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# The Role of *MTHFR* Genotype in Colorectal Cancer Susceptibility in Taiwan

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**Abstract.** *Aim: To evaluate the contribution of methylenetetrahydrofolate reductase (MTHFR) genotype to the risk of colorectal cancer (CRC) in Taiwan. Materials and Methods: In this hospital-based case–control study, the role of MTHFR C677T (rs1801133) and A1298C (rs1801131) genotypes in determining CRC risk were investigated among 362 patients with CRC and an equal number of age- and gender-matched healthy individuals. Results: The percentages of CC, CT and TT genotypes for MTHFR rs1801133 were 64.1%, 29.8% and 6.1% in the CRC group and 51.1%, 37.0% and 11.9% in the control group, respectively (p for trend=0.0006). Analysis of the allelic frequency distribution showed that the variant T allele of MTHFR rs1801133 conferred a lower CRC susceptibility than did the wild-type C allele (odds ratio(OR)=0.66, 95% confidence interval(CI)=0.52-0.84, $p=4.32\times10^{-5}$). For the gene–lifestyle interaction, there were obvious protective effects of MTHFR rs1801133 T allele on the risk of CRC among non-smokers, ever smokers and non-alcohol drinkers, but not drinkers. Conclusion: MTHFR rs1801133 T allele serves as a predictive marker for CRC risk and future studies with larger samples and functional evaluation are warranted to validate the current findings.*

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Colorectal cancer (CRC) incidence and mortality are both third among cancer patients worldwide global and are expected to increase by 60% to more than 2.2 million new cases and 1.1 million deaths by 2030 (1, 2). For many years, the incidence and mortality of CRC has occupied the first and third ranks among the most common types of cancer in Taiwan (3, 4). Etiological studies have attributed more than 85% of CRC risk to lifestyle and environmental factors, particularly meat consumption, cigarette smoking, and exposure to carcinogenic aromatic amines (5, 6). From the viewpoint of epidemiology, about 15-20% of CRC cases have a strong familial history of cancer that have led epidemiologists to seek additional inherited susceptibility factors (7-9). In Taiwan, although specific biomarkers for CRC prediction and detection have been reported in the past decade (4, 10), novel genomic markers of CRC and the interactions among the genomic, lifestyle and environmental risk factors are still of high interest and largely unrevealed.

The protein methylenetetrahydrofolate reductase (MTHFR) is an enzyme in charge of the conversion of 5,10-methylene-tetrahydrofolate into 5-methyltetrahydrofolate, homocysteine re-methylation, and neosynthesis of DNA and RNA (11). The multi-aspect regulatory effects of MTHFR on DNA methylation, DNA replication, DNA repair and cell division make it a potential cancer-predisposing gene. It is reasonable that rapidly proliferating malignancies have a higher requirement for DNA neosynthesis and cell division and could be more susceptible to folate deficiency, resulting in an instable genome with more DNA damage. In a BALB/c

mouse model, low dietary folate and MTHFR deficiency induced the formation of intestinal tumors (12).

The most subtle genetic variations are single nucleotide polymorphisms (SNPs) which determine personal susceptibility to human diseases, such as cancer. In literature, population-based investigations of *MTHFR* variations have focused on examining the genotypes of two SNPs of a catalytic domain, C677T (rs1801133) and A1298C (rs1801131), which may determine the enzymatic activity of encoded *MTHFR* (13, 14). In the case of the MTHFR C677T polymorphism, the cytosine base at position 677 changes to a thymidine base, which in turn affects the amino acid at position 222, as it is changed from alanine to valine. The resultant MTHFR enzyme from the TT variant becomes thermolabile, resulting in decreased activity with elevated temperature (15). The modified protein loses its flavin adenine dinucleotide cofactor more quickly and has a lower stability than the enzyme encoded by CC. The thermolabile effect on TT-encoded MTHFR can be suppressed by the addition of folate, which causes a higher affinity for flavin adenine dinucleotide and an increase in MTHFR stability (15). The second polymorphism, *MTHFR* A1298C, is located in the coding regulatory domain (16). It was reported that enzyme activity of heterozygotes and rare homozygotes of *MTHFR* C677T variant was only 60% and 30%, respectively, of the wild-type (14); for *MTHFR* A1298C, the rare homozygotes have 60% of wild-type activity (14). In 2004, it was reported that colon and breast cancer cells with *MTHFR* 677T variant had lower MTHFR activity than those with *MTHFR* 677C (17). In addition, mutant *MTHFR* 677T increased the sensitivity of cancer cells to the cytotoxicity of 5-fluorouracil (5-FU) (17). In null mouse experiments, the expression of *MTHFR* 677T enhanced the growth rates of xenografts compared to those expressing wild-type *MTHFR* 677C. Furthermore, consistent with the evidence from cell models, the 677T xenografts were also more sensitive to 5-FU treatment than those of 677C in a mouse model (17). Many studies investigating *MTHFR* genetic variants have found positive associations with various types of cancer, such as of breast (18, 19), oral (20, 21), lung (22) and prostate (23) cancer and leukemia (24).

