Genes & Diseases (2021) 8, 931–938

Available online at www.sciencedirect.com

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FULL LENGTH ARTICLE

The combination of methylenehydrofolate reductase C677T polymorphism screening and gastrointestinal tumor markers detection may be an early screening method for gastrointestinal cancer related to helicobacter pylori infection

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Received 24 November 2020; received in revised form 20 February 2021; accepted 21 February 2021
Available online 2 March 2021

KEYWORDS
CA199;
CA724;
CEA;
Gastrointestinal cancer;
Helicobacter pylori;
MTHFR C677T polymorphism

Abstract Methyltetrahydrofolate reductase (MTHFR) is a key enzyme in folate metabolism, and its single nucleotide polymorphism (SNP) site C677T may be associated with gastrointestinal cancer. However, the relationship between MTHFR C677T polymorphism and gastrointestinal tumor markers carcinoma embryonic antigen (CEA), carbohydrate antigen 199 (CA199) and carbohydrate antigen 724 (CA724) in *Helicobacter pylori* (*H. pylori*) infection is not specified. This study aims to identify the association between MTHFR C677T polymorphism and gastrointestinal tumor markers (CEA, CA199 and CA724) in H. pylori infection. The relationship between MTHFR C677T polymorphism and gastrointestinal tumor markers in 58 patients with H. pylori infection and 94 non-infected patients was studied. We found that TT genotype was a susceptibility factor of *H. pylori* infection, which was also associated with increased CEA and CA724 levels. Moreover, there was a negative additive interaction between *MTHFR* gene

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Peer review under responsibility of Chongqing Medical University.

https://doi.org/10.1016/j.gendis.2021.02.011

C677T polymorphism and CEA levels in *H.pylori* infection. Meanwhile, there were significant differences in CEA levels between MTHFR C677T polymorphism and *H.pylori* infection. The presence of T allele led to a decrease in CEA levels when ^{13}C urea breath test (^{13}C-UBT) was positive, while the presence of T allele led to an increase in CEA levels when ^{13}C-UBT was negative. Therefore, we suggest that healthy people should take MTHFR C677T polymorphism screening, combined with ^{13}C-UBT and gastrointestinal tumor markers detection, which can screen out the susceptible population of H. pylori, and help to detect gastrointestinal cancer in the early stage.

Introduction

Even though the knowledge of gastrointestinal cancer is getting more in depth, together with the large amount of funds and resources invested in the research and development of effective treatment for gastrointestinal cancer,[1] in the past few decades, the mortality of gastrointestinal cancer has hardly decreased. So far, gastric cancer and colorectal cancer are still the top ten leading causes of cancer-related deaths in the world.[2,3] Therefore, some scholars and clinical workers also focus on the early screening and diagnosis of gastrointestinal cancer.[4,5] Regarding the 5-year survival rate, patients with early diagnosis and treatment have a higher survival rate than those diagnosed in advanced stage.[6,7]

The etiology of gastrointestinal cancer is multifactorial and co-regulated by different factors.[8,9] Besides the external factors including environmental factors, dietary habits and socio-economic status,[10] it is also related to individual factors, such as age, gender and genetic susceptibility.[9,11] It has been confirmed that *H.pylori* infection is associated with a variety of gastrointestinal diseases and gastrointestinal cancer.[12,13]

MTHFR is a key enzyme in folate metabolism, which resides on chromosome 1 location p36.3, and it appears to be polymorphic such as the gene site C677T, one of the most studied and clinically important variants in exon 4.[14] When the C allele gene at 677 is replaced by T allele, valine is replaced by alanine, which leads to the decrease of enzyme activity. The enzyme activities of heterozygous MTHFR C677T genotype and homozygous MTHFR T677T genotype were 60% and 30% of that of wild-type MTHFR C677C genotype, respectively.[15,16] With the decrease of enzyme activity, folate metabolism is also affected, showing a higher degree of genomic DNA hypomethylation.[17] As one of the characteristics of protooncogenes, hypomethylation leads to gastrointestinal cancer.[18,19] However, the interaction between MTHFR C677T polymorphism and *H.pylori* in gastrointestinal cancers remains unclear.

