



RAPID COMMUNICATION

## Interaction of methylenetetrahydrofolate reductase C677T, cytochrome P4502E1 polymorphism and environment factors in esophageal cancer in Kazakh population

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### Abstract

**AIM:** To evaluate the association and interaction of genetic polymorphisms in methylenetetrahydrofolate reductase (MTHFR) and cytochrome P4502E1 (CYP4502E1), environment risk factors with esophageal cancer (EC) in Kazakh, a high EC incidence area of Xinjiang Uygur Autonomous Region, China.

**METHODS:** A 1:2 matched case-control study was conducted with 120 cases of EC and 240 population- or hospital-based controls. The controls were matched for sex, nationality, area of residence and age within a 5-year difference. MTHFR and CYP4502E1 genotypes were identified by PCR-based restriction fragment length polymorphism (RFLP). A conditional logistic regression model was established to identify risk factors. The strata method was adopted in interaction analysis.

**RESULTS:** Low consumption of green vegetables and fresh fruits, alcohol drinking, and unsafe water (shallow well, or river) were found to be the risk factors for EC. Individuals with the MTHFR677 (C/T + T/T) genotype had a 2.62-fold (95% CI: 1.61-4.28) risk of developing EC compared with those who carried the C/C genotype. Individuals with the CYP4502E1C1/C1 genotype had a 3.00-fold (95% CI: 1.82-4.96) risk compared with those who carried the CYP4502E1 (C1/C2 + C2/C2) genotype.

Gene-environment interaction analysis showed that MTHFR677 gene polymorphism was correlated with consumption of green vegetables and fresh fruit, while CYP4502E1 C1/C1 was correlated with alcohol drinking and unsafe drinking water. MTHFR and CYP4502E1 analysis of gene-gene interaction showed that individuals with the MTHFR677 (C/T + T/T) and CYP4502E1C1/C1 genotypes had a 7.41-fold (95% CI: 3.60-15.25) risk of developing EC compared with those who carried the MTHFR677C/C and CYP4502E1 RsaI C1/C2 + C2/C2 genes, and the interaction rate was higher than that of the two factors alone.

**CONCLUSION:** Low consumption of green vegetables and fresh fruits, alcohol drinking, and unsafe water (shallow well, or river) and polymorphisms in MTHFR and CYP4502E1 genes are important risk factors for EC. There is a synergistic interaction among polymorphisms in MTHFR and CYP4502E1 genes and environment factors. MTHFR and CYP4502E1 genes can be used as biomarkers for prevention of EC in Kazakh, Xinjiang Uygur Autonomous Region, China.

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**Key words:** Kazakh; Esophageal Cancer; Methylenetetrahydrofolate reductase C677T; Cytochrome P4502E1; Genetic polymorphism; Environment risk factors; Interaction; Case control study

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### INTRODUCTION

Kazakh is an ethnic group with a high incidence and mortality rate of esophageal cancer (EC) in China. A national survey in 1990-1992 showed that the age-adjusted

mortality rate was 68.88 per 100 000 population for EC in Kazakh, and the rate was 14.95 per 100 000 population in Chinese nationality. The mortality rate for the same Kazakh population was 67.60 per 100 000 population in 2004–2005. The mortality rate of esophageal cancer in Kazakh has never been decreased over the last 15 years. Different risk factors for esophageal cancer have been reported in the world<sup>[1–5]</sup>. It was reported that deficiency in folate is caused by low consumption of green vegetables and fresh fruits, unsanitary drinking water, smoking, alcohol drinking, fast and irregular eating, eating of peppery food, frequent engorgement, eating out of date cake, history of esophagus or stomach illness and family history of EC are the risk factors for EC in Kazakh<sup>[6]</sup>. EC is caused by multi-factors, including environmental risk factors and genetic factors. In recent years, environmental and genetic susceptibilities and their interactions were used in evaluating the risks of EC<sup>[7–11]</sup>. Primary candidates for gene-environment interaction studies are those encoding enzymes related to the metabolism of established risk factors for cancer.

