Relationship between MTHFR gene polymorphism and risk of thrombosis in postoperative patients with colorectal cancer

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Abstract. An association between the methylenetetrahydrofolate reductase (MTHFR) C667T genotype and the risk of colorectal cancer, as well as a link between MTHFR gene polymorphism and thrombosis, have been revealed. However, the connection between MTHFR gene polymorphism and the risk of thrombosis in patients with colorectal cancer has remained to be fully elucidated. The present study investigated the link between MTHFR gene polymorphism and basic clinical data, postoperative D-dimer (DDi), postoperative thromboelastogram and postoperative thrombosis in 591 patients who underwent surgery for colorectal cancer. Postoperative DDi, thromboelastogram and postoperative thrombosis were not significantly different among patients with colorectal cancer and different MTHFR genotypes. While the results were 'negative', the present study may help physicians understand that it is not necessary to detect MTHFR polymorphism for therapeutic purposes. Regarding the danger of venous thrombosis, more focus should be placed on the standardized procedural enforcement system for deep vein thrombosis prevention for patients undergoing pelvic and abdominal surgery.

Introduction

Folic acid metabolism requires the conversion of 5-methylene tetrahydrofolic acid to 5-methyltetrahydrofolic acid by methylenetetrahydrofolate reductase (MTHFR) (1). The gene polymorphism of MTHFR has been linked to an increased risk of colorectal cancer (2,3). MTHFR may have a significant role in folic acid metabolism, converting 5,10-methylene tetrahydrofolic acid to 5-methyltetrahydrofolic acid, which supplies methyl groups for the conversion of homocysteine to methionine (4). The mutation at the MTHFR C677T location decreases MTHFR activity, resulting in normal hypomethylation of the gene, which in turn results in aberrant methylation (5). By contrast, 5-methylene tetrahydrofolic acid is necessary for the conversion of deoxyuric acid to thymic acid. A decrease in MTHFR enzyme activity results in aberrant thymidylate production, which in turn results in the incorrect binding of uracil to DNA, which damages DNA and causes single- and double-strand breaks (6,7). DNA damage has an essential role in the development and incidence of cancer (8). Patients with colorectal cancer are in a state of hypercoagulability, which means they have an increased tendency to form blood clots. This is due to a number of factors associated with the cancer itself, including the release of pro-coagulant factors by the tumor cells and the activation of the coagulation system by the body in response to the cancer (9).

In addition, previous research has revealed that the MTHFR gene polymorphism is associated with thrombosis (10). The mutation generates an imbalance between vasodilator and contractile factors, which increases the risk of developing deep vein thrombosis (DVT) (11). The MTHFR C677T mutation results in the conversion of alanine to valine, which decreases the active site of MTHFR and causes homocysteine to be converted to methionine (5). Within the blood, a chemical called homocysteine is formed when the amino acid methionine is naturally broken down (metabolized) to be excreted in the urine. During this breakdown process, homocysteine may be recycled by the body to be used to build other proteins. Vitamins B12, B6 and folate are required to do this recycling and the MTHFR enzyme is needed for recycling to be efficient (12). Homocysteine cannot be efficiently recycled if an individual does not have sufficient vitamin B12, B6 or folate. Mutations of the gene responsible for the MTHFR enzyme may result in an enzyme that is not optimally active and, consequently, homocysteine levels are increased. Mutations of the gene responsible for the MTHFR enzyme may result in an enzyme that is not optimally active and, consequently, homocysteine levels are increased (13). Continuous accumulation of homocysteine generates excessive oxygen free radicals that harm the structure and function of vascular endothelial cells, leading to a reduction in the release of the endothelial relaxation factor and prostaglandins, as well as to thrombosis (14).

Patients with colorectal tumors face the contradictory risks of thrombus and bleeding after surgery and a surgeon must find a balance between both. One patient at our department suffered an acute pulmonary embolism (PE) after undergoing

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surgery for colorectal cancer. In this case, the rescue failed and the patient died as a result. Due to this event, the present study was conceived, focusing on the management of the postoperative thrombus risk in patients with colorectal cancer. Due to being hypercoagulable and bedridden for the majority of the time after surgery, patients with colorectal cancer are more likely to have thrombosis and experience acute PEs (15).