As important indicators of gastrointestinal cancer screening, the increase of CEA, CA199 and CA724 indicates the occurrence of gastrointestinal cancer.[20,21] Therefore, this study analyzes the independent and mutual relationships between MTHFR C677T polymorphism and gastrointestinal tumor markers (CEA, CA199 and CA724) with or without *H.pylori* infection. We aim to explore the significance of MTHFR C677T polymorphism, CEA, CA199, CA724 and *H.pylori* infection in early screening for gastrointestinal cancer.

Materials and methods

Study population

Data and blood sample were collected from January 2016 to October 2020 in the Health Management Center of the First Affiliated Hospital of Chongqing Medical University (CQMU). Inclusion criteria: subjects aged >18 without cancer history, and underwent gastrointestinal tumor markers (CEA, CA199 and CA724) test and (^{13}C-UBT) during health checkup. Exclusion criteria: patients diagnosed with cancer before, or patients received or were under treatment for *H.pylori* eradication, or subjects with ethnic minorities or incomplete information. Finally, a total of 152 subjects were included in the study, of which 58 (38.20%) patients were ^{13}C-UBT positive and 94 (61.8%) were ^{13}C-UBT negative. All participants had signed the informed consent before health checkup, and this study has been approved by the ethics committee of the clinical college of CQMU (NO.2020-089).

Genotyping of MTHFR C667T polymorphism

An amount of 3—5 ml peripheral venous blood was collected and placed in the EDTA anticoagulant tube for genotyping. Genomic DNA (batch number: q5502) was extracted from 200 μL anticoagulant peripheral blood with the adoption of whole blood genomic DNA rapid extraction kit (Tiangen Biology Inc.). DNA sequencing for determining the results of MTHFR C667T polymorphism was read after PCR amplification and purification (Fig. 1).[22]

^{13}C-UBT

In the morning fasting state, all subjects used ^{13}C-UBT bottom gas collection bag (Shenzhen CNUO Haidway Biotechnology Co., LTD., China) to collect the exhaled gas before taking medicine, and then took 75 mg of ^{13}C capsule orally. After 30 min, the matching sample gas collection bags were used to collect the breath after taking the medicine. The breath test tester (HCBT-01, Shenzhen CNUO

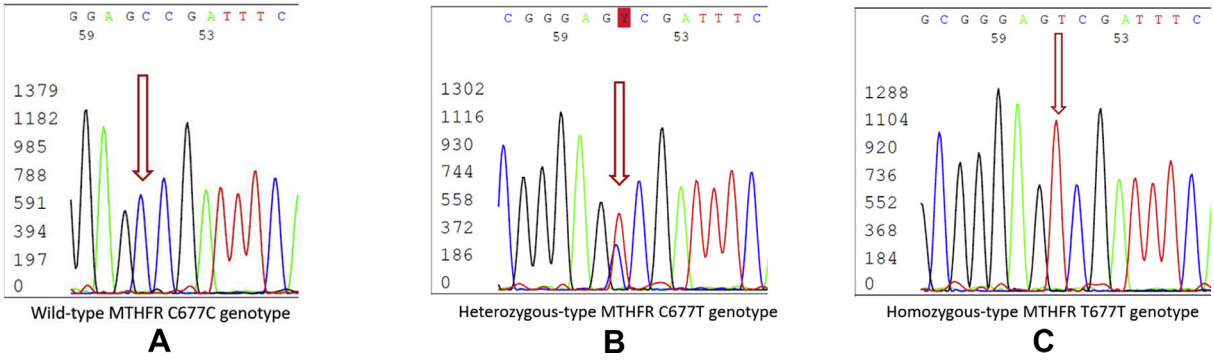

Figure 1 MTHFR C677T polymorphism sequencing map. **(A)** MTHFR C677T wild-type: CC genotype. **(B)** MTHFR C677T heterozygous: CT genotype. **(C)** MTHFR C677T homozygous: TT genotype. The ordinate represents was the signal intensity of gene, and the abscissa represents the position of base.

