# Methylenetetrahydrofolate Reductase Genotypes, Dietary Habits and Susceptibility to Stomach Cancer

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**OBJECTIVE** To study the relation among methylenetetrahydrofolate reductase (MTHFR) C677T genotypes, dietary habits and the risk of stomach cancer (SC).

**METHODS** A case-control study was conducted with 107 cases of SC and 200 population-based controls in Chuzhou district, Huaian, Jiangsu province, China. The epidemiological data were collected, and DNA of peripheral blood leukocytes was obtained from all of the subjects. MTHFR genotypes were detected by PCR-RFLP.

**RESULTS** (1) The prevalence of the MTHFR C/T or T/T genotypes was found to be significantly different between controls (68.5%) and SC cases (79.4%, $P$=0.0416), the increased risk had an adjusted OR of 1.79 (95%CI: 1.01-3.19). (2) Among subjects who had a low intake of garlic or Chinese onion, MTHFR C/T or T/T genotypes significantly increased the risk of developing SC. Among non-tea drinkers or among subjects who had a frequent intake of meat, the carriers of the MTHFR C/T or T/T genotypes had a higher risk of SC than individuals with the C/C type MTHFR.

**CONCLUSION** The polymorphism of MTHFR C677T was associated with increased risk of developing SC, and that individuals with differing genotypes may have different susceptibilities to SC, based on their exposure level to environmental factors.

**KEYWORDS: methylenetetrahydrofolate reductase genotypes, dietary habits, stomach cancer.**

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in the metabolism of folate, an enzyme which contributes to DNA methylation and DNA synthesis. MTHFR converts 5,10-methylenetetrahydrofolate(a donor for methylating dUMP to dTMP in DNA synthesis) to 5-methytetrahydrofolate (a primary methyl donor for the remethylation of homocysteine to methionine).[1,2] The MTFHR gene is located on chromosome 1p36.3 and contains 11 exons. A common polymorphism of this gene(MTFHR C677T) associated with reduced MTHFR activity has been identified. MTFHR C677T is located in exon 4 at the folate binding site, converting an alanine into a valine residue in the enzyme. Individuals with the variant MTHFR T/T genotype have ~30% of the in vitro enzyme activity seen in those with the C/C wild genotype. Heterozygotes (C/T) show ~65% of normal enzyme activity. [3] A MTHFR C677T mutation leads to decreased formation of 5-methyltetrahydrofolate, and parallel increased homocysteine, 5,10- methylenetetrahydrofolate plasma level and DNA hypomethylation.[4,5] Thus, MTHFR C677T mutation may

play a role in the etiology of cancer via effects on DNA methylation and nucleotide synthesis.

Stomach cancer is one of the most common cancers in China. The etiology of stomach cancer is not well established, although nutritional, microbial and genetic factors have been suggested in a multi-step and multifactorial process. Because MTHFR is an enzyme in folate metabolism and folates may be supplied by food, we hypothesize that dietary habits may be modify the relation between MTHFR C677T polymorphism and the risk of stomach cancer. To clarify the association between MTHFR C677T polymorphism, dietary habits and the risk of stomach cancer, we performed a population-based case-control study in Huaian, Jiangsu, China.

## MATERIALS AND METHODS

### Study subjects and sample collection

We recruited cases from patients who visited Chuzhou District Municipal Hospital of Huaian City, and who were histopathologically diagnosed as having primary stomach cancer from September, 1999 to September, 2000. Physicians at the hospital asked eligible patients to participate in our study, and doctors or nurses interviewed the subjects and collected their blood samples from a peripheral vein after obtaining oral informed consent. The questionnaire elicited information about subjects' smoking status, alcohol drinking, tea consumption and dietary habits before the onset of disease. Population-based controls were selected from healthy residents in the villages or towns where the patients resided, using regisration records of the local government. The data were organized after adjustment for gender, ethnicity and age (grouped according to 5 years).

Doctors at public health centers asked eligible residents for their participation, and preformed interviews and blood collection in the same way. The eligible subjects comprised 107 patients with stomach cancer (84 males and 23 females, mean age ± SD: 61.35 ± 7.75 years and 60.17 ± 9.89 years, respectively) and 200 controls (132 males and 68 females, mean age ± SD: 60.76 ± 8.46 years and 58.72± 9.54 years, respectively). The distributions of age and sex showed no significant difference between patients and controls.