Methylenetetrahydrofolate reductase (MTHFR), a key enzyme in folate metabolism, which catalyzes 5, 10-methylene-tetrahydrofolate to 5-methyltetrahydrofolate<sup>[12]</sup>. A substitution of C to T at nucleotide 677 in MTHFR results in an alanine to valine substitution, which alters enzyme activity<sup>[13]</sup>. The *MTHFR C677T* polymorphism influences DNA methylation through an interaction with folate<sup>[14]</sup>. Alteration in DNA methylation, disruption of DNA integrity and DNA repair are believed to enhance carcinogenesis by altering the expression of critical tumor suppressor genes and proto-oncogenes<sup>[15]</sup>. Cytochrome P4502E1 (CYP4502E1), a member of the cytochrome P450 superfamily, is involved in the metabolic activation of many low molecular weight compounds, such as N-nitrosamines, aniline, vinyl chloride, and urethane<sup>[16–17]</sup>. N-nitrosamines present in tobacco and diet are well-recognized carcinogens involving cancer development at various sites, including the esophagus and stomach<sup>[18–19]</sup>.

The high mortality rate in Kazakh population indicates that environment factors play an important role in the development of EC. However, only a few individuals in the high-risk Kazakh population develop EC, although all residents share very similar environment-related risk factors and life style, suggesting that host susceptibility factors, such as the MTHFR677 and CYP4502E1 gene polymorphisms, may play an important role in the increased risk for EC. This study analyzed the gene-environment and gene-gene interaction among the MTHFR and CYP4502E1 gene polymorphisms, and environmental risk factors for EC in order to determine their relevance to EC prevention.

## MATERIALS AND METHODS

### Specimens

The 120 cases of esophageal cancer (confirmed by pathological diagnosis) came from inpatients and outpatients of six hospitals in the north of Xinjiang Uygur Autonomous Region between March 2005 and May 2007. Two hundred

and forty population- or hospital-based controls were randomly selected and matched for sex, nationality, residence and age within a 5-year difference. The controls were confirmed to have no history of cancer and digestive system diseases. Specially trained interviewers administered a standardized questionnaire that included demographic characteristics (sex, age, area of birth and residence), life-style, smoking status, drinking alcohol status, history of stomach or esophagus diseases and family history of EC. Blood samples were collected and DNA was extracted for genotyping of *MTHFR* and *CYP4502E1* genes.

### Genotyping of MTHFR and CYP2E1

Sites *MTHFR C677T* and *CYP4502E1* genotypes were analyzed by PCR-based restriction fragment length polymorphism (RFLP). The primers for *MTHFR*/*CYP4502E1* were synthesized by Shengong Biotechnology Company (Shanghai, China). The sequences of PCR primers for the *C677T* site are 5'-TTTGAGGCTGACCTG AAGCACTTGAAGGAG-3' and 5'-GAGTGTAGCCCTGGATGGGAAAGATCCCG-3'<sup>[20,21]</sup>. PCR was carried out in 25.0 µL reaction mixture containing 2.5 µL 10 × PCR buffer, 1.5 µL MgCl<sub>2</sub> (25 mmol/L), 0.5 µL dNTP (10 mmol/L), 0.5 µL forprimer (20 µmol/L), 0.5 µL revprimer (20 µmol/L), 0.2 µL Taq DNA polymerase (5 U/µL), 1.5 µL template DNA, and 17.8 µL nuclease free water. The reaction was initially carried out at 94°C for 5 min followed by 35 cycles at 94°C for 1 min, at 61°C for 1 min, at 72°C for 1 min, and a final extension at 72°C for 8 min. The PCR products were digested with Hinf I at 37°C for 13 h. The digested products were separated by electrophoresis on a 2.0% agarose gel. The 677C/C wild-type homozygotes were identified by the presence of only a 173-bp fragment; 677C/T heterozygotes were identified by the presence of 173-, 125-, and 48-bp fragments, and 677T/T homozygotes were identified by the presence of 125- and 48-bp fragments.