Haidway Biotechnology Co., LTD., China) was used to calculate the ^{13}C-UBT exceeding the baseline δ value. Diagnostic criteria: Delta over baseline (DOB) $\geq$ 4 was positive, and DOB $<$ 4 was negative. Diagnostic accuracy:[23] sensitivity was 98%, and specificity was 98%.[24]

Detection methods of human body index and biochemical index

The weight and height of the subjects were measured by a computer human scale (SK-X80/TCS-160D-W/H, Shenzhen Shuangjia Electronics Co., LTD., China). Blood pressure was measured by pulse wave medical sphygmomanometer (RBP-9001, Shenzhen Ruiguang Kangtai Technology Co., LTD.) during quiet sitting.[25]

All subjects fasted for more than 8 h before blood samples collection, and peripheral venous blood was collected for analysis under the fasting state from 7:30 AM to 10:00 AM.[25] CEA, CA199 and CA724 were tested by chemiluminescence immunoassay on an automatic chemiluminescence immunoassay analyzer (Cobas 8000 e602, Roche Diagnostics Inc). White blood cell (WBC), Red blood cell count (RBC) and hemoglobin (HB) were analyzed on a five classification hematology analyzer (XE-2100, Sysmex Inc.). All blood biochemical tests have passed ISO15189 certification (No. ML00036).

Statistical analysis

IBM SPSS statistics 23 (IBM, Chicago, USA) was used to conduct the Chi-square goodness-of-fit test for calculating the Hardy–Weinberg equilibrium, and Chi-square test or Fisher's exact test was used to compare categorical variables between groups. Univariate logistic regression was used to analyze the distribution of MTHFR C677T polymorphism and ^{13}C-UBT results. Multivariate logistic regression with forward LR variable selection was used to calculate the odd ratio (OR) and 95% confidence interval (CI) to evaluate the association between ^{13}C-UBT positive and risk factors. Multivariate analysis of variance was used to analyze the interaction between MTHFRC677T polymorphism and ^{13}C-UBT results on gastrointestinal tumor markers. Bootstrap method was used to calculate 95% CI, and SAS 9.4 (SAS Institute Inc., Cary, NC, USA) was used to calculate the statistical interaction between categorical variables and continuous variables by logistic regression.[26] The synergy index S (S) = 1 and the relative excess risk of interaction (RERI) = 0 were defined as no interaction, S $>$ 1 and RERI $>$ 0 were defined as positive additive interactions, and S $<$ 1 and RERI $<$ 0 were defined as negative additive interactions. The attributable proportion of interaction (AP) was defined as the proportion of all cases attributable to the interactions between two factors. $P < 0.05$ was considered statistically significant.

Results

Anthropometric measurements, blood biochemical indexes and genotype of MTHFR C677T polymorphism in ^{13}C-UBT positive and negative groups

The positive rate of ^{13}C-UBT in males was 43.4%, which was higher than that in females (26.1%, $P = 0.044$). The positive rate of ^{13}C-UBT in the group aged $>$60 (71.4%) was significantly higher than that in the other age groups ($P = 0.027$). In ^{13}C-UBT positive group, CEA and CA724 levels were significantly higher than those in ^{13}C-UBT negative group (both $P < 0.05$), but there was no significant difference of CA199 levels between the two groups. The diastolic blood pressure (DBP) and systolic blood pressure (SBD) in ^{13}C-UBT positive group were higher than those in^{13}C-UBT negative group ($P = 0.017$ and $P = 0.023$, respectively). The mean WBC of these two groups was within the normal range, and that of the ^{13}C-UBT positive group ($6.00 \pm 1.34\ 10^9$/L) was higher than the ^{13}C-UBT negative group ($5.54 \pm 1.42\ 10^9$/L, $P = 0.045$), Table 1.

