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REVIEW ARTICLE

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Methylenetetrahydrofolate reductase polymorphisms and colorectal cancer prognosis: A meta-analysis

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Abstract

Background: The present study focused on understanding the prognostic value of the methylenetetrahydrofolate reductase (*MTHFR*) single nucleotide polymorphisms rs1801133 (C667T) and rs1801131 (A1298C) in patients with colorectal cancer (CRC).

Methods: A systematic literature search was conducted in March 2016. Databases, including Medline, EMBASE, Cochrane and Chinese databases (including CNKI, Wanfang and VIP), were searched to identify the relevant articles describing *MTHFR* polymorphisms in patients with CRC. Data regarding overall survival (OS), progression-free survival (PFS) and disease-free survival (DFS) were collected and analysed.

Results: Twenty-four studies with 5423 patients with CRC were included. Significant differences in OS, PFS and DFS were not observed among the different comparisons of patients carrying different alleles of the *MTHFR* rs1801133 polymorphism (including TT versus CC, TT versus CT + CC, CT + TT versus CC and CT versus CC). Compared with patients with the rs1801131 CA + AA genotypes, patients with the CC genotype had a shorter OS (hazard ratio = 1.85; 95% confidence interval = 1.30–2.65) and DFS (hazard ratio = 2.16; 95% confidence interval= 1.19–3.93). Significant differences in OS, PFS and DFS were not observed among the other patient groups (including CC versus AA, CC + CA versus AA and CA versus AA). Subgroup analysis

of rs1801133 and rs1801131 showed that patients with CRC from Asian regions and Western regions demonstrated similar results.

Conclusions: The *MTHFR* rs1801133 polymorphism was not associated with the prognosis of patients with CRC; however, rs1801131 may be associated with the prognosis of patients with CRC. Well-designed prospective studies are necessary to obtain a better understanding of the prognostic value of rs1801133 and rs1801131.

KEYWORDS

colorectal cancer, gene polymorphism, meta-analysis, methylenetetrahydrofolate reductase, prognosis

1 | INTRODUCTION

As the third most commonly diagnosed cancer, colorectal cancer (CRC) has a worldwide incidence of over 1.3 million and a mortality rate of approximately 50%.[1-3] Although the incidence of CRC has decreased in recent years because of improvements in its early diagnosis and treatment,[1] the number of CRC cases continues to increase worldwide. Despite recent advances in treatment modalities, the 5-year survival rate of patients with advanced CRC is not satisfactory as a result of recurrence and drug resistance.[4]

Methylenetetrahydrofolate reductase (MTHFR) is required for folate metabolism, intracellular homeostasis and DNA synthesis. It converts 5,10-methylenetetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate (5-MTHF), which is the major circulating form of folate in the blood and provides methyl groups to convert homocysteine into methionine. MTHFR contributes to the imbalance in methylation reactions, leading to genomic DNA hypomethylation, and influences folate metabolism.[5,6]

The two most common loci for *MTHFR* single nucleotide polymorphisms (SNPs) are rs1801133 (C677T) and rs1801131 (A1298C).[7] Both are associated with a deficiency in enzymatic activity.[8] The *MTHFR* rs1801133 polymorphism is a point mutation at the position 677C>T, in which alanine is replaced with valine.[9] The *MTHFR* rs1801131 polymorphism is a point mutation at position 1298A>C, in which glutamate is replaced with valine.[10] The rs1801133 and rs1801131 polymorphisms reduce the activity of the MTHFR enzyme and increase the homocysteine level in the blood, which may be a risk factor for cancer.[11]

Recently, some meta-analyses have reported significant correlations between the *MTHFR* rs1801133 and rs1801131 polymorphisms and tumour responses to chemoradiotherapy and short-term clinical benefits.[12-14] For example, two meta-analyses were performed to investigate the associations between *MTHFR* polymorphisms and the response of patients with CRC to chemotherapy.[13,14] A meta-analysis was conducted to investigate the associations between *MTHFR* polymorphisms and short-term clinical benefits (complete or partial response, relapse or progression) of chemotherapy in patients with CRC.[12] These meta-analyses only focused on the short-term prognostic effects of *MTHFR* polymorphisms on patients with CRC.