However, the connection between MTHFR gene polymorphism and the risk of thrombosis in patients with colorectal cancer remains uncertain. In the present study, the MTHFR gene polymorphism of 591 patients with colorectal cancer who were pathologically diagnosed after surgery was analyzed. The aim of the present study was to investigate the association between MTHFR gene polymorphisms and the risk of venous thrombosis in patients with colorectal cancer and to provide a theoretical basis for early intervention in patients with a high risk of thrombosis after surgery for colorectal cancer.

Patients and methods

Patients and data gathering. Patients underwent laparoscopic or da Vinci robotic surgery for colorectal cancer at the Department of Colorectal Surgery [Sir Run Run Shaw Hospital (SRRSH), Zhejiang University School of Medicine, Hangzhou, China], between January 2020 and December 2021. Written informed consent and informed consent for biospecimen collection were obtained, which was approved by the ethics committee of SRRSH (Zhejiang University School of Medicine, Hangzhou, China) and the patients had provided basic clinical data, including age, gender and body mass index (BMI). The present study excluded patients undergoing emergency surgery, non-minimally invasive colorectal cancer surgery and non-oncology surgery.

Measuring the parameters of D-dimer (DDi), DVT, PE and thromboelastography (Detailed in below). DDi and thromboelastography were determined by collecting venous blood from patients on the first day after the operation, following the manufacturers' protocols using the STA-Liatest D-Di assay (automatic coagulation analyzer for DDi test; STA R MAX; STAGO; Diagnostica), thromboelastography kaolin detection kit (coagulation method; no. 20212400053; Changshu ChangJiang Biotechnology Co. Ltd) and thromboelastography (for thromboelastography test; TCA-6000; no. 20142220297; Zhejiang Shengyu Medical Technology Co. Ltd). In order to monitor the occurrence of DVT events, not all patients underwent color Doppler ultrasonography (IE33; Philips Medical Systems) of both lower extremities and lung CT pulmonary angiogram (CTPA). A significant increase in DDi was generally associated with obvious clinical symptoms (16), such as bilateral lower limb edema, chest tightness and shortness of breath, and a significant decrease in blood oxygen saturation was examined further using arterial blood gas analysis, color Doppler ultrasonography of both lower extremities or lung CTPA determined by clinicians.

Identification of MTHFR polymorphisms. On the first day of routine postoperative blood testing, 2 ml of peripheral blood was collected in EDTA anticoagulant tubes, DNA was extracted and purified using a DNA extraction kit (cat. no. 20160167; Changsha Sanji Bio) and PCR primers were designed using the whole gene sequence of MTHFR as a template in the conserved region of the C677T locus (17). The primer sequences used were as follows: MTHFR-forward primer, 5'-TGTCATCCC TATTGGCAGGTTAC-3'; MTHFR-reverse primer, 5'-GCC TTCACAAAGCGGAAGAAT-3'; and MTHFR-sequencing primer, 5'-TGCGTGATGATGAAAT-3'. The PCR amplification (Changsha Sanji Biotechnology Co. Ltd) was followed by pyrophosphate sequencing using PyroMark Q24 (QIAGEN GmbH). The associated analysis software (PyroMark Q24 software v2.0.8) was used to examine the data and the sequences of the genes were identified.

Statistical analysis. SPSS (version 24; IBM Corp.) was utilized for statistical data analysis. The Chi-square test was used to analyze categorical variables, one-way ANOVA (with Tukey's post-hoc test and the least-significant difference post-hoc test) was used to analyze continuous variables between multiple groups with a normal distribution (expressed as mean ± standard deviation) and the Kruskal-Wallis test (with the Bonferroni-Dunn's post-hoc test) was used to analyze continuous variables between multiple groups with a non-normal distribution (expressed as the median ± interquartile range). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Association of MTHFR gene variants with the clinical features of patients with colorectal cancer. With the approval of the ethics committee of SRRSH affiliated to the Medical College of Zhejiang University (Hangzhou, China), 591 patients who underwent surgery for colorectal cancer between January 2020 and December 2021 were enrolled, including 361 males and 230 females, with a mean age of 64.39 years and a mean BMI of 23.11 kg/m^2. As indicated in Table I, the distribution of the MTHFR gene among the patients enrolled in the entire observation and study cycle was the CC type in 219 (37.1%) cases, CT type in 289 (48.9%) cases and TT type in 83 (14%) cases. The representative chromatograms from the pyrosequencing of the CC, CT and TT variants are presented in Fig. 1. The association between the MTHFR gene polymorphism genotype distribution and the age, sex and BMI of the patients was investigated. According to the data, there was no significant difference in the age (P=0.229), sex (P=0.283) or BMI (P=0.908) of the patients (Table I).