The genotypes of MTHFR of all studied subjects were CC (36.84%), CT (46.71%) and TT (16.45%). The genotypes distribution of ^{13}C-UBT positive group ($\chi^2 = 2.980$, $P = 0.225$) and ^{13}C-UBT negative group ($\chi^2 = 1.116$, $P = 0.572$) were accorded with Hardy–Weinberg equilibrium test. By

Table 1 The characteristics of anthropometric measurements, biochemical indexes and MTHFR C677T genotype in 13C-UBT positive and negative groups.

Variable	^{13}C-UBT positive group (n = 58,%)	^{13}C-UBT negative group (n = 94,%)	P value
Gender			0.044
Male	46 (43.4)	60 (56.6)	
Female	12 (26.1)	34 (73.9)	
Age, mean,(SD)	51.34 (11.50)	48.95 (9.66)	0.169
Age subgroup			0.027
<40	8 (36.4)	14 (63.6)	
40–60	40 (34.5)	76 (65.5)	
>60	10 (71.4)	4 (28.6)	
DBP(mmHg), mean,(SD)	79.89 (12.29)	75.14 (11.36)	0.017
SBP(mmHg), mean,(SD)	127.49 (20.23)	120.37 (17.35)	0.023
CEA(ng/ml), mean,(SD)	2.08 (1.04)	1.69 (0.74)	0.008
CA199(U/ml),median,(P25,P75)	9.20 (5.80,15.25)	9.65 (6.48,13.50)	0.764
CA724(U/ml),median,(P25,P75)	3.70 (1.60,5.93)	2.10 (1.08,4.23)	0.004
WBC(10^9/L), mean,(SD)	6.00 (1.34)	5.54 (1.42)	0.045
RBC(10^12/L), mean,(SD)	5.05 (0.55)	4.94 (0.58)	0.244
Hb(g/L), mean,(SD)	153.12 (12.88)	150.11 (15.48)	0.216
MTHFR C677T genotype			0.091
CC	22 (39.3)	34 (60.7)	
CT	22 (31.0)	49 (69.0)	
TT	14 (56.0)	11 (44.0)	

Abbreviations: SD standard deviation, P25 and P75 the 25% percentile and the 75% percentile of the percentiles, DBP diastolic blood pressure, SBP systolic blood pressure, CEA carcinoma embryonic antigen, CA199 carbohydrate antigen199, CA724 carbohydrate antigen724, WBC white blood cell, RBC red blood cell, Hb haemoglobin, ^{13}C-UBT ^{13}C urea breath test.
P value < 0.05 was considered statistically significant.

comparing three genotypes frequencies between ^{13}C-UBT positive group and negative group, we found that the TT genotype was more frequent than CC and CT genotype in ^{13}C-UBT positive group ($P = 0.028$), Table 2.

Risk factors of ^{13}C-UBT positive filtrated by univariate and multiple logistic regression analysis

Univariate analysis showed that gender, age, DBP, SBP, CEA, CA724, WBC and TT, CC and CT genotype were risk factors for ^{13}C-UBT positive ($P < 0.05$). After multivariate analysis, only CEA, CA724 and TT, CC and CT genotype were the risk factors for ^{13}C-UBT positive ($P < 0.05$), Table 3.

MTHFR C677T polymorphism and CEA levels had negative additive interaction effect on ^{13}C-UBT positive

By analyzing the additive interactions effect between MTHFR C677T polymorphism and DBP, CEA, CA724 on ^{13}C-UBT results, only MTHFR C677T polymorphism had negative additive interaction with CEA levels ($P = 0.026$). The RERI was −1.407 (95% CI = −3.737–0.277) and S was 0.468 (95% CI = 0.072–0.910). The interaction between MTHFR C677T polymorphism and CEA was 63.1% (95% CI = −1.941–0.079) without considering other factors, Table 4.

