Association of MTHFR C677T polymorphism with severity and localization of chronic atrophic gastritis: a case control study

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Abstract

Background

Previous reports indicate that the methylenetetrahydrofolate reductase (MTHFR) 677C > T polymorphism plays a role in gastric cancer. However, whether it influences the development and progression of atrophic gastritis remains unclear. We aim to determine the possible association between the MTHFR 677C > T polymorphism and the severity of atrophic gastritis.

Methods

A total 128 patients with atrophic gastritis were included in the study. The presence and severity of gastric atrophy was assessed by histology using OLGA and OLGIM Gastritis Staging System. MTHFR 677C > T genotyping was performed by digital fluorescence molecular hybridization. Categorical variables were analyzed by percentages using the χ^2 test.

Results

In this study, the TT genotype was significantly more frequent among patients aged ≤ 44 years (age ≤ 44 years vs. >44 years, P = 0.039). Patients with the TT genotype showed a higher ratio of incisura with atrophy or intestinal metaplasia (TT vs. CC + CT, P = 0.02). Furthermore, the TT genotype was associated with more severe lesions compared with the CC + CT genotypes (TT vs. CC + CT for atrophy: odds ratio [OR] = 2.18, P = 0.07; for intestinal metaplasia: OR = 3.39, P = 0.02; for moderate-to-severe lesions: OR = 3.84, P = 0.02). OLGA and OLGIM stages III-IV were observed more frequently in patients with the TT genotype compared with the CC + CT genotypes (for OLGA: OR = 3.98, P = 0.004; for OLGIM: OR = 2.45, P = 0.04).

Conclusions

The MTHFR 677C > T TT genotype showed an increased risk of moderate-to-severe lesions by OLGA and OLGIM stages, and these results suggest that the MTHFR C677T polymorphism may serve as a predictive marker for precancerous gastric lesions, especially in patients aged ≤ 44 years.

Introduction

Gastric cancer is one of the most common cancers and the third leading cause of cancer-related death worldwide [1]. According to the Correa's theory, chronic inflammation of the gastric mucosa induces a cascade of precancerous conditions (chronic atrophic gastritis [AG], intestinal metaplasia [IM], and dysplasia), which may result in the development of gastric cancer[2]. AG is a chronic disorder characterized by the loss of the oxyntic glands and is accompanied by fibrosis or fibromuscular

proliferation in the lamina propria, or their replacement with pseudo-pyloric or IM[3, 4]. Therefore, early supervision of AG could reduce the incidence of gastric cancer[5].

Cancer development is a result of intricate interactions between genetic and environmental factors. Epigenetic changes such as DNA methylation have a role in cancer development and progression[6]. 5,10-Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate metabolism and is involved in DNA, RNA, and protein methylation[7]. The polymorphism C677T in the MTHFR gene has been shown to be associated with various tumors, as this change in sequence reduces the activity of this enzyme[8, 9]. Indeed, individuals with the TT and CT genotypes have mildly higher homocysteine levels than CC homozygotes[10]. In addition, hyperhomocysteinemia is an emerging risk factor for various precancerous conditions according to its observed effects on morbidity and mortality among patients[11, 12]. Previous studies have shown that gastric diseases may be a contributing factor to hyperhomocysteinemia, possibly via nutrient malabsorption[13]. As a result, AG patients with the TT genotype have a much higher risk of gastric cancer. Therefore, the MTHFR C677T polymorphism might be useful in predicting the development and severity of gastrointestinal cancer, especially in Asian populations[14–16]. However, the role of the MTHFR C677T polymorphism in gastric precancerous lesions is still unclear. Therefore, in view of the fact that the Operative Link on Gastritis Assessment (OLGA) and Operative Link on Gastric Intestinal Metaplasia Assessment (OLGIM) staging systems ranks the risk for gastric cancer in progressive stages (0-IV)[17], in this study, we investigated the association between the MTHFR C677T polymorphism and AG in Chinese patients using OLGA and OLGIM stages.

Materials And Methods

Trial design and subjects

This study was designed as a single-center, cross sectional observational trial. Consecutive patients who underwent endoscopy for evaluation of the cause of any abdominal symptom or screening for upper gastrointestinal cancer were recruited in the First Affiliated Hospital of Nanjing Medical University from November 2018 to December 2019. A total of 128 patients suffering from chronic AG were diagnosed for the first time and had not received any previous treatments, and each diagnosis was confirmed after endoscopy by pathological examination. The exclusion criteria were as follows: (1) *Helicobacter pylori* positivity (an independent contributing factor to the development of AG); (2) previous *H. pylori* eradication; (3) intake of antibiotics, proton pump inhibitors, or H2-receptor blockers within the previous month; and (4) use of drugs affecting the plasma folic acid level. *H. pylori* infection was assessed by the ^{13}C-urea breath test (UBT). The study protocol was reviewed and approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University. Written informed consent was obtained from each participant. This trial was completed and registered with ClinicalTrials.gov (ChiCTR1900020815,Chinese Clinical Trial Registry).