No meta-analysis has been performed investigating the association between these *MTHFR* polymorphisms and survival (e.g. overall survival [OS], progression-free survival [PFS] or disease-free survival [DFS]). By systematically reviewing recent publications, we conducted a meta-analysis according to the guidelines of the PRISMA statement.[15] The aim was to explore whether the *MTHFR* rs1801133 and rs1801131 polymorphisms might affect the prognosis of patients with CRC and whether these SNPs are potentially useful as predictive biomarkers.

2 | MATERIALS AND METHODS

2.1 | Literature search strategy

A comprehensive literature search was performed independently by two investigators (XLC and YMW) from the inception of each database up to 14 March 2016. The databases included PubMed, EMBASE, the Cochrane Central Register of Controlled Trials and Chinese databases (including CNKI, Wanfang and VIP). The search terms included the keywords: colorectal cancer (including colorectal cancer, colon cancer, rectal cancer), MTHFR (including MTHFR and methylenetetrahydrofolate reductase) and prognosis (including prognosis, prognoses, predictive, biomarker, marker, survival, log rank, Kaplan–Meier and Cox). The detailed search strategy is documented in the Supporting information (Doc. S1). Google Scholar was also used to search for relevant articles. Systematic reviews and meta-analyses of *MTHFR* polymorphisms and CRC were manually screened for potentially eligible articles.

Duplicate articles that were obtained from multiple databases were deleted. The abstract of each article was extracted and screened by two of three investigators (FZ, TGY and GT), and the full texts of potentially eligible articles were reviewed for data analysis. Next, two of three investigators (FZ, TGY and GT) independently reviewed and confirmed the eligibility of the articles. Any disagreement was recorded and resolved by consensus under the guidance of a fourth investigator (XLC). The cross-referencing strategy was adopted until the two investigators reached a consistent result.

2.2 | Inclusion criteria for the studies

This meta-analysis includes articles reporting the patient's CRC prognosis and *MTHFR* genotype. The inclusion criteria comprised: (i) a diagnosis of CRC, colon cancer, rectal cancer or metastatic CRC (mCRC); (ii) rs1801133 or rs1801131 polymorphisms identified by polymerase chain reaction (PCR) or polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP); and (iii) data describing OS, DFS and/or PFS with hazard ratios (HRs), 95% confidence intervals (CIs) or the relevant information (e.g. survival curves) were provided. Articles published in abstract form were included only when sufficient outcome data were presented or when the authors were willing to provide detailed results from the study. If several articles from the same patient population were reported, the most recent or most detailed study was included.

2.3 | Data extraction and quality assessment

For each article, two of three investigators (FZ, TGY and GT) independently extracted the required data according to a predefined protocol. The extracted data comprised: authors' names, year of publication, patient characteristics (cancer type, sample size, gender and mean age), therapy (surgery, chemotherapy and radiotherapy), characteristics of *MTHFR* polymorphisms (rs1801133 or rs1801131, sample source, sample content, test method and cut-off values) and prognostic outcomes (HRs and their 95% CIs for OS, PFS and DFS). If the data from any of the above categories were unavailable in the text, the corresponding record was marked as "NR (not reported)". Differences in data extraction were resolved by cross-checking until a consensus was reached.

2.4 | Statistical analysis

Four genetic models existed for rs1801133: TT versus CC (TT/CC, additive model), TT versus CT and CC (TT/CT + CC, recessive model), TT and CT versus CC (TT + CT/CC, dominant model) and CT versus CC (CT/CC, heterozygous model). For rs1801131, the four models included CC versus AA (CC/AA), CC versus CA and AA (CC/CA + AA), CC and CA versus AA (CC + CA/AA) and CA versus AA (CA/AA). None of the included articles reported data about the allele model (wild-type allele versus mutant-type allele) for rs1801133 and rs1801131. Therefore, the allele model was not included in our meta-analysis. OS, PFS and DFS were analysed separately.