Table 2 Genotype frequencies of MTHFR C677T polymorphism between ^{13}C-UBT positive and negative groups.

MTHFR C677T Polymorphism	^{13}C-UBT positive group (n = 58,%)	^{13}C-UBT negative group (n = 94,%)	OR (95%CI)	P value
CC vs. CT+TT				0.827
CC	22 (39.3)	34 (60.7)	1.078 (0.548–2.122)	
CT+TT	36 (37.5)	60 (62.5)	Reference	
CT vs. CC+TT				0.090
CT	22 (31.0)	49 (69.0)	0.561 (0.288–1.094)	
CC+TT	36 (44.4)	45 (55.6)	Reference	
TT vs. CC+CT				0.028
TT	15 (57.7)	11 (42.3)	2.632 (1.113–6.225)	
CC+CT	43 (34.1)	83 (65.9)	Reference	

Abbreviations: OR odds ratio, 95%CI 95% confidence interval, ^{13}C-UBT ^{13}C urea breath test.
P value < 0.05 was considered statistically significant.

Table 3 Univariate and multivariate analysis of risk factors in ^{13}C-UBT positive and negative groups.

variable	Univariate Logistic Regression		Multivariate Logistic Regression	
	OR (95% CI)	*P* value	Adjusted OR (95% CI)	*P* value
Gander Male *vs*. Female	2.172 (1.014—4.654)	0.046		
Age <40 *vs*. 60	0.229 (0.054—0.973)	0.046		
40—60 *vs*. >60	0.211 (0.062—0.714)	0.012		
DBP	1.035 (1.005—1.066)	0.020	1.032 (1.000—1.064)	0.052
SBP	1.021 (1.002—1.040)	0.027		
CEA	1.674 (1.126—2.487)	0.011	1.746 (1.128—2.700)	0.012
CA724	1.077 (1.012—1.145)	0.019	1.096 (1.024—1.173)	0.009
WBC	1.272 (1.001—1.617)	0.049		
MTHFR C677T *vs*. MTHFR C677 C/T	2.632 (1.113—6.225)	0.028	2.678 (1.044—6.874)	0.040

Abbreviations: OR odds ratio, 95%CI 95% confidence interval, DBP diastolic blood pressure, SBP systolic blood pressure, CEA carcinoma embryonic antigen, CA724 carbohydrate antigen724, WBC white blood cell.
P value < 0.05 was considered statistically significant.

Statistical interaction between MTHFR C677T polymorphism and ^{13}C-UBT results in CEA, CA199 and CA724 levels

After correcting the influence of other factors, the results of factorial analysis showed that the CEA levels of male (2.13 ± 0.11 ng/ml) was significantly higher than those of female (1.66 ± 0.14 ng/ml, $P = 0.002$). Meanwhile, the CEA levels of the group aged >60 (2.41 ± 0.22 ng/ml) were significantly higher than those of other groups (aged <40: 1.51 ± 0.18 ng/ml, aged 40—60: 1.78 ± 0.09 ng/ml, $P = 0.009$), Table 5. There was a significant interaction between MTHFR C677T polymorphism and ^{13}C-UBT results in CEA levels. The presence of T allele led to a decrease in CEA levels when ^{13}C-UBT was positive, while the presence of T allele led to an increase in CEA levels when ^{13}C-UBT was negative ($P = 0.004$), Table 5, Fig. 2A. The CA199 levels of female (15.63 ± 2.30 U/ml) were significantly higher than those of male (9.25 ± 1.84 U/ml, $P = 0.010$), and the CA724 levels of UBT positive (5.85 ± 0.93 U/ml) were significantly higher than those of negative (3.18 ± 0.92 U/ml, $P = 0.020$). However, there was no significant interaction between MTHFR C677T polymorphism and UBT results in CA199 and CA724 levels, Table 5 and Fig. 2B and C.

