer history, diet, alcohol consumption, obesity, tumor site, size, grade, histologic type, TNM stage, and carcinoembryonic antigen (CEA) level, and so on (50-53). But these kinds of epidemiological studies always lack genetic data for any genetic-linked analysis. In the present study, we combined numerous clinical indexes with genotyping data for analysis, and come up with the findings that *MTRR* rs1801394 G/G genotype can interact with non-

Table V. *Odds ratio for MTRR rs1801394 genotype and colorectal cancer after stratification by smoking status.*

Genotype	Non-smokers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value	Smokers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value
	Controls	Cases				Controls	Cases			
A/A	156	177	1.00 (ref)	1.00 (ref)		46	57	1.00 (ref)	1.00 (ref)	
A/G	99	84	0.74 (0.52-1.07)	0.70 (0.48-1.12)	0.1150	32	31	0.78 (0.42-1.47)	0.72 (0.36-1.28)	0.4423
G/G	23	10	**0.38 (0.18-0.83)**	**0.36 (0.15-0.80)**	**0.0123***	6	3	0.40 (0.10-1.70)	0.35 (0.08-1.55)	0.2989
Total	278	271				84	91			

[a]By multivariate logistic regression analysis; [b]by multivariate logistic regression analysis after adjusted for confounding factors age, sex, alcohol consumption and BMI status; *Bold values indicate statistical significance; CI, confidence interval; aOR, adjusted odds ratio.

Table VI. *Odds ratios for MTRR rs1801394 genotype and colorectal cancer after stratification by alcohol drinking status.*

Genotype	Non-drinkers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value	Drinkers, n		OR (95% CI)[a]	aOR (95% CI)[b]	*p*-Value
	Controls	Cases				Controls	Cases			
A/A	170	202	1.00 (ref)	1.00 (ref)		32	32	1.00 (ref)	1.00 (ref)	
A/G	119	104	0.74 (0.53-1.03)	0.71 (0.47-1.02)	0.0702	12	11	0.92 (0.35-2.38)	0.88 (0.32-2.12)	0.8581
G/G	22	12	**0.46 (0.22-0.95)**	**0.42 (0.18-0.83)**	**0.0336***	7	1	0.14 (0.02-1.23)	0.11 (0.02-1.12)	0.0626
Total	311	318				51	44			

[a]By multivariate logistic regression analysis; [b]by multivariate logistic regression analysis after adjusted for confounding factors age, sex, smoking and BMI status; *Bold values indicate statistical significance; CI, confidence interval; aOR, adjusted odds ratio.

Table VII. *Correlation between MTRR rs1801394 genotypes and clinicopathological properties of 362 colorectal cancer patients.*

Indexes		Number of cases	MTRR rs1801394 genotypes			*p*-Value[a]
			A/A (%)	A/G (%)	G/G (%)	
Age (years)	≤60	95	60 (63.2)	31 (32.6)	4 (4.2)	
	>60	267	174 (65.2)	84 (31.4)	9 (3.4)	0.8996
Sex	Male	203	129 (63.5)	65 (32.0)	9 (4.5)	
	Female	159	105 (66.0)	50 (31.5)	4 (2.5)	0.6041
BMI	<24	193	120 (62.2)	67 (34.7)	6 (3.1)	
	≥24	169	114 (67.5)	48 (28.4)	7 (4.1)	0.4093
Tumor size	<5 cm	195	121 (62.1)	63 (32.3)	11 (5.6)	
	≥5 cm	167	113 (67.7)	52 (31.1)	2 (1.2)	0.0664
Location	Colon	257	164 (63.8)	83 (32.3)	10 (3.9)	
	Rectum	105	70 (66.7)	32 (30.5)	3 (2.8)	0.8214
Lymph node involvement	Negative	210	129 (61.4)	71 (33.8)	10 (4.8)	
	Positive	152	105 (69.1)	44 (28.9)	3 (2.0)	0.1861

[a]Based on Chi-square test without Yates's correction.

smoking (Table V) and non-alcohol drinking habits (Table VI) to affect CRC risk. It is interesting, but complicated to figure out how *MTRR* rs1801394 G/G genotype can affect non-smokers and non-alcohol drinkers with their CRC risk, but not affect smokers and alcohol drinkers. We temporarily found no significant interactions between *MTRR* rs1801394 genotype and those clinical indexes (Table VII). However, some indexes such as tumor size (p=0.0664), may have significant results when larger sample size could be collected for bringing up a conclusive finding.

In conclusion, we provided evidence for the significant association of *MTRR* rs1801394 with Taiwan CRC risk. Our results also suggest that the G/G genotype of *MTRR* rs1801394 may have its protective effects specifically among

non-smokers and non-alcohol drinkers. It can serve as a useful genomic marker for Taiwan CRC risk.

Conflicts of Interest

The Authors declare no conflicts of interest regarding this study.

Authors' Contributions

Research design: Wu MH, Chen CH, Bau DT, Pei JS and Chang WS; patient and questionnaire summaries: Wu MH, Chen CP and Yueh TC; experimental work: Huang TH, Chang WS and Tsai CW; statistical analysis: Wang ZH, Mong MC and Yang YC; article writing: Chang WS and Bau DT; review and revision: Bau DT and Chang WS.

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