Abstract. The 5,10-methyl-tetrahydrofolate reductase (MTHFR) enzyme plays a critical role in folate and homocysteine metabolism, and its gene, MTHFR, displays common genetic polymorphisms that influence its activity. Clinical implications of MTHFR polymorphisms have been reported for several diseases, including a variety of solid tumors such as colorectal cancer (CRC). Here, the role of the 677C→T polymorphism of MTHFR was evaluated by genotyping 369 patients and 170 healthy controls from the Mexican population. The observed genotype frequencies for the controls and patients, respectively, were: 18.8% and 32% for 677TT; 34.7% and 34% for 677CC; 46.4% and 34% for 677CT. The odds ratio (OR) was 2.0 (95% confidence intervals CI; 1.3-3.3) (p<0.05). The data suggested that the 677C→T polymorphism in MTHFR contributes significantly to the risk of CRC susceptibility in the Mexican population.

Colorectal cancer (CRC) is one of the leading causes of death in American countries (1). CRC invariably shows a progression through several clinical and histopathological steps that are the outcome of genetic changes involving the activation of oncogenes or inactivation of tumor-suppressor genes (2). To date, little is known about the etiology of CRC. Nonetheless, genetic susceptibility and environmental risk factors have recently been described (2). Folates are involved in a large number of biochemical processes and genetic or dietary deficiencies of these vitamins lead to hyperhomocysteinemia. This leads to the impairment of several cellular functions, such as cytokine expression, the bioavailability of nitric oxide, the induction of oxidative stress, apoptosis activation, DNA methylation and nucleotide synthesis (3, 4). A relationship between plasma folate levels, content of uracil, and DNA damage in dividing cells renders MTHFR a suitable candidate for studies of CRC susceptibility (5-7). Specifically, 5,10-methyl-tetrahydrofolate reductase (MTHFR) plays an important role in cellular DNA methylation, catalyzing the conversion of 5, 10-methyl-THF to 5-methyl-THF. It also has an important regulatory function in folate metabolism, where it directs the folate pool to remethylate homocysteine at the expense of DNA and RNA synthesis (8, 9). The single nucleotide polymorphism g.677C→T (p. Ala222Val) of the MTHFR gene encodes a thermolabile variant of the protein that reduces its global activity by 50% (10).

Evidence of an association between the g.677C→T and g.1298A→C MTHFR polymorphisms and an increased risk of developing malignancies (including CRC) has recently been reported (11-14). Osiyan et al. reported that individuals with the g.677TT and g.1298CC polymorphisms in MTHFR had an increased risk of CRC (15). Rayn et al. found a high frequency of the T allele in CRC patients (16), and Urlich et al. found a novel mechanism by which MTHFR polymorphisms can affect the risk of CRC (17). More recently, studies aiming to establish correlation between C677T polymorphism and CRC yield not conclusive data, because some of them suggest this allele increase risk for CRC (18, 19), but other authors conclude C677T does not affect this risk (20).

It has also been observed that the frequency of the 677C→T polymorphism varies among diverse ethnic groups. For example, the frequencies of 677TT in the Dutch and Canadian populations are 8.5% and 12%, respectively (21, 22). Several reports studying the general Mexican population have estimated it, as one of the highest homozygote...
prevalences of the 677TT polymorphism (23-26). However, there are no descriptions of an association of the MTHFR 677C→T polymorphism and adult Mexican individuals affected with CRC. Here a possible role for the 677C→T polymorphism of MTHFR, as a risk factor for the development of CRC in the Mexican population, is reported.

Materials and Methods

DNA was extracted by standard protocols (27) from peripheral blood lymphocytes collected from 170 healthy individuals and 369 patients with CRC from the metropolitan area of Guadalajara and its surroundings. The patients were recruited from January 2006 to July 2008; healthy individuals were recruited from volunteer blood donors. All the samples were taken after obtaining appropriate written informed consent. Efforts were taken to ensure that siblings of those already sampled were excluded. Clinical and demographical data were obtained by questionnaire; all the patients were interviewed to investigate occupational exposure and chemotherapy agent use was recorded. Clinical and demographical data were obtained by questionnaire; all the patients were interviewed to investigate occupational exposure and chemotherapy agent use was recorded. MTHFR gene amplification was performed by PCR using the following primers: 5'–GTTGAGGTTGCTGAAAGG–3' and 5’–CAAAGAAAAGCTGCGTGATG–3'. The reactions were performed in a total PCR volume of 15 μL containing 200 μM dinucleotide triphosphates (dNTPs) (Invitrogen, Los Angeles, CA, USA), 2.5 pmol of primers, 3.0 mM MgCl₂, and 1.5 U Taq polymerase (Invitrogen, Los Angeles, CA, USA). The PCR conditions were 94°C (1 min), 57°C (1 min), and 72°C, (1 min). By this procedure a fragment of 158 bp was obtained. For allele discrimination, the amplified product was subjected to restriction enzyme analysis with HinfI (New England Biolabs, Beverly, MA, USA) according to the manufacturer’s instructions. The samples were separated using 6% polyacrylamide electrophoresis gels (29:1), followed by silver staining (28). This revealed the 158 bp fragment for the wild type, three fragments of 158, 130, and 28 bp for the heterozygous, and two fragments of 130 and 28 bp for the homozygous variants.