Assessment and grading of AG

All pathological diagnoses were made by histological examination of gastric biopsy samples (corpus, antrum and incisura) following the updated Sydney System[18]. Biopsies were fixed in 10% formalin, sectioned, and stained using hematoxylin and eosin. Endoscopic gastric atrophy was assessed according to the Kimura-Takemoto classification[19]. The stage of gastritis was calculated by OLGA and OLGIM staging systems[17], in which a higher stage number represents a more severe lesion. Biopsies were assessed by two independent pathologists who were blinded to all other patient characteristics. In case of disagreement, the biopsies were re-examined by a third pathologist until agreement was reached.

Determination of plasma folic acid, pepsinogen I, pepsinogen II, gastrin-17, and homocysteine levels

Blood samples were obtained from 128 patients for the measurement of folic acid, pepsinogen I, pepsinogen II, gastrin-17, and homocysteine concentrations using routine tests performed in the Department of Laboratory Medicine, the First Affiliated Hospital of Nanjing Medical University. Pepsinogen I, pepsinogen II, and gastrin-17 levels in the serum samples were determined using a GastroPanel ELISA kit (Biohit HealthCare, Helsinki, Finland) according to the manufacturer's instructions. The absorbance in each sample was determined at 450 nm using a microplate reader (SpectraMax® Plus 384; Molecular Devices, LLC, Sunnyvale, CA, USA). Assay results were then analyzed using GastroSoft 1.51b for Excel (Biohit HealthCare) to obtain the serum sample concentrations. Plasma homocysteine levels were measured by high-performance liquid chromatography, and plasma folic acid levels by radioimmunoassay. The normal ranges for homocysteine and folic acid adopted by our laboratory were 5.0–15.0 μmol/L and 7.0–45.1 nmol/L, respectively. High homocysteine plasma levels were defined as a fasting serum total homocysteine level greater than 15.0 μmol/L. Folates were defined as low at a concentration less than 6.0 ng/mL.

DNA extraction and genotyping of MTHFR polymorphism

Genomic DNA was extracted from blood samples using a column extraction kit (QIAGEN Inc., Valencia, CA, USA). The DNA content was quantified using a Nanodrop spectrophotometer (BioLab). For MTHFR C677T genotyping, digital fluorescence molecular hybridization (DFMH) was performed using a commercial kit from Sino Era Genotech (Beijing, China), according to the manufacturer's instructions. DFMH genotyping uses hybridization of molecular beacon probes specific for each allele (C or T). The samples were then analyzed with the TL998A real-time PCR system (Tianlong, Xi'an, China).

Statistical analysis

Categorical variables were analyzed by percentages using the χ^2 test. Continuous variables were described by mean values with standard deviations and were compared between groups using Student's t-test. Associations between the clinical parameters were evaluated by Spearman's rank test. The agreement between endoscopic and histological findings regarding the grade of AG was assessed based on the kappa value. Results were considered statistically significant if the P-value was less than 0.05. The Statistical Package for the Social Sciences (SPSS Inc., Chicago, IL, USA) software version 25.0 was used for the statistical analyses.

Results

TT genotype is more frequent among younger AG patients

The study group consisted of 128 AG patients (50.00% men, age range 27–80 years, mean age 55.1 ± 10.2 years). The clinical characteristics of patients are shown in Table 1. The genotypes and frequencies observed in our population were TT in 21.88% (28/128) of patients, CT in 53.91% (69/128), and CC in 24.22% (31/128). This distribution followed the Hardy-Weinberg equilibrium (P = 0.817). Generally, the allele frequencies of the MTHFR C677T genotypes should be stable for people of all age groups. However, in patients 44 years or younger (≤ 44 years), the frequency of the TT genotype was significantly higher than that in older patients greater than 44 years (41.18% vs. 18.92%; P = 0.039; Fig. 1). For the 17 patients aged 27–44 years, the MTHFR C677T genotypes and frequencies were TT in 41.18% (7/17), CT in 29.41% (5/17), and CC in 29.41% (5/17). In the 111 patients older than 44 years, the genotypes and frequencies were TT in 18.92% (21/111), CT in 57.66% (64/111), and CC in 23.42% (26/111). In addition, the pepsinogen I to pepsinogen II ratio (PGR) was significantly higher among patients aged 44 years and older compared to that among patients older than 44 years (13.0 ± 4.2 vs. 10.9 ± 3.9; P = 0.045).