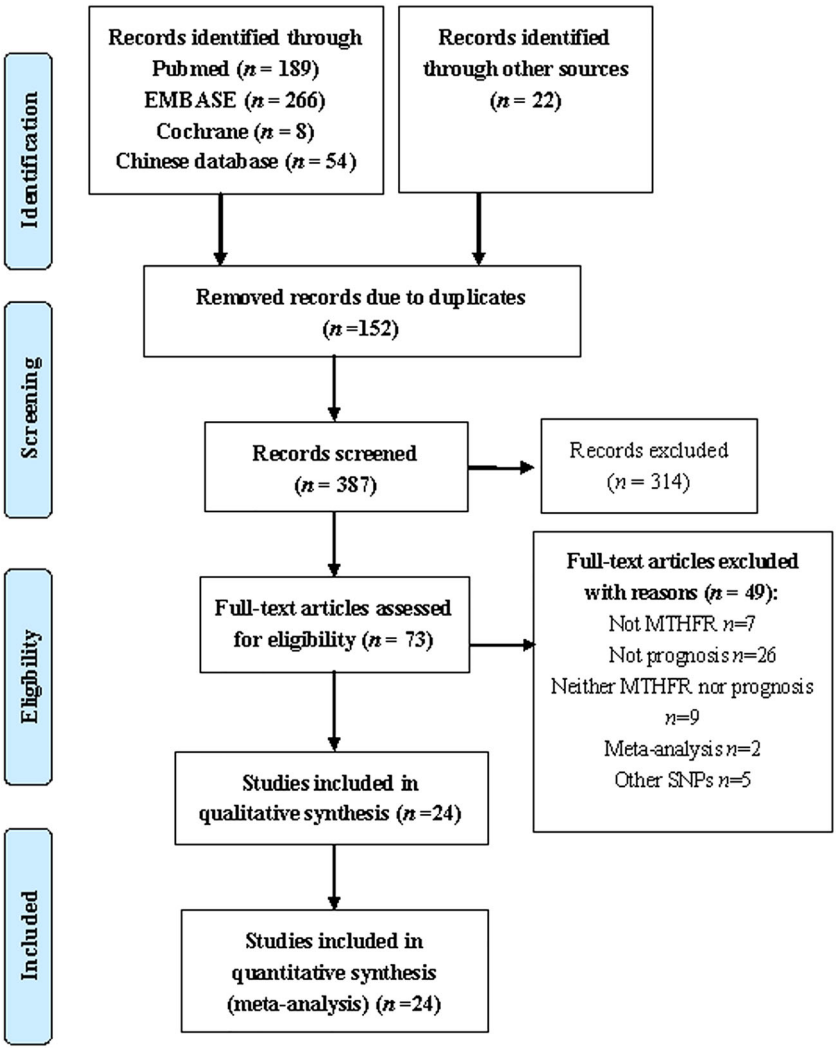

FIGURE 1 Flow chart of the search strategy

TABLE 1 Characteristics of the included articles

Reference	Year of publication	Country	Time	Patients	Sample size	Number of males	Mean age (range, years)	Stage	Surgery	Chemotherapy	Radiotherapy	Median (range) follow-up (months)
Afzal *et al.*[36]	2009	Denmark	1996–2003	CRC	331	166	61 (NR)	II–IV	NR	5-FU + LV	NR	120 (NR)
Budai *et al.*[29]	2012	Hungary	2006–2008	CRC	85	NR	NR (NR)	IV	NR	5-FU + LV + CPT-11 + BEV	NR	NR (NR)
Castillo-Fernández *et al.*[33]	2010	Mexico	1998–2004	mCRC	29	11	55.9 (NR)	IV	NR	5-FU + FA	NR	NR (NR)
Cecchin *et al.*[21]	2015	Italy	2003–2007	CRC	112	62	65 (30–85)	II, III	All	5-FU/CAPE	NR	80 (10–185)
Chua *et al.*[37]	2009	Britain	1999–2000	mCRC	118	80	61 (31–75)	IV	NR	5-FU + LV + OX	NR	NR (NR)
Custodio *et al.*[23]	2014	Spain	2004–2009	CRC	202	115	63.8 (23–85)	II, III	All	5-FL + OX	None	51.4 (7–96)
Delgado-Plasencia *et al.*[27]	2013	Spain	1990–2003	CRC	50	28	NR (NR)	NR	All	5-FU-based	NR	NR (NR)
Dong *et al.*[20]	2016	China	2012–2013	CRC	81	44	56.2 (27–76)	IV	NR	5-FU + LV + OX	NR	14 (5–20)
Etienne *et al.*[43]	2004	France	NR	mCRC	98	57	64 (40–82)	IV	NR	5FU + FA	NR	NR (NR)
Fernández-Peralta *et al.*[34]	2010	Spain	1992–1996	CRC	143	81	67.3 (NR)	NR	All	5-FU-based	NR	44.3 (NR)
Gusella *et al.*[35]	2009	Britain	1999–2008	CRC	130	84	64.7 (34–84)	B, C*	NR	5-FU + LV	NR	45.6 (4.8–120.0)
Huang *et al.*[31]	2011	China	2005–2009	mCRC	157	85	62.5 (36–82)	IV	NR	5-FU + LV + OX	NR	35 (8–56)
Jang *et al.*[24]	2014	Korea	1996–2009	CRC	372	215	62.1 (NR)	I–IV	All	5-FU-based	NR	34 (4–173)
Kim *et al.*[32]	2010	Korea	1995–2004	CRC	103	49	57.0 (NR)	II–IV	All	5-FU/5-FU + OX	NR	62.2 (18–121)
Negandhi *et al.*[25]	2013	Canada	1999–2003	CRC	784	327	61.4 (20.7–75.0)	I–IV	All	5-FU (partial)	NR	76.8 (4.8–130.8)
Qiu *et al.*[28]	2013	China	2004–2006	CRC	76	48	57 (21–75)	I–III	All	5-FU + OX + LV	NR	NR (37–67)
Ruzzo *et al.*[40]	2007	Italy	NR	mCRC	166	87	66 (NR)	IV	NR	5-FU + LV + OX	NR	24 (NR)
Ruzzo *et al.*[39]	2008	Italy	NR	mCRC	146	80	61 (38–75)	IV	NR	5-FU + LV + CPT-11	NR	NR (NR)
Sharma *et al.*[38]	2008	Australia	2002–2003	mCRC	54	35	72 (42–86)	IV	NR	CAPE	NR	NR (NR)