The allele frequencies were obtained by direct counting. The Hardy-Weinberg equilibrium was tested by a goodness-of-fit Chi-square test to compare the observed genotype frequencies with the expected frequencies among the control subjects. Odds ratios (OR) and 95% confidence intervals (CI) were also calculated. A two-sided p<0.05 was assumed to be statistically significant. All the statistical analyses were performed with the Statistical Analysis System software SSPS 13.0 (Chicago, IL, USA).

Results

Table I shows the comparative epidemiological data for the CRC patients and controls. In the CRC group, the observed average age was 59.26 years (range: 18 to 91 years). Sixteen (59/369) of these patients had a positive family history for cancer. The interaction of the genotype g.677C→T between the patients and controls showed statistically significant differences with male gender (OR 2.5 [CI 95% 1.3-5.2]; p<0.005), age (the stratified group ≥50 years old, based on the average of CRC symptom onset from the literature) (OR 2.1 [CI 95% 1.2-4.0];
Table III. Genotype distribution of the 677C→T MTHFR polymorphism in CRC patients with respect to gender, age, smoking status and family history.

<table>
<thead>
<tr>
<th>Gender</th>
<th>Patients (n=369)</th>
<th>Controls (n=170)</th>
<th>(CI 95% Low-high)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>Male</td>
<td>Female</td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td>677TT</td>
<td></td>
<td>48</td>
<td>69</td>
<td>18</td>
</tr>
<tr>
<td>*677CT +677CC</td>
<td>122</td>
<td>130</td>
<td>75</td>
<td>63</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;50 years</td>
<td>≥50 years</td>
<td>&lt;50 years</td>
<td>≥50 years</td>
</tr>
<tr>
<td>677TT</td>
<td></td>
<td>23</td>
<td>94</td>
<td>13</td>
</tr>
<tr>
<td>*677CT +677CC</td>
<td>63</td>
<td>189</td>
<td>59</td>
<td>79</td>
</tr>
</tbody>
</table>

Smoking status

<table>
<thead>
<tr>
<th>patients</th>
<th>smoking status</th>
<th>non-smoking</th>
<th>smoking</th>
<th>ex-smoking</th>
<th>non-smoking</th>
<th>smoking</th>
<th>ex-smoking</th>
<th>OR (CI 95% Low-high)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>677TT</td>
<td></td>
<td>41</td>
<td>57</td>
<td>19</td>
<td>18</td>
<td>9</td>
<td>5</td>
<td>3.0 (1.4-7.4)</td>
<td>0.005</td>
</tr>
<tr>
<td>*677CT +677CC</td>
<td>93</td>
<td>124</td>
<td>35</td>
<td>61</td>
<td>58</td>
<td>19</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
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Family history

<table>
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<tr>
<th>patients</th>
<th>yes</th>
<th>no</th>
<th>yes</th>
<th>no</th>
</tr>
</thead>
<tbody>
<tr>
<td>677TT</td>
<td>22</td>
<td>97</td>
<td>3</td>
<td>29</td>
</tr>
<tr>
<td>*677CT +677CC</td>
<td>37</td>
<td>213</td>
<td>11</td>
<td>127</td>
</tr>
</tbody>
</table>

*This reference category combined the two genotypes, because the odds ratio (OR) showed an equal risk for each group. CI: confidence interval, 
1Male patients vs. male controls, 2≥50 years patients vs. ≥50 years controls, 3smoking patients vs. smoking controls, 4Non-familial antecedents of cancer patients vs. No familial antecedents of cancer controls.

Table IV. Genotype distribution of the 677C→T MTHFR polymorphism in CRC patients with respect to gender, age, smoking status, and familial history of cancer combined.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Controls</th>
<th>OR (CI 95% Low-high)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>677TT, male, ≥50 years, smoker, non familial history of cancer</td>
<td>18</td>
<td>7</td>
<td>10.4 (3.59-32.2)</td>
</tr>
<tr>
<td>677CC, 677CT, female, &lt;50 years, non-smokers, family history of cancer</td>
<td>101</td>
<td>25</td>
<td></td>
</tr>
</tbody>
</table>

Table V. Multivariate logistic regression analysis of the study groups.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Beta</th>
<th>SE</th>
<th>Wald</th>
<th>df</th>
<th>Significance</th>
<th>OR</th>
<th>Lower</th>
<th>Upper</th>
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<tbody>
<tr>
<td>677TT/677TT</td>
<td>1.2</td>
<td>0.265</td>
<td>22.41</td>
<td>1</td>
<td>0.000</td>
<td>3.5</td>
<td>2.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Age stratified</td>
<td>1.5</td>
<td>0.246</td>
<td>38.85</td>
<td>1</td>
<td>0.000</td>
<td>4.6</td>
<td>2.8</td>
<td>7.5</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.5</td>
<td>0.263</td>
<td>33.86</td>
<td>1</td>
<td>0.000</td>
<td>4.6</td>
<td>2.7</td>
<td>7.7</td>
</tr>
<tr>
<td>Family history of cancer</td>
<td>0.95</td>
<td>0.35</td>
<td>7.4</td>
<td>1</td>
<td>0.007</td>
<td>2.6</td>
<td>1.3</td>
<td>5.1</td>
</tr>
</tbody>
</table>