### DNA extraction and genotyping of the MTHFR C677T

Whole blood was collected into EDTA-coated tubes, centrifuged for 15 min, and the buffy coat layer isolated. Genomic DNA was extracted from 200 μl of the buffy coat layer using a Qiagen QIAamp DNA Blood Mini Kit. MTHFR C677T genotypes were determined by the PCR-RFLP method. The primers were 5'-TGAAGGAGAAGGTGTCTGCGGGA-3' and 5'-AGGACGGTGCGGTGAGAGTG-3'.

Reactions were carried out in a total volume of 25 μl containing each primer 0.5 μl (25pmol/μl); 4× dNTP 2.5 μl; *Taq* DNA polymerase, 1 unit; genomic DNA 1μl ; 10 × buffer (containing $MgCl_2$) 2.5 μl . The cycling conditions were: initial melting at 94℃ for 2 min, then 40 amplification cycles of 94℃ for 30 s, 62℃ for 30 s, 72℃ for 30 s, and 72℃ for 7min. After amplification, the PCR product was digested with HinfI restriction enzyme at 37℃ for 3 hr. The digestion products were analysed by electrophoresis in 3% agarose gels containing ethidium bromide. Wild-type (C/C) individuals were identified by only a 198-bp fragment, heterozygotes (C/T) by both the 175 and 198-bp fragments, and homozygote variants (T/T) by the 175-bp fragment.

### Statistical analysis

The SAS statistical package was used for data analysis. Associations between the MTHFR C677T polymorphism and stomach cancer risk were estimated by OR, using the unconditional logistic regression model. ORs were adjusted for age (continuous), sex, smoking and drinking habits, and consumption of some foods to control for the effects of potential confounding factors.

## RESULTS

### Distribution of MTHFR genotypes in patients and controls

The frequency of MTHFR variant genotypes (C/T+T/T) among the patients (79.4% ) was significantly higher compared with the controls (68.5% , $\chi^2$=4.15, $P$=0.0416). Using MTHFR C/C genotype as a referent, MTHFR C/T or T/T genotype were associated with an increased OR for stomach cancer. The crude OR was 1.78 (95% CI: 0.99-3.22). After adjustment for sex, age, smoking and alcohol drinking, the OR was 1.79 (95% CI: 1.01-3.19) (Table 1).

### MTHFR genotypes and the risk of stomach cancer among individuals with different dietary habits

Among non-tea drinkers,individuals with the MTHFR

**Table 1. MTHFR C677T genotypes and risk for stomach cancer**

| MTHFR genotypes | Controls | | Cases | | $OR_1$(95%CI) | $OR_2$(95%CI) |
|---|---|---|---|---|---|---|
| | n | % | n | % | | |
| C/C | 63 | 31.5 | 22 | 20.6 | 1.00 | 1.00 |
| C/T | 99 | 49.5 | 61 | 57.0 | 1.76(0.95~3.29) | 1.94(1.05~3.59) |
| T/T | 38 | 19.0 | 24 | 22.4 | 1.81(0.84~3.89) | 1.29(0.88~1.88) |
| C/T+T/T | 137 | 68.5 | 85 | 79.4 | 1.78(0.99~3.22) | 1.79(1.01~3.19) |

$OR_1$: Not adjusted; $OR_2$: Adjusted for sex, age and habits of smoking and alcohol drinking.

**Table 2. Association between polymorphism of MTHFR C677T and susceptibility to stomach cancer according to dietary habits**