Table 1
Patients characteristics

Characteristic	CC	CT	TT
Number, n **(%)**	31 (24.22%)	69 (53.91%)	28 (21.88%)
Age (years), mean ± SD	55 ± 10	56 ± 9	54 ± 13
27–44, *n* (%)	5(29.41%)	5(29.41%)	7(41.18%)
45–62, *n* (%)	17(21.52%)	48(60.76%)	14(17.72%)
63–80, *n* (%)	9(28.13%)	16(50.00%)	7(21.88%)
Male	15	34	15
Family history of gastric cancer in first-degree relatives	7	19	4
Smoking status			
Never	23	50	19
Current/Former	8	19	9
Alcohol status			
Never	23	53	19
Current/Former	8	16	9
Gastrin-17 (pmol/L)	8.2 ± 20.6	5.8 ± 11.4	3.8 ± 6.6
Pepsinogen I (μg/L)	104.8 ± 54.8	103.6 ± 69.5	81.1 ± 31.3
Pepsinogen II (μg/L)	10.5 ± 6.3	10.1 ± 8.6	8.4 ± 3.8
PGR	11.3 ± 4.7	11.4 ± 4.0	10.5 ± 3.4
BMI (kg/m^2)	22.4 ± 2.8	22.3 ± 2.8	22.6 ± 2.5

SD: standard deviation; PGR: pepsinogen I and pepsinogen II ratio; Hcy: homocysteine; BMI: body mass index.

AG may be main cause of hyperhomocysteinemia in AG patients rather than MTHFR polymorphism

As shown in Table 2, the mean levels of Hcy in patients with the CC, CT, and TT genotypes were 11.7 ± 5.4 μmol/L, 12.9 ± 5.6 μmol/L and 13.5 ± 6.0 μmol/L, respectively. The highest levels of Hcy were observed in patients with the TT genotype followed by those with the CT and CC genotypes ($P > 0.05$). Also, no statistical difference was observed in the incidence of hyperhomocysteinemia (> 15 μmol/L) among patients with the different MTHFR C677T genotypes ($P = 0.82$). However, folic acid deficiency (≤

6 ng/mL, as defined in ref [20]) was observed more often in patients with the TT genotype compared with the CT and CC genotypes (P = 0.001).

Table 2
Baseline folic acid and Hcy levels in AG patients stratified by MTHFR C677T genotypes.

Characteristic	CC	CT	TT	P
Folic acid (nmol/L)	42.5 ± 12.6	36.9 ± 13.9	32.6 ± 17.7	> 0.05
Hcy (μmol/L)	11.7 ± 5.4	12.9 ± 5.6	13.5 ± 6.0	> 0.05
Hyperhomocysteinemia				
Yes	8	22	8	0.819
No	23	47	20	
Folic acid deficiency				
Yes	1	9	11	0.001
No	30	60	17	

As shown in Table 3, in our population, 29.69% (38/128) of AG patients had hyperhomocysteinemia and 16.41% (21/128) of AG patients had folic acid deficiency. We found that patients with folic acid deficiency had a significantly higher incidence of hyperhomocysteinemia compared with patients without folic acid deficiency (52.38% [11/21] vs. 25.23% [27/107], P = 0.013).

Table 3
Association between hyperhomocysteinemia and folic acid deficiency

		Hyperhomocysteinemia		
		No	Yes	P
Folic acid deficiency	No	80	27	0.013
	Yes	10	11	

Association between high-risk OLGA/OLGIM stages III-Ⅳ and MTHFR C677T polymorphism

The results regarding the influence of the MTHFR C677T polymorphism on lesion status in the gastric mucosa of AG patients are presented in Table 4. The antrum region showed the highest frequency of atrophy or IM (86.72%, 111/128), followed by the incisura (37.50%, 48/128) and corpus (8.59%, 11/128). No association was observed between the MTHFR C677T polymorphism and lesions in the corpus and antrum (P > 0.05). However, in the incisura part of the stomach, patients with the TT genotype showed a higher susceptibility to develop lesions including atrophy or IM (CC + CT vs. TT: 40.00% [40/100] vs.

64.29% [18/28], P = 0.02). OLGA and OLGIM stages III-IV were observed more frequently in patients with TT genotype compared with the CC + CT genotypes (for OLGA: CC + CT vs. TT: 16.50% [17/103] vs. 44.00% [11/25], P = 0.003; for OLGIM: CC + CT vs. TT: 16.05% [13/81] vs. 31.91% [15/47], P = 0.036).

Table 4

Baseline features of lesion status in the gastric mucosa of patients with AG stratified by MTHFR C677T genotype

	Genotypes				C allele dominance					
	CC	CT	TT	P	CC + CT	TT	P	OR	95%CI	P
Lesions in biopsies										
Incisura										
yes	10	30	18	0.04	40	18	0.02			
no	21	39	10		60	10				
Antrum										
yes	28	62	21	0.15	90	21	0.06			
no	3	7	7		10	7				
Corpus										
yes	1	5	5	0.17	6	5	0.22			
no	30	64	23		94	23				
Atrophy										
Absent/Mild	19	43	12	0.19	62	12	0.07	2.18	0.93–5.09	0.07
Moderate/Severe	12	26	16		38	16				
IM										
Absent/Mild	14	34	6	0.04	48	6	0.01	3.39	1.27–9.06	0.02
Moderate/Severe	17	35	22		52	22				
Moderate-to-severe lesions										
Absent/Mild	11	28	4	0.04	39	4	0.01	3.84	1.24–11.90	0.02
Moderate/Severe	20	41	24		61	24				
OLGA										

Lesions included atrophy or intestinal metaplasia; atrophy: atrophy located in any one biopsy; intestinal metaplasia: intestinal metaplasia located in any one biopsy; moderate-to-severe lesions: moderate to severe intestinal metaplasia, moderate to severe atrophy or low-grade intraepithelial neoplasia in any one location; CI: confidence interval.