In this study, we analyzed the genetic polymorphisms of both *MTHFR* C677T and A1298C using representative CRC samples (control/case=362/362) in order to examine the association between *MTHFR* genotypes and CRC in a Taiwanese population, and summarize the relevant and updated literature about CRC and *MTHFR* genotypes.

## Materials and Methods

*Investigated population*. The investigated population included 724 individuals (362 patients with CRC and 362 controls). Patients diagnosed with CRC were recruited at the outpatient clinics of general surgery at the China Medical University Hospital, Taichung, Taiwan by the team of Drs. LB Jeng and MD Yang. The clinical characteristics of patients, including histological details, were all graded and defined by expert surgeons as previous published (4, 10). All participants completed a self-administered questionnaire and provided a 5-ml sample of peripheral blood for genotyping. An equal number of non-cancer healthy volunteers (n=362) were selected as controls by matching for age, gender and some indulgences after initial random sampling from the Health Examination Cohort of the Hospital with the help of colleagues in the Department of Family Medicine. The exclusion criteria for the control group included previous malignancy, metastasized cancer from other or unknown origin and any familial or genetic diseases. This study was approved by the Institutional Review Board of the China Medical University Hospital (IRB project identification coding number: DMR99-IRB-108) and written informed consent was obtained from all participants with the help of Tissue Bank of China Medical University Hospital. Selected patient characteristics extracted from personal questionnaires is summarized in Table I.

Table I. *Summary of basic and clinical data of the 362 patients with colorectal cancer and 362 matched non-cancer healthy controls.*

| | Controls (n=362) | | Cases (n=362) | | |
|---|---|---|---|---|---|
| Characteristic | n | % | n | % | *p*-Value[a] |
| Age (years) | | | | | |
| ≤60 | 93 | 25.7% | 95 | 26.2% | |
| >60 | 269 | 74.3% | 267 | 73.8% | 0.8654 |
| Gender | | | | | |
| Male | 209 | 57.7% | 203 | 56.1% | |
| Female | 153 | 42.3% | 159 | 43.9% | 0.6525 |
| Smoking | | | | | |
| Non-smoker | 278 | 76.8% | 271 | 74.9% | |
| Smoker | 84 | 23.2% | 91 | 25.1% | 0.6525 |
| Drinking | | | | | |
| Non-drinker | 311 | 85.9% | 318 | 87.8% | |
| Drinker | 51 | 14.1% | 44 | 12.2% | 0.4410 |
| Tumor size (cm) | | | | | |
| <5 | | | 195 | 53.9% | |
| ≥5 | | | 167 | 46.1% | |
| Location | | | | | |
| Colon | | | 257 | 71.0% | |
| Rectum | | | 105 | 29.0% | |
| Lymph node metastasis | | | | | |
| Negative | | | 210 | 58.0% | |
| Positive | | | 152 | 42.0% | |

SD, Standard deviation. [a]Based on chi-square test without Yates' correction.

*Genotyping conditions*. The genomic DNA from peripheral blood leucocytes of each recruited subject was prepared with the QIAamp Blood Mini Kit (Blossom, Taipei, Taiwan) and stored at −80˚C until processed as per our recent publications (25-28). The methodology for *MTHFR* C677T (rs1801133) and A1298C (rs1801131) genotyping, including the designing of the specific primers and the selection of restriction enzymes, were performed at the Terry Fox Cancer Research Laboratory. Briefly, the sequences for forward and reverse primer pairs for *MTHFR* C677T were: forward 5'-TGA AGG AGA AGG TGT CTG