Association of MTHFR gene polymorphisms with thrombotic events and the monitoring of dynamic DDi. Clinically, the DDi in the peripheral blood of the patients was screened on the first postoperative day and the test was repeated as needed when DDi was >5 μg/ml. In addition, an ultrasound of the deep veins in the lower legs and, if necessary when obvious chest tightness, pulmonary CTPA was performed. From the time of surgery until the time of discharge, patients were observed to assess and document the incidence of thrombosis, including DVT, and PE. DVT and PE was experienced in 48/591 and 4/591 cases, respectively. As presented in Table II, there was no statistically significant difference between the proportion of patients with different MTHFR genotypes and the proportion

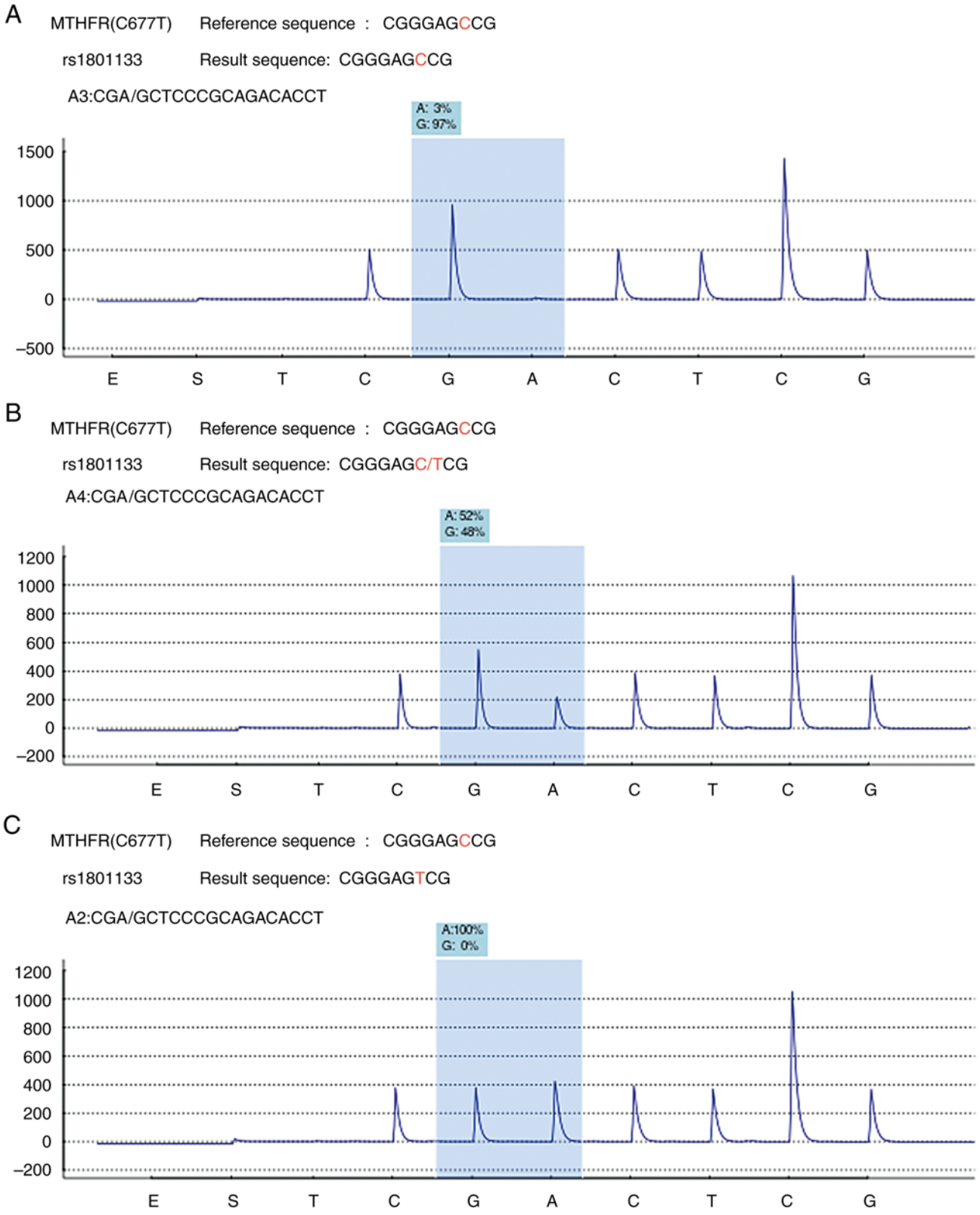

Figure 1. Representative chromatograms from the pyrosequencing of the (A) CC, (B) CT and (C) TT variants. MTHFR, methylenetetrahydrofolate reductase.

of patients that experienced thrombotic events. In addition, there was no statistically significant difference between the patients with different MTHFR genotypes and DDi levels on the first postoperative day. There was also no statistically significant association between the highest postoperative DDi levels and the MTHFR genotype.

Relationship between thromboelastography and MTHFR genotype. On the first postoperative day, the thromboelastogram of the peripheral blood of the patients was examined, which included five indices: The creatine kinase (CK) reaction time (CK-R) value, the CK kinetics (CK-K) value, the CK angle of clot formation (CK-Angle), the CK maximum

Table I. Association of MTHFR gene variants with the clinical features of patients with colorectal cancer.

	MTHFR genotype			
Characteristic	CC (n=219)	CT (n=289)	TT (n=83)	P-value
Sex				0.283
Female	94 (15.9)	104 (17.6)	32 (5.4)	
Male	125 (21.2)	185 (31.3)	51 (8.6)	
Age, years	63.83±11.92	65.2±11.61	63.05±11.53	0.229
BMI, kg/m2	23.02±3.17	23.16±3.63	23.13±4.21	0.908

Values are expressed as n (%) or the mean ± standard deviation. BMI, body mass index; MTHFR, methylenetetrahydrofolate reductase.