The *CYP4502E1* was identified by amplifying genomic DNA with the forward primer 5'-CCAGTC GAGTCTACATTGTCA-3' and the reverse primer 5'-TTCATTCTGTCTTCTAACTGG-3'. PCR was performed in 25.0 µL mixture containing 2.5 µL 10 × PCR buffer (including MgCl<sub>2</sub>), 2.0 µL dNTP (2.5 mmol/L), 0.5 µL forprimer (20 µmol/L), 0.5 µL revprimer (20 µmol/L), 0.2 µL Taq DNA polymerase (5 U/µL), 2.0 µL template DNA, and 17.3 µL nuclease free water. The reaction was initially carried out at 95°C for 8 min followed by 35 cycles at 94°C for 1 min, at 56°C for 1 min, at 72°C for 1 min, and a final extension at 72°C for 8 min. The PCR products were digested with restriction enzyme RsaI at 37°C for 4 h. The digested products were separated by electrophoresis on a 2.0% agarose gel. Three genotypes of *CYP4502E1* resulting from digestion with the restriction enzyme RsaI were found: common homozygote C1/C1, heterozygote C1/C2, and rare homozygote C2/C2.

### Quality control

DNA extraction and PCR were conducted at different time points. The genotypes of DNA samples were iden-

**Table 1** Environmental risk factors for EC in Kazakh population

| Environmental risk factors      | Case <i>n</i> (%) | Control, <i>n</i> (%) | <i>P</i> <sup>1</sup> | OR (95% CI) <sup>1</sup> |
|---------------------------------|-------------------|-----------------------|-----------------------|--------------------------|
| Consumption of green vegetables |                   |                       |                       |                          |
| Frequently                      | 42 (35.00)        | 126 (52.50)           | -                     | 1.00                     |
| Occasionally                    | 45 (37.50)        | 90 (37.50)            | 0.106                 | 2.54 (0.91-2.59)         |
| Less                            | 33 (27.50)        | 24 (10.00)            | 0.000                 | 4.66 (2.33-9.30)         |
| Consumption of fresh fruits     |                   |                       |                       |                          |
| Frequently                      | 13 (10.83)        | 71 (29.58)            | -                     | 1.00                     |
| Occasionally                    | 71 (59.17)        | 145 (60.42)           | 0.003                 | 2.79 (1.43-5.43)         |
| Less                            | 36 (30.00)        | 24 (10.00)            | 0.000                 | 9.03 (3.90-20.89)        |
| Smoking status                  |                   |                       |                       |                          |
| Never                           | 50 (41.67)        | 100 (41.67)           | -                     | 1.00                     |
| Former                          | 29 (24.17)        | 42 (17.50)            | 0.309                 | 1.39 (0.74-2.62)         |
| Current                         | 41 (34.16)        | 98 (40.83)            | 0.509                 | 0.83 (0.47-1.45)         |
| Alcohol drinking frequency      |                   |                       |                       |                          |
| Never                           | 50 (41.67)        | 152 (63.33)           | -                     | 1.00                     |
| 1-2 times/wk                    | 34 (28.33)        | 61 (25.42)            | 0.002                 | 3.28 (1.54-7.01)         |
| 3-4 times/wk                    | 29 (24.17)        | 21 (8.75)             | 0.000                 | 7.31 (3.14-17.03)        |
| ≥ 5 times/wk                    | 7 (5.83)          | 6 (2.50)              | 0.005                 | 6.00 (1.71-21.02)        |
| Drinking water source           |                   |                       |                       |                          |
| Safe water                      | 43 (35.83)        | 163 (67.92)           | -                     | 1.00                     |
| Shallow well water              | 20 (16.67)        | 25 (10.41)            | 0.002                 | 2.88 (1.47-5.66)         |
| River water                     | 57 (47.50)        | 52 (21.67)            | 0.000                 | 4.40 (2.55-7.60)         |

<sup>1</sup>ORs, 95% CIs and *P* values were calculated in a conditional logistic regression model.

tified without knowledge of the case-control status, and a 10% random sample set of case and controls was genotyped by different investigators and the reproducibility was 100%. Each PCR was performed with the controls as blank (without DNA template), positive and negative controls, respectively. When any of these controls failed, PCR was re-conducted. Twenty percent of the questionnaires were re-administered by different investigators and the consistency was 100%.