| Dietary habits | MTHFR | Controls(n) | Cases(n) | $OR_1$ | 95%CI | $OR_2$ | 95%CI |
|---|---|---|---|---|---|---|---|
| Tea drinking | | | | | | | |
| drinking | C/C | 34 | 13 | 1.00 | | 1.00 | |
| | C/C+T/T | 69 | 32 | 1.21 | 0.53~2.80 | 0.98 | 0.37~2.56 |
| no drinking | C/C | 29 | 9 | 1.00 | | 1.00 | |
| | C/T+T/T | 68 | 53 | 2.51 | 1.03~6.28 | 3.19 | 1.20~8.46 |
| Raw vegetables | | | | | | | |
| frequently | C/C | 29 | 10 | 1.00 | | 1.00 | |
| | C/T+T/T | 70 | 47 | 1.95 | 0.81~4.74 | 1.80 | 0.96~3.38 |
| almost never | C/C | 33 | 12 | 1.00 | | 1.00 | |
| | C/T+T/T | 63 | 38 | 1.66 | 0.72~3.87 | 2.03 | 0.79~5.23 |
| Fruit | | | | | | | |
| ≥1time/week | C/C | 20 | 7 | 1.00 | | 1.00 | |
| | C/T+T/T | 45 | 17 | 1.08 | 0.35~3.41 | 1.01 | 0.24~4.20 |
| <1 time/week | C/C | 41 | 15 | 1.00 | | 1.00 | |
| | C/T+T/T | 92 | 65 | 1.93 | 0.94~4.00 | 2.10 | 0.98~4.48 |
| Tomatoes | | | | | | | |
| ≥1time/week | C/C | 15 | 5 | 1.00 | | 1.00 | |
| | C/T+T/T | 33 | 17 | 1.55 | 0.42~5.88 | 0.66 | 0.13~3.49 |
| <1 time/week | C/C | 47 | 17 | 1.00 | | 1.00 | |
| | C/T+T/T | 104 | 67 | 1.78 | 0.91~3.53 | 2.03 | 0.98~4.19 |
| Meat | | | | | | | |
| ≥1time/week | C/C | 41 | 10 | 1.00 | | 1.00 | |
| | C/T+T/T | 89 | 52 | 2.40 | 1.05~5.59 | 2.80 | 1.15~6.82 |
| <1 time/week | C/C | 21 | 11 | 1.00 | | 1.00 | |
| | C/T+T/T | 48 | 32 | 1.27 | 0.50~3.27 | 0.92 | 0.32~2.67 |
| Soybean products | | | | | | | |
| ≥1time/week | C/C | 46 | 15 | 1.00 | | 1.00 | |
| | C/T+T/T | 102 | 55 | 1.65 | 0.81~3.41 | 1.91 | 0.86~4.25 |
| <1 time/week | C/C | 16 | 7 | 1.00 | | 1.00 | |
| | C/T+T/T | 34 | 29 | 1.95 | 0.64~6.11 | 1.55 | 0.46~5.29 |

$OR_1$: Not adjusted; $OR_2$: Adjusted for sex, age, smoking, alcohol drinking, consumption of garlic and Chinese onions and all foods in the table.

**Table 3. Association between polymorphism of MTHFR C677T and susceptibility of stomach cancer according to consumption of allium vegetables**

| Dietary habits | MTHFR | Controls(n) | Cases(n) | $OR_1$ | 95%CI | $OR_2$ | 95%CI |
|---|---|---|---|---|---|---|---|
| Garlic | | | | | | | |
| ≥1time/week | C/C | 38 | 18 | 1.00 | | 1.00 | |
| | C/T+T/T | 95 | 60 | 1.33 | 0.67-2.68 | 1.23 | 0.62-2.44 |
| <1 time/week | C/C | 15 | 3 | 1.00 | | 1.00 | |
| | C/T+T/T | 24 | 18 | 3.75 | 0.83-19.3 | 9.94 | 1.26-78.1 |
| Chinese onions | | | | | | | |
| ≥1time/week | C/C | 39 | 17 | 1.00 | | 1.00 | |
| | C/T+T/T | 89 | 50 | 1.29 | 0.63-2.65 | 1.19 | 0.58-2.45 |
| <1 time/week | C/C | 15 | 3 | 1.00 | | 1.00 | |
| | C/T+T/T | 27 | 27 | 5.00 | 1.16-24.7 | 12.9 | 2.02-82.6 |

$OR_1$: Not adjusted; $OR_2$: Adjusted for sex, age, smoking, alcohol and tea drinking, and consumption of raw vegetables, fruit, tomatoes, meat and soybean products.