Table II. Association of MTHFR gene polymorphisms with thrombotic events and monitoring of the dynamic D-dimer.

	MTHFR genotype			
Characteristic	CC (n=219)	CT (n=289)	TT (n=83)	P-value
DVT				0.574
No	201 (34)	268 (45.3)	74 (12.5)	
Yes	18 (3)	21 (3.6)	9 (1.5)	
PE				0.608
No	217 (36.7)	288 (48.7)	82 (13.9)	
Yes	2 (0.3)	1 (0.2)	1 (0.2)	
DDi 1st day	2.35±2.52	2.19±2.19	2.46±2.5	0.583
DDi highest time	2.97±3.45	2.65±2.65	3.38±3.34	0.146

Values are expressed as n (%) or the mean ± standard deviation. DDi 1st day: The DDi values are generally determined by collecting venous blood from patients on the first day following the operation. DDi highest time: The DDi values were highest on the first day after surgery until discharge. DVT, deep vein thrombosis; PE, pulmonary embolism; DDi, D-dimer; MTHFR, methylenetetrahydrofolate reductase.

amplitude (CK-MA) and the CK composite coagulation index (CK-CI). Each of these indices were compared with the MTHFR genotype distribution to determine whether there were any differences (Table III). Among the MTHFR CC genotype, CT genotype and TT genotype, the CK-R values (mean ± standard deviation) were 4.44±1.21, 4.46±0.97 and 4.40±0.99 min, respectively. CK-K values were 1.36±0.44, 1.32±0.33 and 1.42±0.53 min for the MTHFR CC genotype, CT genotype and TT genotype respectively. The CK-Angle values were 73.80±4.35, 74.09±3.58 and 73.39±4.92 degrees for the MTHFR CC genotype, CT genotype and TT genotype, respectively. The CK-MA values were 64.18±5.71, 65.18±5.20 and 64.02±6.62 mm for the MTHFR CC genotype, CT genotype and TT genotype, respectively. The mean ± standard deviation CK-CI values were 2.25±1.59, 2.41±1.31 and 2.2±1.61 for the MTHFR CC genotype, CT genotype and TT genotype, respectively. The analysis revealed that there were no statistically significant differences in the thromboelastography indexes among patients with colorectal cancer and different MTHFR genotypes (all P>0.05), indicating that differences in the MTHFR genotypes of patients with colorectal cancer do not result in differences in their coagulation function.