	Genotypes				C allele dominance					
I-II	26	60	17	0.119	86	14	0.003	3.98	1.54–10.23	0.004
III-	5	9	11		17	11				
OLGIM										
I-II	20	48	13	0.162	68	32	0.036	2.45	1.05–5.76	0.039
III-	11	21	15		13	15				

Lesions included atrophy or intestinal metaplasia; atrophy: atrophy located in any one biopsy; intestinal metaplasia: intestinal metaplasia located in any one biopsy; moderate-to-severe lesions: moderate to severe intestinal metaplasia, moderate to severe atrophy or low-grade intraepithelial neoplasia in any one location; CI: confidence interval.

For moderate-to-severe lesions (moderate-to-severe IM, moderate-to-severe atrophy or low-grade intraepithelial neoplasia in any one location), TT homozygous patients were at an increased risk compared with CC + CT patients (P = 0.01). In addition, TT homozygous patients had an increased risk of IM at any location compared with CC + CT patients (P = 0.01). Although not statistically significant (P = 0.07), a trend towards a higher frequency of more severe atrophy at any location was observed in those with the TT genotype (CC + CT vs. TT: 38.00% vs. 57.14%).

The MTHFR C677T polymorphism was an independent predictor of the severity of lesions as shown in Table 4 (TT vs. CC + CT for atrophy: odds ratio [OR] = 2.18; 95% confidence interval [CI], 0.93–5.09; P = 0.07; for IM: OR = 3.39; 95% CI, 1.27–9.06; P = 0.02; for moderate-to-severe lesions: OR = 3.84; 95% CI, 1.24–11.90; P = 0.02; for OLGA: OR = 3.98; 95% CI, 1.54–10.23; P = 0 .004; and for OLGIM: OR = 2.45; 95% CI, 1.05–5.76; P = 0.039).

Weak correlation between C-1/C-2 of endoscopic atrophy and OLGA stages I-II

The Kimura-Takemoto endoscopic classification (C-1, C-2, C-3, O-1, O-2, O-3) has been widely used in some Eastern countries for the assessment and grading of AG[21]. In our study, the MTHFR C677T polymorphism was an independent predictor of the severity of lesions in patients stratified according to the OLGA and OLGIM systems. However, there was no statistical difference in the severity of endoscopic gastric atrophy between those with the TT and CT + CC genotypes according to the Kimura-Takemoto endoscopic classification (P = 0.40, Fig. 2). In our study, based on the Kimura-Takemoto endoscopic classification, 92.59% patients were C-1 or C-2 and 80.47% patients of patients stratified according to the OLGA system were stages I-II. Based on these classifications, the strength of agreement between the C-1 or C-2 levels on endoscopic atrophy and OLGA stages I-II for the histological atrophy was fair, with a kappa value of 0.29 (95% CI, 0.06–0.50). In addition, correlations of C-1 or C-2 levels on endoscopic atrophy and stages I-II of OLGA were observed (Spearman's rho = 0.31, P = 0.014).

Discussion

DNA methylation is a pivotal epigenetic modification that can be altered in precancerous lesions[22]. As MTHFR is the key gene and metabolite in the one-carbon metabolism pathway that allows for the metabolism of homocysteine and the provision of methyl groups[23, 24], the MTHFR C677T polymorphism may be considered as a reliable factor for predicting the prognosis of gastric precancerous lesions[25, 26]. The reduced activity of the MTHFR enzyme resulting from TT mutation has been associated with alterations in methylation patterns and potentially aberrant DNA synthesis, repair, and chromosomal damage[27]. This study evaluated the degree of atrophy and IM in different biopsies to examine whether the TT genotype confers an increased risk for developing moderate-to-severe lesions (moderate-to-severe atrophy or IM in any one biopsy). In addition, patients with the TT genotype were found to be at a higher risk of OLGA and OLGIM stages III-Ⅳ compared to patients with the CC + CT genotypes. It has been shown previously that OLGA stages I-II are associated with a lower risk while stages III-IV are associated with a higher risk of gastric cancer[28, 29]. Thus, in our study, the TT genotype was a risk factor for gastric precancerous lesions. It is noteworthy to mention that conflicting results have been reported on the influence of the MTHFR C677T polymorphism on precancerous lesions or cancer. Some studies have shown an increased risk of gastric cancer development among Asians and Caucasians[14, 30], while others studies have reported a negative association or null results[31, 32]. Conflicting results indicate that population-specific and geographical factors may account for this phenomenon.

In addition, we recommend the incisura biopsies should be routinely included in the biopsy sampling protocol for patients with the TT genotype for further screening of gastric cancer risk. The incisura is the main region for the early-onset of atrophic-metaplastic transformation[33]. It may undergo more severe atrophic, metaplastic, and chronic inflammatory changes than the antrum and corpus [34, 35].