### Statistical analysis

Statistical analyses were performed using the SPSS software. Cases and controls were compared for any differences in gender and age using  $\chi^2$  test and Mann-Whitney test, respectively. The probability of Hardy Weinberg equilibrium was assessed by  $\chi^2$  test. Conditional logistic regression was employed to calculate the odds ratio (OR) of MTHFR/CYP4502E1 polymorphisms. Gene-environment and gene-gene interactions were calculated by stratified analysis.

## RESULTS

The number of male and female patients with EC was 81 and 39, respectively, who were matched for 162 male and 78 female controls. The mean age ( $\pm$  SD) of cases and controls was  $59.0 \pm 10.0$  years and  $58.4 \pm 10.1$  years, respectively. There was no significant difference in age between cases and controls ( $t = 0.586$ ,  $v = 358$ ,  $P = 0.558$ ).

The distributions of environmental risk factors in

**Table 2** Genotype risk assessment of EC in cases and controls

| Genotype      | Case <i>n</i> (%) | Control <i>n</i> (%) | <i>P</i> <sup>1</sup> | OR (95% CI) <sup>1</sup> |
|---------------|-------------------|----------------------|-----------------------|--------------------------|
| MTHFR677      |                   |                      |                       |                          |
| C/C           | 60 (50.00)        | 170 (70.83)          | -                     | 1.00                     |
| C/T           | 53 (44.17)        | 59 (24.59)           | 0.000                 | 2.69 (1.63-4.44)         |
| T/T           | 7 (5.83)          | 11 (4.58)            | 0.144                 | 2.15 (0.77-5.98)         |
| C/C           | 60 (50.00)        | 170 (70.83)          | -                     | 1.00                     |
| C/T + T/T     | 60 (50.00)        | 70 (29.17)           | 0.000                 | 2.62 (1.61-4.28)         |
| CYP2E1 RsaI   |                   |                      |                       |                          |
| C1/C1         | 94 (78.33)        | 128 (53.33)          | -                     | 1.00                     |
| C1/C2         | 23 (19.17)        | 90 (37.50)           | 0.000                 | 0.37 (0.22-0.62)         |
| C2/C2         | 3 (2.50)          | 22 (9.17)            | 0.009                 | 0.19 (0.05-0.66)         |
| C1/C2 + C2/C2 | 26 (21.67)        | 112 (46.67)          | -                     | 1.00                     |
| C1/C1         | 94 (78.33)        | 128 (53.33)          | 0.000                 | 3.00 (1.82-4.96)         |

<sup>1</sup>ORs, 95% CIs and *P* values were calculated in a conditional logistic regression model.

cases and controls are summarized in Table 1. Low consumption of green vegetables and fresh fruits, alcohol drinking, and unsafe water (shallow well, or river) were found to be the risk factors for EC. The percentage of tobacco smoking in cases and controls was not significantly different.

The genotype distributions of the MTHFR677 and CYP4502E1 RsaI in cases and controls are summarized in Table 2. The observed frequencies of three MTHFR677 genotypes in controls (C/C = 70.83%, C/T = 24.59%, and T/T = 4.58%) were not different from those detected in Hardy-Weinberg equilibrium ( $\chi^2 = 3.34$ ,  $v = 2$ ,  $P = 0.188$ ). However, they were significantly different from those observed in cases (C/C = 50.00%, C/T = 44.17%, and T/T = 5.83%,  $\chi^2 = 15.55$ ,  $v = 2$ ,  $P = 0.000$ ). Subjects who carried the MTHFR677C/T genotype had a 2.69-fold (95% CI: 1.63-4.44) risk of developing EC compared with those who carried the MTHFR677C/C genotype. The MTHFR677T/T genotype, which was rare both in cases and in controls, was associated only with a slightly increased risk of EC, without statistical significance (OR = 2.15, 95% CI = 0.77-5.98). Individuals with the MTHFR677 T allele were more prone to develop EC (OR = 2.62, 95% CI = 1.61-4.28).