Discussion

MTHFR is an enzyme involved in the metabolism of folate, a B vitamin essential to DNA synthesis and repair. MTHFR enzymes are encoded by the MTHFR gene. Variations in this gene, known as polymorphisms, may influence enzyme activity and folate metabolism. The three most common polymorphisms have been extensively studied. At position 677 in the MTHFR gene, the C677T polymorphism changes cytosine to thymine, causing MTHFR enzyme activity to be reduced (18). Several health conditions have been associated with this MTHFR polymorphism, including cardiovascular disease, neural tube defects and certain types of cancer (19). The A1298C polymorphism occurs at position 1,298 of the MTHFR gene, which results in a less pronounced MTHFR enzyme activity reduction compared with the C677T polymorphism. A previous study has suggested that the A1298C polymorphism may increase the risk of depression, schizophrenia and neural tube defects, but it has not been extensively studied (20). Caucasians are more likely to carry the A1298C polymorphism compared with Hispanics and African Americans are more likely to carry the C677T polymorphism (21). It is normal for MTHFR to metabolize homocysteine. However, individuals with the

Table III. Relationship between thromboelastography and the MTHFR genotypes.

	MTHFR genotype			
Characteristic	CC (n=219)	CT (n=289)	TT (n=83)	P-value
CK-R	4.44±1.21	4.46±0.97	4.4±0.99	0.903
CK-K	1.36±0.44	1.32±0.33	1.42±0.53	0.149
CK-Angle	73.8±4.35	74.09±3.58	73.39±4.92	0.413
CK-MA	64.18±5.71	65.18±5.2	64.02±6.62	0.081
CK-CI	2.25±1.59	2.41±1.31	2.2±1.61	0.350

Values are expressed as the mean ± standard deviation. CK-R value: This index measures the time it takes for the clot to form and represents the initiation of clotting. A prolonged CK-R value may indicate a deficiency in clotting factors or an inhibitor of coagulation. CK-K value: This index measures the speed of clot formation and represents the propagation of clotting. A decreased CK-K value may indicate a hypercoagulable state, while an increased CK-K value may indicate a hypocoagulable state. CK-Angle: This index measures the angle of clot formation and represents the kinetics of clot development. A decreased CK-Angle may indicate a hypocoagulable state, while an increased CK-Angle may indicate a hypercoagulable state. CK-MA: This index measures the strength of the clot and represents the final clot strength. A decreased CK-MA may indicate a hypocoagulable state, while an increased CK-MA may indicate a hypercoagulable state. CK-CI: This index measures the rate of fibrinolysis and represents the ability of the clot to dissolve. A decreased CK-CI may indicate a hyperfibrinolytic state, while an increased CK-CI may indicate a hypofibrinolytic state. CK, citrated kaolin; CK-R, reaction time; CK-K, kinetics; CK-Angle, angle of clot formation; CK-MA, maximum amplitude; CK-CI, composite coagulation index; MTHFR, methylenetetrahydrofolate reductase.

C677T or A1298C polymorphisms may accumulate homocysteine in the blood because of reduced MTHFR enzyme activity. Several health conditions have been linked to elevated homocysteine levels, including cardiovascular disease, stroke and Alzheimer's disease. MTHFR polymorphisms C677T and A1298C are relatively common (22); they accounted for 33.54% and may affect MTHFR enzyme activity and folate metabolism, which may increase health risks. Despite this, other genetic and environmental factors may also have a role, so having these polymorphisms does not necessarily mean an individual will develop a particular health condition. Other less common MTHFR polymorphisms have been identified and studied in addition to the three aforementioned ones. Neural tube defects have been linked to the R594Q and R653Q polymorphisms and cardiovascular disease has been linked to the T1317C polymorphism (23).