A cross-sectional serological study performed in Sweden showed an age-related trend with an increasing prevalence of AG in adults aged 35–44 years compared to those older than 44 years[36]. The morbidity age for AG patients seems to be younger than previously thought. Previous studies suggested that the increasing prevalence of overweight and obese patients might contribute to this unexpected trend[36, 37]. In our AG population, we did not find such an association between the severity of AG and overweight or obesity (BMI shown in Table 1, $P > 0.05$). These observations in our study may be due to the fact that we did not establish a control group in the general population for comparison with AG patients, as was done in the study by Song et al[36]. However, when we divided patients into two age groups (27–44 years and 45–80 years), the frequency of the TT genotype was much higher in the younger age group than in the older age group, indicating that AG patients with the TT genotype might have a younger morbidity age and a longer duration of illness. As a result, AG patients with the TT genotype may suffer from more severe gastric diseases. Previous studies have confirmed that aging is an independent risk factor for AG progression to gastric cancer[38]. In general population, the prevalence of AG in persons over 40 years is double that in those under 40 years[39]. In our study, however, the frequency of the TT genotype was lower in patients over 44 years of age. This may be due to some important transition of the dominant

mechanism. Further research on the difference in MTHFR C677T genotype frequency in these two AG age groups is warranted.

Folate deficiencies may increase cancer risk by inducing uracil misincorporation during DNA synthesis, leading to chromosomal damage, DNA strand breaks, and impaired DNA repair, as well as DNA hypomethylation[27]. The data from our study suggest that AG patients with the TT genotype have a higher rate of folate deficiency compared with those with the CC + CT genotypes (P = 0.001), which will theoretically bring a higher rate of hyperhomocysteinemia. However, in our study, this was not the case. No significant difference was observed (P = 0.819), indicating that the AG may be more of a direct cause of hyperhomocysteinemia, which is in good agreement with previous research[13]. This phenomenon suggests that the AG factor may play a more important role in the presence of hyperhomocysteinemia than the MTHFR C677Tgenotype. As a result, AG patients are suggested to receive folic acid supplementation to reduce the risk of gastric cancer.

Although not statistically significant, patients with the TT genotype in our study showed a trend towards a higher frequency of more severe lesions according to the Kimura-Takemoto endoscopic classification. In addition, some studies have reported that the severity of endoscopic gastric atrophy according to the Kimura-Takemoto endoscopic classification is correlated with OLGA and OLGIM stages[21, 40]. In our study, however, the correlation was weak with a kappa value of 0.29.

To our knowledge, our study provides the first observation of an association between the MTHFR C677T polymorphism and gastric precancerous lesions. The results of this study suggest that the TT genotype is associated with more severe lesions. Based on our findings we propose that biopsy of the incisura in AG patients with the TT genotype will be useful for further screening of gastric cancer risk, especially for patients younger than 44 years. AG itself may be a contributing factor towards hyperhomocysteinemia. In addition, patients should be cautious about the potential risk of cardiovascular diseases in view of the association between hyperhomocysteinemia and vascular injury[41].

In conclusion, the effects of the MTHFR C677T polymorphism on gastric precancerous lesions have been systematically examined in this study. Based on our findings, we propose that MTHFR C677T genotyping could be useful in identifying patients at increased risk for moderate-to-severe atrophy or IM. Such screening may be valuable clinically in assessing the risk and prognosis of gastric precancerous lesions. In addition, AG patients should receive appropriate folic acid supplementation to prevent hyperhomocysteinemia. Further standardized research including well-designed and strictly implemented trials are required to confirm that the MTHFR C677T genetic polymorphism is an independent predictor of the severity of AG.

Abbreviations

MTHFR
methylenetetrahydrofolate reductase
AG

atrophic gastritis
IM
intestinal metaplasia
OLGA
Operative Link on Gastritis Assessment
OLGIM
Operative Link on Gastric Intestinal Metaplasia Assessment
UBT
urea breath test
Hcy
homocysteine

Declarations

Availability of data and materials

All data are available without restriction. Researchers can obtain data by contacting the corresponding author. All data generated or analyzed during this study are included in this published article.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Siya Kong, MD; Feng Ye, PhD; Yini Dang, PhD; Yifei Hua, MD and Guoxin Zhang. PhD all contributed to this work. Study concept and design: Siya Kong, Feng Ye and Guoxin Zhang. Acquisition of data: Siya Kong, Yini Dang and Feng Ye. Analysis and interpretation of data: Siya Kong and Feng Ye. Drafting of the manuscript: Siya Kong. Critical revision of the manuscript for important intellectual content: all authors. Statistical analysis: Siya Kong and Yifei Hua. Figures and tables: Siya Kong. Obtained funding: Feng Ye

and Guoxin Zhang. Study supervision: Feng Ye and Guoxin Zhang. All authors had full access to all of the data and approved the final version of this manuscript submitted.

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Ethics approval and consent to participate

Its protocol was reviewed and approved by the ethics committee of the First Affiliated Hospital of Nanjing Medical University. This trial was completed and registered with www.chictr.org.cn (ChiCTR1900020815, Chinese Clinical Trial Registry). Participants provided written informed consent.