p<0.01), tobacco consumption (OR 3.0 [CI 95%: 1.4-7.4]; p<0.005), and positive family history of cancer (OR 2.0 [CI95%: 1.2-3.3]; p<0.005). In the homozygous g.677TT group, an increased risk of developing CRC was detected. Table IV reveals the increased risk of CRC for patients with the g.677TT genotype along with concomitant variables (gender, age, smoker status, and no familial antecedent of cancer) (OR 10.4 [CI 95%: 3.59-32.2] p<0.001). A multivariate logistic regression analysis, in which the groups were considered to be the dependent variable, was
carried out with the following independent variables: g.677TT genotype, stratified age, tobacco consumption and family history of cancer (Table V).

Discussion

In Mexico, as well as many other countries around the world, CRC incidence has increased over the last 50 years. In the present study, 77% of the patients were ≥50 years, consistent with internationally described reports in which the incidence of colon cancer is higher in people over the age of 50 years (29, 30). This may be due to changes in the lifestyle in the Mexican population, such as diet, constant exposure to toxic substances, additives and contaminants. These lifestyle changes, combined with longevity, contribute to CRC incidence in this country.

A slight predominance of men (54%) compared with women (46%) was observed in the CRC group. This was again consistent with findings described in the literature that report an expected men:women ratio of 1.2:1 (31, 32). Recent studies have suggested that the high incidence in men is due to lower participation in the diagnosis of CRC in comparison to women. With regard to the location, gender differences with regard to the proportion of patients with cancer of the distal colon and rectal cancer were observed; these were lower in women than men (33-35). Overall, left CRC was most frequently observed (56%), followed by a rectosigmoidal (37%) and right (7%) localization. Some reports have suggested that the anatomical distribution of CRC is biased in terms of proximal to distal localization. This is probably the result of preventive measures and new techniques and approaches that permit better detection of CRC now being used in developed countries with a high incidence of this disease (36).

With regard to family history of cancer, 16% of the patients had a positive history had cancer (3% of these had grade I CRC, whereas the rest had other types of cancer, data no showed). The frequency was consistent with the overall rates reported in the literature for cancer in control and CRC families (37). Tobacco consumption has been associated with the development of CRC in different studies (38-42), and the present results were consistent with this association. A significant difference was observed in cigarette smokers (49%) compared to the controls (39%) and it has been postulated that tobacco consumption causes chromosomal instability (43).

Little attention has been paid to the role of genetic susceptibility and environmental exposure in the etiology of CRC. In particular, people with an altered ability to activate pro-carcinogens and detoxify carcinogens may have an increased risk of developing cancer (44). Several reports have suggested that reduced activity of MTHFR may decrease the methylation of homocysteine to methionine and in turn the level of S-adenosyl methionine (SAM), resulting in DNA hypomethylation (17, 45). On the other hand, the reduced level of the MTHFR substrate 5,10-methylene-THF, which is required for thymidylate synthesis, could lead to uracil misincorporation into DNA, diminished DNA repair, and an increased frequency of chromosomal breaks and damage (46).

In the present study, a significant difference in the distribution of the 677TT genotype between the CRC patients and the healthy controls was observed (OR 2.0 CI 95% 1.3-3.3; p<0.05). This association was most evident in the patients with a genotype of 677TT/677TT who were male, ≥50 years old, tobacco consumers and not positive for a family history of cancer (OR 2.5 95% CI 1.3-5.2; 2.1 95% CI 1.2-4.0; 3.0 95% CI 1.4-7.4 and 2.0 95% CI 1.2-3.3, respectively; p<0.05). Moreover, analyzing these variables together in patients with the genotype 677TT revealed a strong association with an OR of 10.39 (95% CI 3.59-32.19, p<0.001).

The association between the 677C>T polymorphism and CRC is a contradictory issue; some studies have described a reduction in CRC risk in individuals with the 677TT genotype in comparison to the 677CT and 677CC genotypes. However, it has been shown that this association is influenced by adequate dietary folate intake (44, 45). Other studies suggested an increased risk of CRC in male patients with the 677TT genotype who exhibit high alcohol consumption or poor dietary folate intake (46, 47), suggesting that the risk of CRC conferred by the 677TT polymorphism in MTHFR is related to individual differences in age, gender, diet, alcohol and smoking habits. In this context, it is possible that MTHFR polymorphisms may mediate CRC risk with respect to low folate intake. Unfortunately, the folate status and dietary intake of the individuals were not analyzed in this study. Nonetheless, an emphasis on appropriate dietary folate intake for reducing CRC risk in older men and smokers with the 677TT genotype might be advisable in our populations.

In conclusion, that the 677TT genotype of the MTHFR gene shows significant differences between controls and CRC patients. These differences suggest that the polymorphism may serve as a good marker for men with CRC.

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References


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