Clinicians are paying a growing amount of attention to the problem of postoperative DVT in patients with tumors. Patients with tumors are in a condition of hypercoagulability; by contrast, after major pelvic and abdominal surgery, patients with tumors are susceptible to DVT episodes due to extended bed rest (24). Consequently, the early identification of patients at high risk of DVT and the appropriate treatments are needed. In the present study, the MTHFR gene polymorphisms of 591 patients who had surgery for colorectal cancer were identified and linked with age, gender, BMI and other variables. The results indicated that there was no significant difference between the MTHFR genotype and the age, sex and BMI of the patients. In addition, the expression level of DDi in the different genotypes of these patients were examined on the first postoperative day and during the perioperative period, and it was revealed that there was no link between the DDi expression level and the genotype distribution. In addition, the postoperative thromboelastogram indices of patients with various genotypes were evaluated and revealed that there were no significant variations.

A meta-analysis of 30,650 individuals from 29 studies revealed that the MTHFR 677TT polymorphism may be associated with a lower risk of colorectal cancer; however, this may not apply to all populations (25). Results were positive for whites and Asians but negative for blacks and Hispanics, demonstrating ethnic disparities. There have been several studies investigating the relationship between MTHFR polymorphism and the risk of thrombosis in Asian populations. A meta-analysis of several studies conducted in Asian populations also revealed an association between the MTHFR C677T polymorphism and the risk of venous thrombus embolism (VTE), particularly in East Asian populations (26). Another study revealed that the MTHFR A1298C polymorphism was associated with an increased risk of arterial thromboembolism in Taiwanese patients (27). Overall, while there may be a number of ethnic/regional disparities in the relationship between MTHFR polymorphism and the risk of thrombosis, there is still evidence suggesting a significant association in Asian populations. Compared with the 677CC gene, the 677TT gene is associated with a 20% increased risk of venous thrombosis. However, in North America, the 677TT genotype did not have any effect on venous thrombosis, which may explain the high consumption of folic acid and riboflavin in the region (21). Another investigation revealed that the MTHFR C677T polymorphism was not connected with the incidence of venous thrombosis and concluded that there was no clinical justification for measuring this polymorphism (28). This is consistent with the findings of the present study.

Of note, the present study had certain limitations. To begin with, the limited sample size may limit the confidence of the results. Increasingly, there is growing awareness of deep vein thrombosis (DVT) events occurring in various clinical scenarios, including admission VTE assessments, preoperative evaluations, postoperative checks, and standardized VTE testing before patients are discharged. In line with established guidelines, if ultrasound of both lower extremities

or pulmonary CTPA confirms the presence of DVT or PE, appropriate anticoagulant treatment is administered. However, the adoption of precautionary measures based on specific MTHFR polymorphisms lacks substantial evidential support within this proactive clinical approach. In addition to the introduction of preoperative DVT education, the widespread use of thromboelastic stockings, minimally invasive surgery to decrease trauma and postoperative activities to encourage patients to get out of bed earlier have effectively lowered the incidence of DVT episodes. Even if the MTHFR gene is connected with the risk of venous thrombosis, this association may have been largely obscured by introduction of preoperative DVT education, the widespread use of thromboelastic stockings, minimally invasive surgery to decrease trauma and postoperative activities to encourage patients to get out of bed earlier have effectively lowered the incidence of DVT episodes.

In conclusion, even though the results are 'negative', the present study may help physicians realize that MTHFR polymorphism detection is not necessary for therapeutic purposes. Regarding the danger of venous thrombosis, patients undergoing pelvic and abdominal surgery should pay attention to the standardized procedural enforcement system for DVT prevention.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

EC designed the study, performed the experiments, analyzed and interpreted the data and wrote the manuscript. WZ and LC collected samples and clinical data, analyzed and interpreted the data and wrote the manuscript. GC and FW provided and analyzed the samples and interpreted results and clinical data of the patients. WZ and EC confirm the authenticity of all the raw data. All authors have read and approved the final version of the manuscript.

Ethics approval and consent to participate

Written informed consent was obtained from all patients before specimen collection. This study was approved by the ethics committee of Sir Run Run Shaw Hospital, Zhejiang University (Hangzhou, China; approval no. 20210622-8).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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