C/T or T/T genotype were at a significantly higher risk of developing stomach cancer. After adjusting for sex, age, smoking, alcohol drinking, consumption of garlic, Chinese onion and other foods, the OR for stomach cancer was 3.19 (95% CI: 1.07-6.00). Among tea drinkers, association between MTHFR genotypes and the risk of stomach cancer was not observed. Among subjects who had a lower consumption of raw vegetables, fruit or tomatoes, the MTHFR C/T or T/T genotype tended to increase ORs for stomach cancer, but without statistical significance. Among subjects who frequently consumed meat, individuals with the MTHFR C/T or T/T genotype had a higher risk for stomach cancer (adjusted OR=2.80, 95%CI:1.15-6.82) than individuals with MTHFR C/C genotype (Table 2).

### Intake of allium vegetables, frequency of MTHFR genotypes and the risk of stomach cancer

After adjustment for sex, age, smoking, alcohol drinking, consumption of tea, raw vegetables, fruit, tomatoes, meat and soybean products, among subjects who consumed lower amounts of garlic and Chinese onions, individuals with MTHFR C/T or T/T genotype had a 9.94 (95%CI: 1.26-78.12) and 12.90 (95%CI: 2.02-82.59) fold risk of developing stomach cancer, respectively. But among individuals who frequently consumed garlic and Chinese onions, no significant association was observed between MTHFR genotypes and the risk of stomach cancer (Table 3).

## DISCUSSION

Almost all of the previous investigations have shown a consistent association of dietary habits with the risk of stomach cancer, but studies on the relationship between gene polymorphisms of a metabolic enzyme that acts on dietary components and the risk of stomach cancer are very few. MTHFR is a key enzyme in folate metabolism. A defect in MTHFR could influence both DNA methylation and DNA synthesis. In the present study, we investigated the relationship between the polymorphism of MTHFR C677T and the risk of stomach cancer. We observed that the prevalence of MTHFR C/T and T/T genotypes among stomach cancer patients was significantly higher than that among controls. Individuals with the MTHFR C/T or T/T genotype were at elevated risk for stomach cancer. This result shows not only that polymorphisms of the MTHFR gene are associated with the risk of stomach cancer, but also shows that the nature of folate metabolism may be associated with the risk of stomach cancer.

Because some compounds in the folate cycle, including folate, the vitamins $B_{12}$ and $B_6$, cysteine and methionine can be supplied by food, further study on interactions of these substances is important for less expensive and effective prevention of stomach cancer. In this study, we also investigated the association between the polymorphism of MTHFR C677T and the risk of stomach cancer according to different dietary habits. Among subjects who consumed less allium

vegetables, we observed that individuals with a MTHFR C/T or T/T genotype had a significant higher risk of developing stomach cancer than individuals with a C/C type MTHFR, but among individuals who frequently consumed allium vegetables, the MTHFR genotypes and the risk of stomach cancer had no significant association. In the eighties of the twentieth century, Chinese researchers had discovered that allium vegetables, especially garlic may have a protecting effect against stomach cancer.[6] In our earlier studies in both high and low epidemic areas of cancers in Jiangsu province of China, we also discovered that frequent intake of allium vegetables may significantly decrease the risk of esophageal and stomach cancers.[7-9] A large number of experimental studies have shown that allium vegetables, especially garlic extract, may protect against the development of cancer through a variety of mechanisms. It is currently considered that the action of allium vegetables are through their organosulfur and selenium compounds. Because allium vegetables are rich in folate,[10] it is also possible that protective effect of allium vegetables against stomach cancer may be through the supply of folate and maintaining the balance of folate metabolism.

In the present study, we have also suggested that individuals with a MTHFR C/T or T/T genotype had a significantly higher risk for stomach cancer among non- tea drinkers or among subjects who frequently consumed meat. Among tea drinkers or among subjects who consumed lower amounts of meat, the MTHFR genotypes were not associated with the risk of stomach cancer. These results not only showed that dietary habits were associated with the development of stomach cancer, but also suggested that dietary components might affect gene expression. Because dietary components and their relation with the development of stomach cancer are complex, it is difficult to precisely explain the relationship between the interaction of dietary habits and MTHFR genotypes and the development of stomach cancer. Although the mechanism of the interaction between dietary habits and MTHFR genotypes in the development of stomach cancer is not clear, yet our these results indicate that examining the MTHFR genotype and dietary habits of individuals, at the same time, can be used for screening high-risk individuals for stomach cancer. Furthermore, the results indicate that increased consumption of allium vegetables and tea will benefit individuals with a MTHFR C677T C/T or T/T genotype for the prevention of stomach cancer.

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