Much attention has recently been paid to a possible protective effect of folate in colorectal carcinogenesis. High dietary intake of folate and high levels of plasma or serum folate have been associated with decreased risk of colorectal cancer and adenomas.\(^1,2\) Methylenetetrahydrofolate reductase (MTHFR) plays a pivotal role in folate metabolism, and MTHFR C677T polymorphism determines the activity of the enzyme.\(^3\) The MTHFR mutant homozygous TT genotype was shown to be associated with a decreased risk of colorectal cancer among those with high folate intake.\(^1,2\) On the other hand, studies of colorectal adenomas reported an increased risk associated with the MTHFR TT genotype when folate intake was low and when red blood cell (RBC) or plasma folate levels were low.\(^4-6\)

Previously, we reported that the MTHFR polymorphism was unrelated to colorectal adenoma but there was a small increase in risk for the combination of TT genotype and high alcohol consumption.\(^7\) In this study, we examined the association between plasma folate levels and colorectal adenomas by MTHFR genotype in an Asian population.

### Methods

The study subjects were men aged 47–55 years who received a pre-retirement health examination at the Self Defense Forces (SDF) Fukuoka Hospital from January 1995 to December 1996.
FOLATE, MTHFR, AND COLORECTAL ADENOMAS

or the SDF Kumamoto Hospital from May to December 1996. Screening colonoscopy was a routine procedure in the health examination. Details of the health examination have been described elsewhere. In the consecutive series of 803 men, 778 underwent colonoscopy, and there were 223 cases of histologically confirmed colorectal adenomas and 242 controls with normal total colonoscopy. We excluded 14 cases and 12 controls because they had a prior history of colorectomy, colorectal polyp-ectomy or malignant neoplasms, and a further 32 cases and 38 controls because plasma samples were not available. Finally, 177 cases and 192 controls remained in the analysis.

After an overnight fast, a sample of 7 ml of venous blood was taken for the purpose of research with written informed consent. The MTHFR genotype was determined by the polymerase chain reaction/restriction fragment length polymorphism method, as described previously. Plasma folate concentrations were determined by the chemiluminescent enzyme immuno-assay at an external laboratory (CRC, Inc., Fukuoka, Japan) using commercial reagents (Bayer Medical Co Ltd., East Walpole, MA, USA). A self-administered questionnaire ascertained alcohol use, smoking habit, and other lifestyle characteristics.

Comparison of plasma folate levels was done by t-test following analysis of covariance. Because the distribution of plasma folate levels was skewed to the right, the log-transformed values were used. Logistic regression analysis was used to calculate odds ratios (OR) and 95% CI. Plasma folate levels were dichotomized at the lower tertile (5.50 ng/ml) in the distribution of the controls; this cutoff point was determined a priori so as to distinguish a group with low folate levels. Statistical adjustment was made for hospital, rank in the SDF (three classes), alcohol use (never, past, and current drinking with a consumption of <30, 30–59, or ≥60 ml of alcohol per day), smoking (0, 1–399, 400–799, and ≥800 cigarette-years), and body mass index (kg/m²). Age was not taken into account because the age range was limited to 47–55 years. The interaction was evaluated by a likelihood ratio test. All statistical computations were done by SAS version 6.12 (SAS Institute, Inc., Cary, North Carolina, USA).

Results

Plasma folate levels were slightly lower among adenoma cases than among controls; adjusted geometric means for cases and controls were 6.29 and 6.58 ng/ml, respectively (P = 0.33). Adjusted OR for high (>5.50 ng/ml) versus low folate levels was 0.72 (95% CI: 0.46–1.14). In both cases and controls, plasma folate levels were lower among those with the TT genotype (Table 1). Among men with the TT genotype, adenoma cases had significantly lower folate levels than controls.

Adjusted OR for the CT and TT genotypes as compared with the CC genotype were 0.87 (95% CI: 0.55–1.39) and 1.20 (95% CI: 0.60–2.42), respectively. While there was no material difference in the adjusted OR between low and high folate levels among men with at least one wild allele, the OR increased by approximately twofold for low folate level and decreased by 40% for high folate level among men with the TT genotype as compared with the group of the CC/CT genotypes and low folate level (Table 2).