Table II. *Distribution of methylenetetrahydrofolate reductase (MTHFR) genotypes among the 362 patients with colorectal cancer and 362 matched healthy controls.*

| Genotype | Controls | | Patients | | OR (95% CI) | *p*-Value[a] |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| C677T rs1801133 | | | | | | |
| CC | 185 | 51.1% | 232 | 64.1% | 1.00 (Reference) | |
| CT | 134 | 37.0% | 108 | 29.8% | 0.64 (0.47-0.88) | 0.0064* |
| TT | 43 | 11.9% | 22 | 6.1% | 0.41 (0.24-0.71) | 0.0011* |
| $p_{trend}$ | | | | | | 0.0006* |
| Carrier comparison | | | | | | |
| CC +CT | 319 | 88.1% | 340 | 93.9% | 1.00 (Reference) | |
| TT | 43 | 11.9% | 22 | 6.1% | 0.48 (0.28-0.82) | 0.0063* |
| CC | 185 | 51.1% | 232 | 64.1% | 1.00 (Reference) | |
| CT+TT | 179 | 48.9% | 130 | 35.9% | 0.58 (0.43-0.78) | 0.0003* |
| A1298C rs1801131 | | | | | | |
| AA | 233 | 64.4% | 228 | 63.0% | 1.00 (Reference) | |
| AC | 111 | 30.6% | 117 | 32.3% | 1.08 (0.78-1.48) | 0.6462 |
| CC | 18 | 5.0% | 17 | 4.7% | 0.97 (0.49-1.92) | 0.9195 |
| $p_{trend}$ | | | | | | 0.8866 |
| Carrier comparison | | | | | | |
| AA+AC | 344 | 95.0% | 345 | 95.3% | 1.00 (Reference) | |
| CC | 18 | 5.0% | 17 | 4.7% | 0.94 (0.48-1.86) | 0.8624 |
| AA | 233 | 64.4% | 228 | 63.0% | 1.00 (Reference) | |
| AC+CC | 129 | 35.6% | 134 | 37.0% | 1.06 (0.78-1.44) | 0.6992 |

OR, Odds ratio; CI, confidence interval. [a]Based on Chi-square test without Yates' correction; *statistically significant.

Table III. *Distribution of allelic frequencies for methylenetetrahydrofolate reductase (MTHFR) among the 362 patients with colorectal cancer and 362 matched healthy controls.*

| Allele | Controls, n | % | Cancer patients, n | % | OR (95% CI) | *p*-Value[a] |
|---|---|---|---|---|---|---|
| C677T rs1801133 | | | | | | |
| C | 504 | 69.6% | 572 | 79.0% | 1.00 (Reference) | |
| T | 202 | 30.4% | 152 | 21.0% | 0.66 (0.52-0.84) | $4.32\times10^{-5}$* |
| A1298C rs1801131 | | | | | | |
| A | 577 | 79.7% | 573 | 79.1% | 1.00 (Reference) | |
| C | 147 | 20.3% | 151 | 20.9% | 1.03 (0.80-1.33) | 0.7949 |

[a]Based on Chi-square test without Yates' correction; *statistically significant.

CGG GA-3' and reverse 5'-AGG ACG GTG CGG TGA GAG TG-3'. The primers for *MTHFR* A1298C were: forward 5'- GGG AGG AGC TGA CCA GTG CAG-3' and reverse 5'-GGG GTC AGG CCA GGG GCA G-3'. The following cycling conditions were performed: 5 min of initial denaturation at 95˚C, 35 cycles of 30 s of denaturation at 95˚C, 30 s of annealing at 54˚C and 1 min of elongation at 72˚C, and 7 min of final extension at 72˚C. The 198-bp polymerase chain reaction (PCR) product of *MTHFR* C677T and 138-bp PCR product of *MTHFR* A1298C were subject to enzyme digestion with *Hinf* I and *Fnu4H* I (New England Biolabs, Beverly, MA, USA), respectively for 4 h and then visualized by ethidium bromide-stained 3% agarose gel electrophoresis under UV light. On digestion with Hinf I, the PCR product of *MTHFR* C677T arising from the C allele was uncut (198 bp), whereas the T allele was cut into fragments of 175 bp and 23 bp. On digestion with *Fnu4H* I, the PCR product of *MTHFR* A1298C arising from the A allele was uncut (138 bp), whereas the C allele was cut into fragments of 119 bp and 19 bp. All the genotypic processing was repeated by two researchers independently and blindly; all the genotyping results were 100% concordant.