Discussion

In our study, the infection rate of *H.pylori* was 38.20%, which was lower than 47% reported by Nagy P, Johansson S,

Table 4 The additive interactions between MTHFR C677T polymorphism and risk factors with^{13}C-UBT results.

Exposure	OR (95% CI)	RERI (95% CI)	AP (95% CI)	S (95% CI)	*P* value
MTHFR C677T polymorphism	9.628 (0.261—355.538)	0.155 (−0.016—7.964)	0.015 (−0.04—0.040)	1.019 (0.994—1.045)	0.333
DBP	1.090 (0.993—1.197)				
MTHFR C677T polymorphism & DBP	0.973 (0.929—1.019)				
MTHFR C677T polymorphism	11.020 (2.852—42.584)	−1.407 (−3.737~-0.277)	−0.631 (−1.941~-0.079)	0.468 (0.072—0.910)	0.026
CEA	14.711 (3.712—58.304)				
MTHFR C677T polymorphism & CEA	0.326 (0.168—0.634)				
MTHFR C677T polymorphism	1.044 (0.548—1.988)	0.041 (−0.024—0.153)	0.323 (−0.022—0.152)	1.032 (−1.089—3.199)	0.136
CA724	1.005 (0.837—1.206)				
MTHFR C677T polymorphism & CEA724	1.04 (0.942—1.146)				

Abbreviations: OR odds ratio, 95%CI 95% confidence interval, S synergy index, RERI relative excess risk of interaction, AP attributable proportion, DBP diastolic blood pressure, CEA carcinoma embryonic antigen, CA724 carbohydrate antigen724.
S < 1 and RERI <0 were defined as negative additive interactions. The absolute value of AP was the proportion of all ^{13}C-UBT positive patients that can be attributed to the interaction of the two factors. *P* value < 0.05 was considered statistically significant.

Table 5 The statistical interactions between MTHFR C677T polymorphism and ^{13}C-UBT positive/negative on CEA, CCA199 and CA724 levels.

Variation sources	DOF	Anova sum of squares	Mean square	F value	Pr > F
CEA Level					
Gender	1	6.40495752	6.40495752	9.86	0.002
Age subgroup	2	6.34313373	3.17156687	4.88	0.009
^{13}C-UBT positive/negative	1	0.15807094	0.15807094	0.24	0.623
MTHFR C677T	2	0.23877914	0.11938957	0.18	0.832
MTHFR C677T*^{13}C-UBT positive/negative	2	7.59083895	3.79541948	5.85	0.004
CA199 Level					
Gender	1	1206.172895	1206.172895	6.71	0.010
Age subgroup	2	290.846119	145.423060	0.81	0.447
^{13}C-UBT positive/negative	1	476.913076	476.913076	2.65	0.106
MTHFR C677T	2	87.775419	43.887709	0.24	0.784
MTHFR C677T*^{13}C-UBT positive/negative	2	319.821314	159.910657	0.89	0.413
CA724 Level					
Gender	1	5.1593504	5.1593504	0.15	0.699
Age subgroup	2	26.1993490	13.0996745	0.38	0.684
^{13}C-UBT positive/negative	1	190.8321394	190.8321394	5.55	0.020
MTHFR C677T	2	14.2538377	7.1269189	0.21	0.813
MTHFR C677T*^{13}C-UBT positive/negative	2	62.2266539	31.1133270	0.90	0.407

Abbreviations: DOF degree of freedom, CEA carcinoma embryonic antigen, CA199 carbohydrate antigen 199, CA724 carbohydrate antigen 724. ^{13}C-UBT ^{13}C urea breath test.
Prob > F means *P* value, < 0.05 means significant, rejecting the original hypothesis.