Suh *et al.*[42]	2006	Korea	NR	mCRC	54	30	57.8 (35–39)	II–IV	NR	5-FU + LV + OX	NR	23.6 (6–35)
Taflin *et al.*[30]	2011	Sweden	1999–2006	CRC	649	88	66 (32–82)	III	All	5-FU + LV	NR	70 (NR)
Ulrich *et al.*[22]	2014	America	1994–2000	Rectal cancer	754	482	61 (19–86)	II, III	All	5-FU + LV	All	NR (NR)
Zhang *et al.*[41]	2007	America	1992–2003	mCRC	318	177	58 (25–86)	IV	NR	FU + CPT-11/FU + OX	NR	30 (NR)
Zhu *et al.*[26]	2013	China	2004–2007	CRC	411	245	60 (NR)	I–IV	All	5-FU/5-FU + OX	NR	64 (1–88)

5-FU, 5-fluorouracil; BEV, bevacizumab; CAPE, capecitabine; CPT-11, irinotecan; CRC, colorectal cancer; FA, folinic acid; FU, fluorouracil; LV, leucovorin; mCRC, metastatic CRC; NR, not reported; OX, oxaliplatin;

*, Duke's stage.

The HRs and 95% CIs reflected the effects of rs1801133 and rs1801131 on the prognosis. If these data were available in the collected articles, we extracted these data directly; otherwise, they were calculated from the available numerical data in the articles based on the methods developed by Tierney *et al.*[16]

Pooled HRs and their 95% CIs for OS, PFS and DFS between different genetic models were calculated. The heterogeneity of all HRs was calculated using chi-squared tests. The heterogeneity test with the inconsistency index (I^2) statistic and *Q* statistic was performed. If the HR was homogeneous, then the fixed-effects model was employed for analysis; otherwise, a random-effects model was used. $p < 0.05$ was considered statistically significant. Additionally, an HR > 1 suggested a poor prognosis. Publication bias was evaluated using the methods described by Begg and Mazumdar.[17]

Linkage disequilibrium among the variants can vary across populations.[18,19] For example, Haerian and Haerian[18] showed that rs1801133 and rs1801131 might be CRC susceptibility variants in Americans and Australians, whereas rs1801133 may be more common in the Brazilian and Japanese populations. Based on these results, patients of different ethnicities may carry different rs1801133 and rs1801131 variants. Therefore, a subgroup analysis based on different regions (e.g. Asia and Western countries) was performed. All calculations were performed using STATA, version 12.0 (StataCorp, College Station, TX, USA).