The distribution of the CYP4502E1 RsaI genotypes in controls (C1/C1 = 53.33%, C1/C2 = 37.50%, and C2/C2 = 9.17%) was also in accordance with Hardy-Weinberg equilibrium ( $\chi^2 = 0.921$ ,  $v = 2$ ,  $P = 0.631$ ) and significantly different from that of the CYP4502E1 RsaI genotypes in cases (C1/C1 = 78.33%, C1/C2 = 19.17%, and C2/C2 = 2.50%,  $\chi^2 = 21.79$ ,  $v = 2$ ,  $P = 0.000$ ). The Odds ratio (OR) of developing esophageal cancer for the CYP4502E1 RsaI C1/C2 and C2/C2 genotypes was 0.37 (95% CI = 0.22-0.62) and 0.19 (95% CI = 0.05-0.66), respectively, compared to the CYP4502E1 RsaI C1/C1 genotype. Individuals with the CYP4502E1 RsaI C1/C1 genotype had a 3.00-fold (95% CI = 1.82-4.96) risk of developing EC compared with those who carried the CYP4502E1 RsaI C1/C2 + C2/C2 genotype.

The results of interaction between MTHFR677 gene polymorphism and consumption of green vegetables

**Table 3** Interaction between MTHFR 677 and consumption of green vegetables, fresh fruits in EC

| Consumption of vegetables and fruits | MTHFR 677 genotype | Case (n) | Control (n) | $\chi^2$ | P     | OR (95% CI)       |
|--------------------------------------|--------------------|----------|-------------|----------|-------|-------------------|
| Green vegetables <sup>1</sup>        |                    |          |             |          |       |                   |
| Frequently                           | C/C                | 19       | 89          | -        | -     | 1.00              |
| Occasionally or less                 | C/C                | 41       | 81          | 7.62     | 0.006 | 2.37 (1.27-4.42)  |
| Frequently                           | C/T + T/T          | 23       | 37          | 8.85     | 0.003 | 2.91 (1.42-5.97)  |
| Occasionally or less                 | C/T + T/T          | 37       | 33          | 24.50    | 0.000 | 5.25 (2.66-10.39) |
| Freshfruits <sup>2</sup>             |                    |          |             |          |       |                   |
| Frequently                           | C/C                | 6        | 54          | -        | -     | 1.00              |
| Occasionally or less                 | C/C                | 54       | 116         | 10.90    | 0.001 | 4.19 (1.70-10.34) |
| Frequently                           | C/T + T/T          | 7        | 17          | 4.81     | 0.028 | 3.71 (1.10-12.54) |
| Occasionally or less                 | C/T + T/T          | 53       | 53          | 26.76    | 0.000 | 9.00 (3.57-22.71) |

SIA: Synergic index of addition; RERI: Relative excess risk of interaction; API: Attributable proportion of interaction.  $SIA^1 = 5.25 / (2.37 + 2.91 - 1.00) = 1.23$ ,  $RERI^1 = 5.25 - (2.37 + 2.91) + 1 = 0.97$ ,  $API^1 = [5.25 - (2.37 + 2.91) + 1] / 5.25 = 18.48\%$ ;  $SIA^2 = 9.00 / (4.19 + 3.71 - 1.00) = 1.30$ ,  $RERI^2 = 9.00 - (4.19 + 3.71) + 1 = 2.10$ ,  $API^2 = [9.00 - (4.19 + 3.71) + 1] / 9.00 = 23.33\%$ .