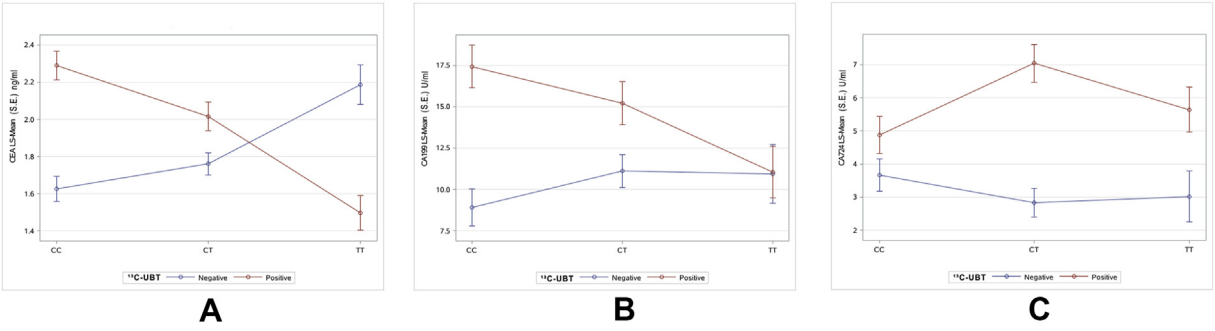

Figure 2 Least Squares means line plot of interaction between MTHFR C677T polymorphism and ^{13}C-UBT results on CEA, CA199, CA724 levels. Main effects of MTHFR C677T polymorphism subgroup was CC genotype, CT genotype and TT genotype. Main effects of ^{13}C-UBT results subgroup was ^{13}C-UBT positive (red line) and ^{13}C-UBT negative (blue line). When the red line and the blue line crossed, there was statistical interaction between MTHFR C677T polymorphism and ^{13}C-UBT results on CEA, CA199, CA724 levels. **(A)** MTHFR C677T polymorphism and ^{13}C-UBT results had statistical interaction on CEA levels ($P = 0.004$). **(B)** MTHFR C677T polymorphism and ^{13}C-UBT results had not statistical interaction on CA199 levels ($P = 0.413$). **(C)** MTHFR C677T polymorphism and ^{13}C-UBT results had not statistical interaction on CA724 levels ($P = 0.407$), but the mean levels of CA724 in ^{13}C-UBT positive (red line) was significantly higher than that in ^{13}C-UBT negative (blue line) ($P = 0.020$).

et al.[27] This may due to the different inclusion criteria of the two studies. MTHFR C677T polymorphism detection and gastrointestinal tumor markers were not routine screening items.[28] People who underwent the screening were likely to be in better socio-economic condition and had reduced infection rates.[29] Meanwhile, the infection rate of *H.pylori* in men was significantly higher than that in women. With the increase of age, the infection rate also increased, which was similar to the previous results.[12,30]

In our study, CC and CT genotypes were predominant in the Han population in Chongqing, which was similar to the results of several studies in Asia.[19,31] TT genotype was more likely to be associated with *H.pylori* infection than CC and CT genotypes (Table 2). This might because of the T SNP mutation, leading to the decrease of MTHFR enzyme activity and affecting the methylation of human gastric mucosa cells, which results in the integrity of gastric mucosa and fosters the colonization of *H.pylori*.[32] And *H.pylori* infection will further affect folic acid absorption and metabolism,[33,34] leading to the damage of gastric mucosa and inducing local chronic inflammation.[13,35] This was consistent with the results of our previous study that WBC in *H.pylori* infected subjects was higher than that in uninfected subjects[23] (Table 1).

Recent studies have shown that MTHFR C677T polymorphism may lead to abnormal DNA and/or RNA synthesis, and can repair abnormalities and chromosome damage in gastric mucosa infected by *H.pylori*,[36] resulting in dysfunction of MTHFR enzyme and then affecting methylation[29,32] and making conditions for other carcinogenic factors to cause abnormal methylation of proto-oncogenes,[17] thus leading to the occurrence and development of gastrointestinal cancer.[37] In our study, *H.pylori* infection was associated with increased CEA and CA724 levels but not associated with CA199 levels (Table 3), which was similar to the study of Xu MY, Cao B, et al.[30] Meanwhile, TT genotype was more closely related to *H.pylori* infection than CC and CT genotypes (Table 3). Therefore, our study suggested that TT genotype was a risk factor for *H.pylori* infection in Chinese Han population in Chongqing.