3 | RESULTS

3.1 | Article characteristics

Figure 1 shows the process used to screen the included articles. The literature search yielded 539 articles, 152 of which were excluded as a result of duplication. The abstracts of 387 articles were reviewed by the investigators, and the 314 articles that failed to meet the

TABLE 2 Information about and results for the rs1801133 and rs1801131 polymorphisms in the included studies

Reference	rs1801133	rs1801131	Test sample	Test content	Test method	Analytical method	Outcome reported
Afzal *et al.*[36]	Yes	Yes	Tumour tissue	DNA	PCR#	Mul	OS*, PFS*
Budai *et al.*[29]	Yes	–	Blood	DNA	PCR	Mul	OS^, PFS^
Castillo-Fernández *et al.*[33]	Yes	–	Tissue	DNA	PCR	Uni	OS^
Cecchin *et al.*[31]	Yes	Yes	Blood or tissue	DNA	PCR#	Mul	DFS*
Chua *et al.*[37]	Yes	–	Tissue	DNA	PCR	Uni	OS^, PFS^
Custodio *et al.*[33]	Yes	–	Tissue	DNA	PCR-RFLP	Uni	DFS^
Delgado-Plasencia *et al.*[27]	Yes	–	Tumour tissue	DNA	PCR-RFLP	Uni	OS^
Dong *et al.*[20]	Yes	–	Tissue	DNA	PCR	Uni	DFS^
Etienne *et al.*[43]	Yes	Yes	Tissue	DNA	PCR	Mul	OS*
Fernández-Peralta *et al.*[34]	Yes	Yes	Blood and tissue	DNA	PCR	Mul	OS*
Gusella *et al.*[35]	Yes	Yes	Blood	DNA	PCR	Uni	OS*, DFS*
Huang *et al.*[31]	Yes	–	Blood	DNA	PCR-RFLP	Uni	OS^, PFS^
Jang *et al.*[24]	Yes	Yes	Blood	DNA	PCR	Mul	OS*, DFS*
Kim *et al.*[32]	Yes	–	Leukocytes	DNA	PCR-RFLP	Uni	OS^
Negandhi *et al.*[25]	–	Yes	Blood	DNA	PCR#	Mul	OS&
Qiu *et al.*[28]	Yes	Yes	Blood	DNA	PCR	Mul	PFS*
Ruzzo *et al.*[40]	Yes	Yes	Blood	DNA	PCR	Mul	PFS*
Ruzzo *et al.*[39]	Yes	Yes	Blood	DNA	PCR	Mul	PFS*
Sharma *et al.*[38]	Yes	–	Blood	DNA	PCR	Uni	OS^
Suh *et al.*[42]	Yes	–	Tissue	DNA	PCR	Uni	OS^
Taflin *et al.*[30]	Yes	–	Blood	DNA	PCR#	Uni	OS^
Ulrich *et al.*[22]	Yes	Yes	Tissue	DNA	PCR#	Mul	OS*
Zhang *et al.*[41]	Yes	Yes	Blood and tissue	DNA	PCR#	Mul	OS*
Zhu *et al.*[26]	Yes	Yes	Blood	DNA	PCR#	Mul	OS*

–, Not available;

*for both rs1801133 and rs1801131;

^for rs1801133 alone;

&for rs1801131 alone. PCR, polymerase chain reaction; PCR#, PCR TaqMan; PCR-RFLP, polymerase chain reaction restriction fragment length polymorphism; Mul, multivariate analysis; Uni, univariate analysis.

inclusion criteria were excluded. The full texts of the remaining 73 articles were retrieved. Finally, twenty-four articles were included in the meta-analysis.[20-43]

Table 1 summarizes the characteristics of the included articles. Among the 24 included articles, seven were conducted in China or Korea,[20,24,26,28,31,32,42] one was conducted in Mexico[33] and the remaining articles were conducted in European or North American countries. All but two eligible articles targeted CRC or mCRC: one addressed rectal cancer[22] and the other studied colon cancer.[28] In total, 5423 patients with CRC were included in our analysis. The sample size of each article ranged from 29 to 784 patients, with a median of 136 patients. All patients received either 5-fluorouracil (5-FU) or 5-FU-based chemotherapy. Information about the rs1801133 and rs1801131 polymorphisms is provided in Table 2.