**Table 4** Interaction between CYP4502E1 RsaI 677 and alcohol drinking, safety drinking water in EC

|                                  |       | CYP4502E1 rsal genotype | Case (n) | Control (n) | $\chi^2$ | P     | OR (95% CI)        |
|----------------------------------|-------|-------------------------|----------|-------------|----------|-------|--------------------|
| Alcohol drinking <sup>1</sup>    | Never | C1/C2 + C2/C2           | 12       | 70          | -        | -     | 1.00               |
|                                  | Yes   | C1/C2 + C2/C2           | 14       | 42          | 2.34     | 0.126 | 1.94 (0.82-4.60)   |
|                                  | Never | C1/C1                   | 38       | 82          | 7.59     | 0.006 | 2.70 (1.31-5.57)   |
|                                  | Yes   | C1/C1                   | 56       | 46          | 31.64    | 0.000 | 7.10 (3.44-14.68)  |
| Safe drinking water <sup>2</sup> | Yes   | C1/C2 + C2/C2           | 9        | 78          | -        | -     | 1.00               |
|                                  | No    | C1/C2 + C2/C2           | 17       | 34          | 11.12    | 0.001 | 4.33 (1.76-10.69)  |
|                                  | Yes   | C1/C1                   | 34       | 85          | 10.11    | 0.001 | 3.47 (1.56-7.69)   |
|                                  | No    | C1/C1                   | 60       | 43          | 46.80    | 0.000 | 12.09 (5.47-26.74) |

SIM: Synergic index of multiplication;  $SIM^1 = 7.10 / (1.94 \times 2.70) = 1.36$ ,  $RERI^1 = 7.10 - (1.94 + 2.70) + 1 = 3.46$ ,  $API^1 = [7.10 - (1.94 + 2.70) + 1] / 7.10 = 48.73\%$ ;  $SIA^2 = 12.09 / (4.33 + 3.47 - 1.00) = 1.78$ ,  $RERI^2 = 12.09 - (4.33 + 3.47) + 1 = 5.29$ ,  $API^2 = [12.09 - (4.33 + 3.47) + 1] / 12.09 = 43.76\%$ .

**Table 5** Interaction between MTHFR 677 and CYP4502E1 RsaI gene polymorphisms in EC

| MTHFR 677 genotype | CYP2E1 rsal genotype | Case (n) | Control (n) | $\chi^2$ | P     | OR (95% CI)       |
|--------------------|----------------------|----------|-------------|----------|-------|-------------------|
| C/C                | C1/C2 + C2/C2        | 13       | 82          | -        | -     | 1.00              |
| C/T + T/T          | C1/C2 + C2/C2        | 13       | 30          | 5.30     | 0.021 | 2.73 (1.14-6.56)  |
| C/C                | C1/C1                | 47       | 88          | 12.91    | 0.000 | 3.37 (1.70-6.68)  |
| C/T + T/T          | C1/C1                | 47       | 40          | 33.44    | 0.000 | 7.41 (3.60-15.25) |

$SIA = 7.41 / (2.73 + 3.37 - 1.00) = 1.45$ ;  $RERI = 7.41 - (2.73 + 3.37) + 1 = 2.31$ ;  $API = [7.41 - (2.73 + 3.37) + 1] / 7.41 = 31.17\%$ .

and fresh fruit are listed in Table 3. Less consumption of green vegetables increased the OR in MTHFR677 T allele carriers (OR = 5.25, 95% CI = 2.66-10.39). The corresponding SIA, RERI and API were 1.23, 0.97, and 18.48%, respectively. Among carriers of the MTHFR677 T allele with occasional or less consumption of fresh fruits was significantly associated with an elevated risk of developing EC (OR = 9.00, 95% CI = 3.57-22.71) and the interaction rate was higher than that of the two factors alone. The corresponding SIA, RERI, and API were 1.30, 2.10, and 23.33%, respectively.

The results of interaction between the CYP4502E1 RsaI gene polymorphism and alcohol drinking as well as

safe drinking water are shown in Table 4. Among carriers of the CYP4502E1C1/C1 genotype, alcohol drinking was significantly associated with an elevated risk of developing EC (OR = 7.10, 95% CI = 3.44-14.68), and the interaction rate was higher than the sum of the two factors alone. The corresponding SIM, RERI, and API were 1.36, 3.46, and 48.73%, respectively. Unsafe water increased the risk of developing EC among carriers of the CYP4502E1C1/C1 genotype (OR = 12.09, 95% CI = 5.47-26.74), and the interaction rate was higher than that of the other two factors. The corresponding SIA, RERI and API were 1.78, 5.29, and 43.76%, respectively.