In our study, MTHFR C677T polymorphism had a negative additive interaction with CEA in *H.pylori* infection (Table 4). Although the CEA levels of all subjects were within the normal range, it still indicated that different genotypes infected with *H.pylori* would cause different degrees of elevated CEA levels. Further analysis showed that the presence of T allele led to a decrease in CEA levels for *H.pylori* infection, while the presence of T allele led to an increase in CEA levels for *H.pylori* non-infection, suggesting that the TT genotype was a risk factor for *H.pylori* infection. However, when *H.pylori* was negative, the TT genotype was a risk factor for elevated CEA levels. This is similar to a study that TT genotype increases moderate to severe atrophic gastritis and gastric mucosal intestinal metaplasia in Chinese Han population without *H.pylori* infection.[38] Although many studies have shown that MTHFR C677T polymorphism was associated with gastrointestinal cancer, the specific relationship was not clear.[18,38,39] We speculated that the specificity of MTFHR C677T polymorphism for gastrointestinal cancer during *H.pylori* infection might be related to the site of cancer origin.[19,40] The previous studies have reported that there was association between MTHFR C677T polymorphism and gastric cancer in Chongqing, and CT and TT genotypes were protective factors for gastric cardia cancer, and TT genotype was a risk factor for gastric body cancer.[41] In addition, our research also found that *H.pylori* infection was still a risk factor for increased CA724 levels. CA724 was suggested for management of gastrointestinal cancer as early as two decades.[21] Therefore, we hypothesized that MTHFR C677T polymorphism might have different effects on cancers in different parts of the gastrointestinal tract in the case of *H.pylori* infection.[41,42] But further animal and clinical trials are required to prove this.

Our study confirmed that *H.pylori* infection was related to gender and age.[38] Our study also found that the blood pressure (BP) in patients with *H.pylori* infection were higher than those without *H.pylori* infection. It was suggested that *H.pylori* infection might be a risk factor for cardiovascular and cerebrovascular diseases,[43,44] although the BP was in the normal range. Previous studies have also shown that *H.pylori* infection was positively correlated with the prevalence of hypertension in Chinese adults.[45] Therefore, further experiments are required to explore whether *H.pylori* infection and MTHFR C677T polymorphism have an interaction effect on cardiovascular and cerebrovascular diseases.

All subjects included in this study were from a healthy population, so it was not possible to assess the effects of MTHFR C677T polymorphism and *H.pylori* on different gastrointestinal benign and malignant diseases. Yet, it indicates that the screening of MTHFR C677T polymorphism, ^{13}C-UBT and gastrointestinal tumor markers (CEA, CA199 and CA724) in healthy population can detect the susceptible population of *H.pylori* and gastrointestinal cancers early. Next, we plan to investigate the specificity and sensitivity of MTHFRC677T polymorphism, ^{13}C-UBT combined with gastrointestinal tumor markers test in *H. pylori* susceptible population and early screening of gastrointestinal cancer. We also plan to further study the influence of MTHFR C677T polymorphism and *H.pylori* infection on benign and malignant gastrointestinal lesions.

Conclusions

Based on the results of this study, we found that TT genotype was a susceptibility factor for *H.pylori* infection. But in the presentation of *H.pylori* infection, MTHFR C677T polymorphism might have different effects on different parts of gastrointestinal cancer. The screening of MTHFR C677T polymorphism, combined with the screening of ^{13}C-UBT and gastrointestinal tumor markers can detect the susceptible population of *H. pylori* and gastrointestinal cancers.

Conflict of Interests

Authors declare no conflict of interests.

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