Table 1  Plasma folate concentrations (ng/ml) according to methylenetetrahydrofolate reductase (MTHFR) genotypes in adenoma cases and controls

<table>
<thead>
<tr>
<th>MTHFR genotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Adjusted geometric mean (95% CI)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Controls</th>
<th>P-value for difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC</td>
<td>6.45 (5.84–7.12)</td>
<td>7.23 (6.37–7.95)</td>
<td>0.11</td>
</tr>
<tr>
<td>CT</td>
<td>6.55 (5.98–7.17)</td>
<td>6.24 (5.71–6.22)</td>
<td>0.19</td>
</tr>
<tr>
<td>TT</td>
<td>4.92 (4.19–5.77)</td>
<td>6.19 (5.14–7.46)</td>
<td>0.045</td>
</tr>
</tbody>
</table>

<sup>a</sup> MTHFR genotype: wild-type homozygotes (CC), heterozygotes (CT), and mutant homozygotes (TT).

<sup>b</sup> Adjusted for hospital, rank in the Self Defense Forces, alcohol use, smoking, and body mass index.

<sup>c</sup> Based on analysis of covariance for the between-genotype variation.

Table 2  Adjusted odds ratios (OR) and 95% CI of colorectal adenomas according to methylenetetrahydrofolate reductase (MTHFR) genotypes and plasma folate levels

<table>
<thead>
<tr>
<th>MTHFR genotype&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Plasma folate&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of cases</th>
<th>No. of controls</th>
<th>Adjusted OR (95% CI)&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>CC + CT</td>
<td>Low</td>
<td>55</td>
<td>56</td>
<td>1.00 (reference)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>96</td>
<td>115</td>
<td>0.86 (0.52–1.41)</td>
</tr>
<tr>
<td>TT</td>
<td>Low</td>
<td>18</td>
<td>8</td>
<td>2.13 (0.82–5.54)</td>
</tr>
<tr>
<td></td>
<td>High</td>
<td>8</td>
<td>13</td>
<td>0.58 (0.21–1.61)</td>
</tr>
</tbody>
</table>

<sup>a</sup> MTHFR genotype: wild-type homozygotes (CC), heterozygotes (CT), and mutant homozygotes (TT).

<sup>b</sup> Low: ≤5.5 ng/ml, high: >5.5 ng/ml.

<sup>c</sup> Adjusted for hospital, rank in the Self Defense Forces, alcohol use, smoking, and body mass index.
genotypes combined. These findings are consistent with results from previous studies in Western populations.\(^1\)\(^2\)\(^4\)\(^6\)

The mechanisms underlying the differential associations between folate levels and colorectal adenomas by MTHFR genotype are rather complex. The folate metabolic pathway is involved in both DNA methylation and de novo nucleotide synthesis.\(^1\)\(^2\)\(^3\) In the presence of high folate levels, the low activity of MTHFR of the TT genotype may be advantageous in terms of nucleotide synthesis due to increased availability of 5,10-methylenetetrahydrofolate, the substrate of MTHFR. On the other hand, under low folate levels, DNA hypomethylation may be more likely to occur because methionine synthesis is decreased due to the lower levels of 5-methyltetrahydrofolate associated with the TT genotype.\(^1\)\(^3\)

There were several limitations in the present study. A single measurement of plasma folate may not be a good indicator of long-term folate intake. Plasma folate levels are affected by the MTHFR genotypes, and thus the use of plasma folate may mask the true association between folate intake and adenomas. We did not ascertain the MTHFR A1298C polymorphism, which is also associated with the enzyme activity\(^9\) and may modify the folate–adenoma relation. Finally, it should be noted that the study subjects were men who had served in the SDF until retirement, and it may be difficult to generalize the present findings to all Japanese men.

**Acknowledgements**

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**KEY MESSAGES**

- It has been suggested, mainly in Western populations, that folate intake may be protective in the development of colorectal cancer and adenomas and that the protective association may differ by genetic polymorphism of methylenetetrahydrofolate reductase (MTHFR), which is a key regulatory enzyme in folate metabolism.
- We examined the relation between plasma folate levels and colorectal adenomas with reference to the effect modification by MTHFR (C677T) polymorphism in 177 cases of colorectal adenomas and 192 controls with normal total colonoscopy in middle-aged Japanese men.
- There was a suggestive, protective association between plasma folate levels and colorectal adenomas in men with the TT genotype, while no such association was observed among those with the CC or CT genotype.
- The findings add to increasing evidence for the interaction between folate and the MTHFR genotype in colorectal carcinogenesis.

**References**


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