*Statistical analyses*. Student's *t*-test was applied for the comparison of ages between the CRC case and the control groups. Pearson's chi-square test was applied to compare the distribution of the *MTHFR* genotypes among the subgroups. The associations between the *MTHFR* genotypes and CRC risk were estimated by computing odds ratios (ORs) and related 95% confidence intervals (CIs) from logistic regression analysis. Statistically, any difference at $p<0.05$ was taken as being significant between the two groups compared.

Table IV. *Odds ratios for methylenetetrahydrofolate reductase (MTHFR) C677T rs1801133 genotype and colorectal cancer risk 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 | | | |
| CC | 144 | 167 | 1.00 (ref) | 1.00 (ref) | | 41 | 65 | 1.00 (ref) | 1.00 (ref) | |
| CT | 102 | 86 | 0.73 (0.51-1.05) | 0.75 (0.50-1.11) | 0.0851 | 32 | 22 | 0.43 (0.22-0.85) | 0.47 (0.31-0.87) | 0.0135* |
| TT | 32 | 18 | 0.49 (0.26-0.90) | 0.52 (0.33-0.88) | 0.0201* | 11 | 4 | 0.23 (0.07-0.77) | 0.31 (0.14-0.73) | 0.0112* |
| Total | 278 | 271 | | | | 84 | 91 | | | |

CI, Confidence interval; [a]OR, adjusted odds ratio. [a]By multivariate logistic regression analysis; [b]by multivariate logistic regression analysis after adjusting for age, gender and alcohol drinking status; *statistically significant.

Table V. *Odds ratios for methylenetetrahydrofolate reductase (MTHFR) rs1801133 genotype and risk of colorectal cancer risk after stratification by alcohol drinking status.*

| Genotype | Non-drinker, 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 | | | |
| CC | 163 | 203 | 1.00 (ref) | 1.00 (ref) | | 22 | 29 | 1.00 (ref) | 1.00 (ref) | |
| CT | 111 | 95 | 0.69 (0.49-0.97) | 0.73 (0.54-0.93) | 0.0317* | 23 | 13 | 0.43 (0.18-1.03) | 0.41 (0.21-1.01) | 0.0564 |
| TT | 37 | 20 | 0.43 (0.24-0.78) | 0.48 (0.33-0.74) | 0.0042* | 6 | 2 | 0.25 (0.05-1.38) | 0.33 (0.11-1.21) | 0.0934 |
| Total | 311 | 318 | | | | 51 | 44 | | | |

CI, Confidence interval; [a]OR, adjusted odds ratio. [a]By multivariate logistic regression analysis; [b]by multivariate logistic regression analysis after adjusting for age, gender and smoking status; *statistically significant.

## Results

The frequency distribution of selected basic characters, including age, gender, smoking and alcohol drinking statuses for the 362 patients with CRC in the case group and 362 non-cancer healthy participants of the control group are summarized and compared in Table I. In addition, clinical characters include tumor size, location, and lymph node metastasis status of the patients are also summarized in Table I. Since we applied frequency matching to recruit non-cancer healthy individuals as controls, there was no difference in the distribution of age or gender between the control and case groups ($p$=0.8654 and 0.6525, respectively) (Table I). There was no difference in the distribution of smoking or drinking subpopulations between the control and case groups ($p$=0.6525 and 0.4410, respectively). There were 195 and 167 patients with tumor size <5 cm and ≥5 cm, respectively. Tumor was located in the colon in 257 patients and in the rectum in 105. There were 152 patients with and 210 without lymph node metastasis (Table I).

The distribution of *MTHFR* C677T and A1298C genotypes among the 326 non-cancer controls and the 326 patients with CRC are presented and statistically analyzed in Table II. The results showed that the genotypes of *MTHFR* C677T were significantly differently distributed between case and control groups ($p$ for trend=0.0006) (Table II). In detail, the *MTHFR* C677T the heterozygous CT and homozygous TT were associated with lower CRC risk than the wild-type CC genotype ($p$=0.0064 and 0.0011, respectively; Table II). In the recessive model, there was a significant association between the TT genotype of *MTHFR* C677T and CRC risk, compared with CC+CT genotypes ($p$=0.0063; Table II). In the dominant model, there was also a significant association between the CC genotype of *MTHFR* C677T and CRC risk, compared with CT+TT genotypes ($p$=0.0003; Table II). The genotypes of *MTHFR* A1298C were not differentially distributed between case and control groups in any model (Table II).