Consent for publication

Not applicable.

Publisher's Note

References

1. Bray F, et al. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
2. Rugge M, et al. Precancerous lesions in the stomach: from biology to clinical patient management. Best Pract Res Clin Gastroenterol. 2013;27(2):205–23.
3. Neumann WL, et al. Autoimmune atrophic gastritis–pathogenesis, pathology and management. Nat Rev Gastroenterol Hepatol. 2013;10(9):529–41.
4. Rugge M, et al. Gastric mucosal atrophy: interobserver consistency using new criteria for classification and grading. Aliment Pharmacol Ther. 2002;16(7):1249–59.
5. Kaji K, et al. Correction: Grading of Atrophic Gastritis is Useful for Risk Stratification in Endoscopic Screening for Gastric Cancer. Am J Gastroenterol. 2019;114(5):836.
6. Schubeler D. Function and information content of DNA methylation. Nature. 2015;517(7534):321–6.
7. Tran P, et al. Multiple transcription start sites and alternative splicing in the methylenetetrahydrofolate reductase gene result in two enzyme isoforms. Mamm Genome. 2002;13(9):483–92.

8. Yang B, et al. Geographical and ethnic distribution of MTHFR gene polymorphisms and their associations with diseases among Chinese population. Clin Genet. 2017;92(3):243–58.
9. Liew SC, Gupta ED. Methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism: epidemiology, metabolism and the associated diseases. Eur J Med Genet. 2015;58(1):1–10.
10. Hiraoka M, Kagawa Y. Genetic polymorphisms and folate status. Congenit Anom (Kyoto). 2017;57(5):142–9.
11. Sun A, Yu-Fong J, Chang, Chiang CP, *Smart link of chronic atrophic gastritis with atrophic glossitis, gastric parietal cell antibody positivity, vitamin B12 deficiency, and hyperhomocysteinemia*. J Formos Med Assoc, 2020. **doi**: **10.1016/j.jfma.2020.01.007**.
12. Hasan T, et al. Disturbed homocysteine metabolism is associated with cancer. Exp Mol Med. 2019;51(2):21.
13. Santarelli L, et al. Atrophic gastritis as a cause of hyperhomocysteinaemia. Aliment Pharmacol Ther. 2004;19(1):107–11.
14. Xia LZ, et al. Methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer susceptibility. World J Gastroenterol. 2014;20(32):11429–38.
15. Xie SZ, et al. Association between the MTHFR C677T polymorphism and risk of cancer: evidence from 446 case-control studies. Tumour Biol. 2015;36(11):8953–72.
16. Chen L, et al. Association between the MTHFR C677T polymorphism and gastric cancer susceptibility: A meta-analysis of 5,757 cases and 8,501 controls. Oncol Lett. 2015;10(2):1159–65.
17. Rugge M, Genta RM. Staging gastritis: an international proposal. Gastroenterology. 2005;129(5):1807–8.
18. Dixon MF, et al., *Classification and grading of gastritis. The updated Sydney System. International Workshop on the Histopathology of Gastritis, Houston 1994*. Am J Surg Pathol, 1996. **20**(10): p. 1161-81.
19. Kimura K, et al. Gastritis in the Japanese stomach. Scand J Gastroenterol Suppl. 1996;214:17–20. discussion 21 – 3.
20. de Benoist B. Conclusions of a WHO Technical Consultation on folate and vitamin B12 deficiencies. Food Nutr Bull. 2008;29(2 Suppl):S238-44.
21. Kono S, et al. Can endoscopic atrophy predict histological atrophy? Historical study in United Kingdom and Japan. World J Gastroenterol. 2015;21(46):13113–23.
22. Kulis M, Esteller M. *DNA methylation cancer* Adv Genet. 2010;70:27–56.
23. Yan S, et al. MTHFR C677T polymorphism contributes to the risk for gastric cancer. Tumour Biol. 2014;35(3):2123–32.
24. Mazzuca F, et al. Effect of MTHFR Polymorphisms on Gastrointestinal Cancer Risk in Italy. World J Oncol. 2015;6(4):394–7.
25. Boccia S, et al. Meta- and pooled analyses of the methylenetetrahydrofolate reductase C677T and A1298C polymorphisms and gastric cancer risk: a huge-GSEC review. Am J Epidemiol.