A significant interaction between the MTHFR 677 genotype and CYP4502E1 RsaI genotype was found in EC risk (Table 5). Individuals who had both MTHFR677 (C/T + T/T) and CYP4502E1 RsaI C1/C1 genotypes had a 7.41-fold risk of developing EC (95% CI = 3.60-15.25) compared with those who carried MTHFR677C/C and CYP4502E1 RsaI C1/C2 + C2/C2 genotypes. The corresponding SIA, RERI and API were 1.45, 2.31 and 31.17%, respectively.

## DISCUSSION

The results of the current study indicate that MTHFR677 and CYP4502E1 RsaI gene polymorphisms are the susceptibility factors for EC<sup>[22,23]</sup>. Cohort studies that simultaneously consider multiple genetic and environmental



factors possibly involved in esophageal carcinogenesis are needed to ascertain not only the relative contribution of these factors to tumor development but also the contributions of their putative interactions<sup>[24]</sup>. We observed a significant risk of having the MTHFR677 C/T + T/T and CYP4502E1C1/C1 genotypes in EC. There is a synergistic interaction among polymorphisms in MTHFR and CYP4502E1 genes and environment factors.

The studies conducted in high-risk areas showed the MTHFR677T allele increases the risk of developing EC<sup>[25,26]</sup>. However, no risk change has been observed among Caucasians in Germany and Japan<sup>[27,28]</sup>. Regional differences in folate consumption among populations may explain this inconsistency in the impact of T alleles<sup>[25]</sup>, suggesting that gene-nutrient environment interactions between folate consumption and impact of the MTHFR 677T allele vary with folate intake. When folate intake is sufficient, individuals with the MTHFR CT or TT genotype may have a decreased risk of developing cancer, since decreased MTHFR activity associated with the 677TT polymorphism can lead to elevation in 5,10-methylene-tetrahydrofolate, facilitating DNA synthesis, while adequate provision of methyl donors can be ensured. In contrast, in the presence of low folate, DNA methylation and DNA synthesis/repair may be impaired, initiating carcinogenesis. Deficiency in folate is caused by low consumption of vegetables and fruit in the Kazakh population and MTHFR677 C/T + T/T genotype has a synergistic interaction with less consumption of vegetables and fruit in EC. Our results are consistent with the reported findings<sup>[25,26]</sup>.

Over-representation of variant CYP4502E1 RsaI alleles has been reported in gastric cancer<sup>[29]</sup> and a lower frequency of the RsaI variant allele has also been observed in patients with EC than in controls<sup>[30]</sup>. Individuals with the variant RsaI allele (c1/c2 or c2/c2) have a lower basal CYP450 2E1 activity. It was reported that the Cyp4502E1 C2/C2 genotype is associated with the decreased enzyme activity<sup>[31]</sup>, adding biological plausibility to the protective effect of CYP4502E1 C2/C2 genotype observed in this study. Our observation is in agreement with the finding of recent studies showing that the C1/C1 genotype of CYP4502E1 is associated with the increased risk of developing EC<sup>[32-35]</sup>. However, contrary results have also been reported elsewhere<sup>[36-38]</sup>. Studies have shown inconsistent findings regarding the association between the CYP4502E1 polymorphism and EC. The reasons for these inconsistent findings are unknown. However, it may be due to the differences in ethnicity and life-style which can lead to variations in enzyme activity. The present findings indirectly support the hypothesis that environmental exposure to carcinogens plays a role in the etiology of EC. The CYP4502E1 polymorphism is involved in metabolism of various nitrosamines. Cigarette smoking, and alcohol drinking, unsafe drinking water containing chemicals including nitroso compound, were found to be the risk factors for EC in this study. A significant gene-environment interaction between the CYP4502E1 polymorphism and alcohol drinking and unsafe water was also observed in this study.