To confirm the results shown in Table II, analysis of allelic frequency distribution for the *MTHFR* C677T and A1298C SNPs was further conducted and the results are presented in Table III. Supporting the findings that genotype of *MTHFR* C677T was associated with CRC risk, the variant allele T was found at a frequency of 21.0% in the patient group, significantly lower than that of 30.4% in the non-cancer healthy control group ($p=4.32\times10^{-5}$, Table III). On the contrary, there was no significant difference in the allelic frequencies of *MTHFR* A1298C between the case and control groups (Table III).

Since smoking and alcohol drinking habits are well-known risk factors for CRC in Taiwan, we were interested in investigating the interactions between the novel genetic marker of *MTHFR* C677T and personal cigarette smoking and alcohol

drinking habits. Among non-smokers, those with TT genotype at *MTHFR* C677T were at 0.49-fold odds of having CRC ($p$=0.0201), conferring a protective effect, compared with those with CC genotype (Table IV). After adjusting for age, gender and alcohol drinking status, statistical significance still existed at a similar level ($p$=0.0112; Table IV). The protective effects are very similar to those with CT and TT at *MTHFR* C677T among smokers (Table IV). On the other hand, among non-drinkers, those with CT and TT genotypes at *MTHFR* C677T were at 0.69 and 0.43-fold odds of having CRC ($p$=0.0317 and 0.0042, respectively), conferring a protective effect, while this was not the case for ever drinkers (Table V). After adjusting for age, gender and smoking status, results were equally significant (Table V).

## Discussion

In literature, the genotypes of *MTHFR* may be associated with prognosis of chemotherapy in patients with CRC (29). Similar to the report of Robien and colleagues mentioned in the introduction, people carrying the common T variant of *MTHFR* genotypes have reduced MTHFR enzyme activity, ~50% that of normal, with subsequently increased plasma homocysteine concentrations (30). The first study to evaluate the association between *MTHFR* C677T polymorphism and CRC was performed by Chen and colleagues, reporting that the *MTHFR* C677T variant affected enzyme activity and was involved in abnormal methylation as well as influencing DNA synthesis, leading to colorectal tumorigenesis (31). In 2001, Junker *et al*. also reported that the MTHFR enzyme activity with the TT and CT genotypes were 30% and 65% of that associated with the CC genotype (32). In the present study, the relative percentages of *MTHFR* C677T taints were all significantly lower in patients with CRC compared with those who were healthy controls; however, such a difference in genotypic distribution was not observed for *MTHFR* A1298C (Table II). In addition, a lower percentage of individuals bearing the T allele at *MTHFR* C677T was observed in patient group than that in control group (Table III). Furthermore, the genotypes of *MTHFR* C677T may conduct its protective influence for both smokers and non-smokers, and those non-drinkers (Table V).

Despite our efforts to conduct accurate and comprehensive genotyping and related analysis, there are some limitations that should be noted. Firstly, the lack of recorded follow-up limited the analysis of the correlation of prognostic indices, such as survival rates. From the viewpoint of prognosis, Wu and colleagues found that TT-homozygosity at *MTHFR* C677T was significantly associated with higher survival rates for patients with CRC (29, 33). Secondly, lack of collection of both tumor and non-tumor samples limited the study of differential expression of *MTHFR* mRNA and protein levels among the participants, in addition to the inter-individual difference of the patients with CRC. Further molecular investigations of the genotype–phenotype correlation may help in understanding the contribution of *MTHFR* C677T genotype not only to overall cell proliferation, DNA neo-synthesis and DNA repair capacity, but also regarding personal susceptibility to CRC and other types of cancer. From the viewpoint of folate metabolism, the serum monitoring of folate levels should be taken into consideration by patient care systems since Chiang *et al*. reported that higher serum folate concentration was significantly correlated with increased CRC risk in individuals with adenomatous polyps, while it had no effect on CRC risk in healthy controls (34). Thirdly, the relatively small sample size, especially in subgroup analysis, such as those shown in Tables IV and V, may have caused bias and reduced the statistic power of our estimates. The strongest evidence supporting our findings comes from meta-analyses showing that *MTHFR* 677 homozygous mutation TT genotype was revealed to be a protective genotype for CRC with statistical significance in a pooled population (35).

In conclusion, this study provides evidence that the T allele at *MTHFR* C677T rs1801133 may serve as a protective factor which interacts with smoking and alcohol habits to determine personal susceptibility to CRC. At the same time, overall monitoring of alterations of cell proliferation, DNA neo-synthesis, DNA repair capacity and folate metabolism for each individual according to their *MTHFR* genotype may help us provide a better quality care and prognosis for patients with CRC.

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