2008;167(5):505–16.
26. Tang M, et al. The methylenetetrahydrofolate reductase (MTHFR) C677T polymorphism and tumor risk: evidence from 134 case-control studies. Mol Biol Rep. 2014;41(7):4659–73.
27. Blount BC, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci U S A. 1997;94(7):3290–5.
28. Lahner E, et al. Chronic atrophic gastritis: Natural history, diagnosis and therapeutic management. A position paper by the Italian Society of Hospital Gastroenterologists and Digestive Endoscopists [AIGO], the Italian Society of Digestive Endoscopy [SIED], the Italian Society of Gastroenterology [SIGE], and the Italian Society of Internal Medicine [SIMI]. Dig Liver Dis. 2019;51(12):1621–32.
29. Rugge M, et al. Operative link for gastritis assessment vs operative link on intestinal metaplasia assessment. World J Gastroenterol. 2011;17(41):4596–601.
30. Lin J, et al. Aberrant DNA methylation of the P16, MGMT, and hMLH1 genes in combination with the methylenetetrahydrofolate reductase C677T genetic polymorphism and folate intake in gastric cancer. Genet Mol Res. 2014;13(1):2060–8.
31. Yoo JY, et al. Association Study between Folate Pathway Gene Single Nucleotide Polymorphisms and Gastric Cancer in Koreans. Genomics Inform. 2012;10(3):184–93.
32. Kim JK, et al. Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of stomach cancer in a Korean population. Anticancer Res. 2005;25(3b):2249–52.
33. Rugge M, et al. Atrophic gastritis: pathology and endoscopy in the reversibility assessment. Gut. 2003;52(9):1387–8.
34. Varbanova M, et al. Impact of the angulus biopsy for the detection of gastric preneoplastic conditions and gastric cancer risk assessment. J Clin Pathol. 2016;69(1):19–25.
35. Isajevs S, et al. The effect of incisura angularis biopsy sampling on the assessment of gastritis stage. Eur J Gastroenterol Hepatol. 2014;26(5):510–3.
36. Song H, et al. Increase in the Prevalence of Atrophic Gastritis Among Adults Age 35 to 44 Years Old in Northern Sweden Between 1990 and 2009. Clin Gastroenterol Hepatol. 2015;13(9):1592–600.
37. Hirabayashi M, et al. Effect of body-mass index on the risk of gastric cancer: A population-based cohort study in A Japanese population. Cancer Epidemiol. 2019;63:101622.
38. Sonnenberg A, Genta RM. Changes in the Gastric Mucosa With Aging. Clin Gastroenterol Hepatol. 2015;13(13):2276–81.
39. de Vries AC, et al. Epidemiological trends of pre-malignant gastric lesions: a long-term nationwide study in the Netherlands. Gut. 2007;56(12):1665–70.
40. Quach DT, et al. The severity of endoscopic gastric atrophy could help to predict Operative Link on Gastritis Assessment gastritis stage. J Gastroenterol Hepatol. 2011;26(2):281–5.
41. Fu Y, Wang X, Kong W. Hyperhomocysteinaemia and vascular injury: advances in mechanisms and drug targets. Br J Pharmacol. 2018;175(8):1173–89.