Selection bias and/or systematic error may occur in

a case-control study because of inappropriate selection of subjects and other confounding factors. However, our study including a relatively large number of cases diagnosed in hospitals was matched for potential confounding variables. Solid and reproducible genotyping techniques can minimize systematic errors in measurement. For these reasons, the findings of our study could not solely attribute to bias.

In summary, MTHFR677 and CYP4502E1 RsaI gene polymorphisms are significantly correlated with EC, and MTHFR677 C/T or T/T genotype and CYP4502E1 C1/C1 wild type increase the susceptibility to EC in the Kazakh population of Xinjiang Uygur Autonomous Region, China. Interaction between MTHFR677 C/T + T/T genotype and less consumption of green vegetables and fresh fruits, as well as between CYP4502E1 C1/C1 and alcohol and unsafe drinking water is associated with the risk of developing EC. Gene-gene interaction between MTHFR 677 and CYP4502E1 RsaI can serve as a useful biomarker for prevention of EC in the Kazakh population.

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## COMMENTS

### Background

Kazakh is an ethnic group with a high incidence and mortality rate of esophageal cancer (EC) in China. Epidemiological studies have demonstrated deficiency in folate caused by low consumption of green vegetables and fresh fruits, alcohol drinking, unsanitary drinking water (containing chemicals including nitroso compound) are the main risk factors for EC in Kazakh population. Genetic polymorphisms in the MTHFR677 and CYP4502E1 genes affect the metabolism of folate, alcohol, and N-nitrosamines. There have been some studies on the roles of folate and MTHFR677 genes, alcohol and the CYP2E1 genes in EC in Chinese Han population. However, their results were conflicting and little study in Kazakh population. Therefore, the aim of the present study was to evaluate the association and interaction of MTHFR and CYP4502E1 and environment risk factors with EC in Kazakh population.

### Research frontiers

Accumulating evidence from prior epidemiologic studies shows an association between deficiency in folate caused by low consumption of green vegetables and fresh fruits, alcohol drinking, unsanitary drinking water and EC in Kazakh population. The genetic polymorphisms of MTHFR affect the metabolism of folate and CYP4502E1 genetic polymorphisms also affect the metabolism of alcohol and N-nitrosamines. Polymorphisms in the MTHFR and CYP4502E1 genes are associated with the risk of EC in Kazakh.

### Innovations and breakthroughs

This is the first study to show significant interactions of MTHFR677 and CYP4502E1 and consumption of green vegetables and fresh fruits, alcohol drinking, and unsafe waters (shallow well, or river) with EC in Kazakh population. Synergistic interactions were found in MTHFR677 gene polymorphism with consumption of green vegetables and fresh fruit, CYP4502E1 gene polymorphism with alcohol drinking and unsafe drinking water, MTHFR677 with CYP4502E1 genotypes for EC in Kazakh population, and the interaction rate was higher than that of the two factors alone.

### Applications

The detection of MTHFR677 and CYP4502E1 genotypes may become a useful biomarker for EC in Kazakh population, and also help clinicians to diagnose EC earlier.

### Terminology

Methylenetetrahydrofolate reductase (MTHFR), a key enzyme in folate metabolism responsible for circulating form of folate, 5-methyl-tetrahydrofolate,

which converts methionine to S-adenosylmethionine, the universal methyl donor for various intracellular methylation reactions, particularly DNA methylation. The cytochrome P450 2E1 (CYP450E1), a member of the cytochrome P450 superfamily metabolizes a range of small organic compounds, including aniline and benzene as well as N-nitrosamines. Individuals frequently encounter different environmental conditions, and the physiological and behavioral responses to these conditions can depend on an individual's genetic makeup. This phenomenon is known as gene-environment interaction.

### Peer review

This paper is interesting, and would accumulate new data on interaction of genetic polymorphisms and environment factors for esophageal cancer in Kazakh population.

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