3.2 | Meta-analysis of rs1801133

Twenty-three of the included articles assessed the association between rs1801133 and survival time. According to the heterogeneity analysis, all of the articles were homogeneous and the fixed-effect model was adopted. Compared with patients carrying the CC genotype, patients carrying the TT genotype did not show an increased HR for OS (HR = 1.17; 95% CI = 0.99–1.40), PFS (HR = 0.90; 95% CI = 0.70–1.15) or DFS (HR = 1.23; 95% CI = 0.93–1.62) (Table 3). Additionally, significant differences in OS (HR = 1.07; 95% CI = 0.76–1.49), PFS (HR = 0.91; 95% CI = 0.53–1.55) and DFS (HR = 1.27; 95% CI = 0.86–1.88) were not observed between patients carrying the TT genotype and patients carrying the CT + CC genotypes (Table 3). In the comparison of patients carrying the TT + CT genotypes with patients carrying the CC genotype, the pooled HRs of OS, PFS and DFS were 1.09 (95% CI = 0.90–1.31), 1.12 (95% CI = 0.85–1.48) and 1.02 (95% CI = 0.69–1.51), respectively (Table 3). Significant differences in OS, PFS and DFS were not observed between patients carrying the CT genotypes and patients carrying the CC genotypes (Table 3).

Subgroup analysis revealed similar results for patients with CRC from Asian regions or Western regions (Table 4). For example, the HR of the TT versus CC genotype for patients from Asian regions was 1.06 (95% CI = 0.75–1.49) and the value for patients from Western regions was 1.22 (95% CI = 0.99–1.50).

TABLE 3 Results of the meta-analysis of the *MTHFR* rs1801133 polymorphism

	Number of articles	Number of patients	HR (95% CI)	Heterogeneity (I^2, p)
TT/CC				
OS	11	2,526	1.17 (0.99–1.40)	0.0%, 0.957
PFS	5	672	0.90 (0.70–1.15)	0.0%, 0.778
DFS	3	1,256	1.23 (0.93–1.62)	11.1%, 0.325
TT/CT + CC				
OS	5	840	1.07 (0.76–1.49)	2.1%, 0.395
PFS	1	331	0.91 (0.53–1.55)	–
DFS	3	686	1.27 (0.86–1.88)	34.4%, 0.218
TT + CT/CC				
OS	8	1,574	1.09 (0.90–1.31)	40.9%, 0.106
PFS	3	284	1.12 (0.85–1.48)	52.3%, 0.123
DFS	2	448	1.02 (0.69–1.51)	0.0%, 0.387
CT/CC				
OS	10	2,369	1.12 (0.96–1.31)	0.0%, 0.673
PFS	2	203	0.96 (0.68–1.36)	47.0%, 0.170
DFS	3	1,256	1.10 (0.90–1.35)	0.0%, 0.478

TT/CC: TT genotype versus CC genotype; TT/CT + CC: TT genotype versus (CT + CC) genotype; TT + CT/CC: (TT + CT) genotype versus CC genotype; CT/CC: CT genotype versus CC genotype. –, not available.

3.3 | Meta-analysis of rs1801131

Thirteen articles assessed the association between rs1801131 and survival time. Significant differences in OS, PFS and DFS were not observed between patients carrying the CC genotype and patients

TABLE 4 Results for the subgroup analysis of the *MTHFR* rs1801133 polymorphism in different geographic regions