Figures

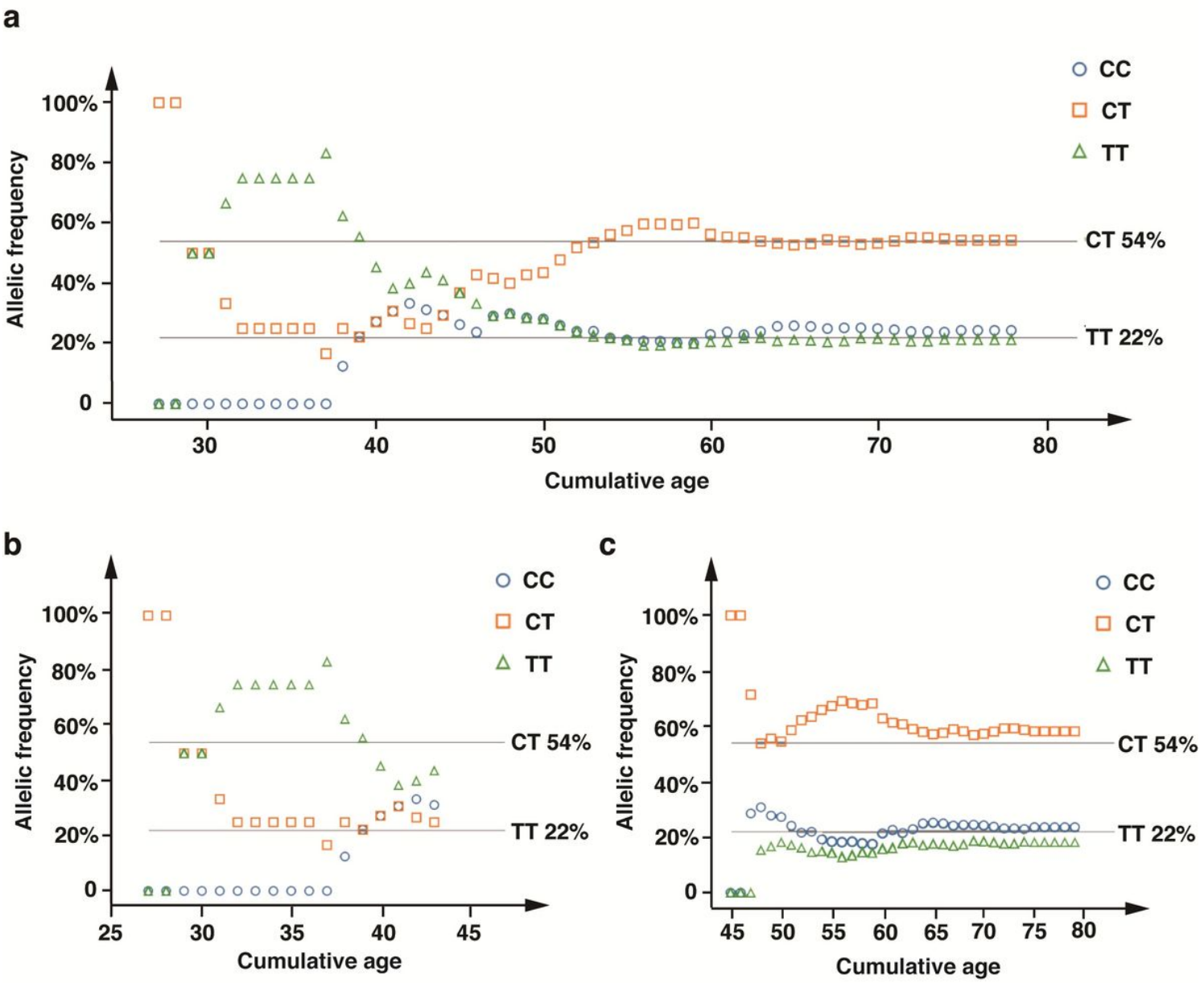

Figure 1

MTHFR C677T allelic frequency (Y-axis) with respect to cumulative age (X- axis) in different patient age groups. The MTHFR C677T allelic frequency in all patients included in the study. The frequency of the TT genotype was significantly higher among patients aged ≤44 years than among patients over 44 years (41.18% vs. 18.92%; P=0.039). The two horizontal lines represent the genotype frequencies in 128 patients in our study (TT=21.88%, CT=53.91%). b. The MTHFR C677T allelic frequency in patients aged 27–44 years. c. MTHFR C677T allelic frequency in patients aged 45–80 years.

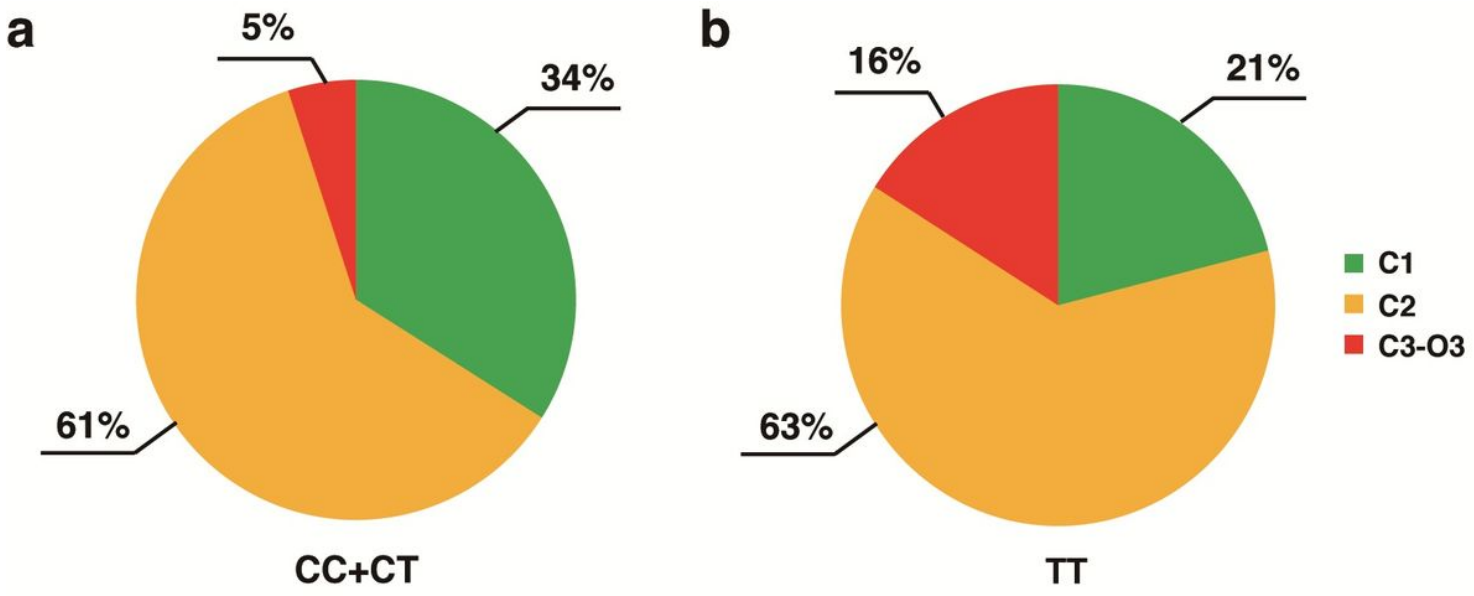

Figure 2

Distributions of patients with different Kimura-Takemoto endoscopic classifications among different MTHFR C677T genotypes. Patients with the TT genotype showed a trend toward a higher frequency of C-2 or C3-O3 lesions according to the Kimura-Takemoto endoscopic classification (CC+CT vs. TT: 66% vs. 79%, P=0.29).