	Subgroup	Number of articles	Number of patients	HR (95% CI)
TT/CC				
OS	Asian	4	994	1.06 (0.75–1.49)
	Western	7	1,532	1.22 (0.99–1.50)
PFS	Asian	1	157	1.53 (0.36–6.51)
	Western	4	515	0.88 (0.68–1.14)
DFS	Asian	1	372	0.71 (0.33–1.53)
	Western	2	884	1.33 (0.99–1.79)
TT/CT + CC				
OS	Asian	2	426	0.94 (0.59–1.51)
	Western	3	414	1.19 (0.78–1.81)
PFS	Asian	–	–	–
	Western	1	331	0.91 (0.53–1.55)
DFS	Asian	1	372	0.86 (0.45–1.64)
	Western	2	314	1.59 (0.98–2.58)
TT + CT/CC				
OS	Asian	3	426	1.13 (0.76–1.69)
	Western	5	995	1.07 (0.87–1.33)
PFS	Asian	1	81	1.81 (0.99–3.32)
	Western	2	203	0.98 (0.72–1.34)
DFS	Asian	2	448	1.02 (0.69–1.51)
	Western	–	–	–
CT/CC				
OS	Asian	3	837	1.22 (0.89–1.67)
	Western	7	1,532	1.09 (0.92–1.30)
PFS	Asian	–	–	–
	Western	2	203	0.96 (0.68–1.36)
DFS	Asian	1	372	0.89 (0.53–1.50)
	Western	2	884	1.14 (0.92–1.43)

–, Not available.

carrying the AA genotype (Table 5). Compared with patients with the CA + AA genotypes, patients with the CC genotype had a shorter OS (HR = 1.85; 95% CI = 1.30–2.65) and DFS (HR = 2.16; 95% CI = 1.19–3.93) (Figure 2 and Table 5). Significant differences in OS, PFS and DFS were not observed between patients with the CC + CA genotypes and patients with the AA genotype (Table 5). Significant differences in OS, PFS and DFS were not observed between patients with the CA genotype and patients with the AA genotype (Table 5).

Subgroup analysis revealed similar results for patients with CRC from Asian regions and Western regions (Table 6). For example, the HR of the CC versus AA genotype in patients from Asian regions was 0.81 (95% CI = 0.51–1.29) and the HR for this same comparison of patients from Western regions was 1.25 (95% CI = 0.82–1.91).

TABLE 5 Results of the meta-analysis of the *MTHFR* rs1801131 polymorphism

	Number of articles	Number of patients	HR (95% CI)	Heterogeneity (I^2, p)
CC/AA				
OS	7	2,867	1.13 (0.81–1.59)*	50.9%, 0.057
PFS	2	312	0.89 (0.58–1.37)	0.0%, 0.660
DFS	3	1,256	0.78 (0.53–1.13)	0.0%, 0.738
CC/CA + AA				
OS	3	1,254	1.85 (1.30–2.65)	0.0%, 0.584
PFS	–	–	–	–
DFS	2	484	2.16 (1.19–3.93)	0.0%, 0.337
CC + CA/AA				
OS	5	1,948	1.11 (0.85–1.45)*	62.3%, 0.031
PFS	2	412	0.79 (0.55–1.14)	0.0%, 0.547
DFS	1	372	0.92 (0.55–1.54)	–
CA/AA				
OS	6	2,549	0.97 (0.84–1.12)	0.0%, 0.507
PFS	2	312	0.97 (0.60–1.57)	0.0%, 0.933
DFS	3	1,256	0.88 (0.73–1.07)	0.0%, 0.974

CC/AA: CC genotype versus AA genotype; CC/CA + AA: CC genotype versus (CA + AA) genotype; CC + CA/AA: (CC + CA) genotype versus AA genotype; CA/AA: CA genotype versus AA genotype. –, not available.

*Results from the random-effects model.

4 | DISCUSSION

Our meta-analysis highlighted the long-term prognostic effects (including OS, PFS and DFS) of *MTHFR* polymorphisms on patients with CRC. The rs1801131 polymorphism may predict the prognosis. Compared with patients with the CA + AA genotypes, patients with the CC genotype had a shorter OS (HR = 1.85) and DFS (HR = 2.15). However, significant differences were not observed among the other comparisons (CC versus AA, CC + CA versus AA and CA versus AA). Other researchers also reported similar results. For example, rs1801131 appears to be a potential prognostic factor for patients with gastric cancer.[13,44,45]

The *MTHFR* rs1801131 polymorphism may predict the prognosis; the possible explanations are described below. As a crucial enzyme in metabolism, MTHFR catalyses the transformation of 5,10-MTHF into 5-MTHF.[25,46-48] Notably, 5,10-MTHF mainly synthesizes purines and thymidine. Furthermore, 5-MTHF participates in the synthesis of *S*-adenosyl-methionine, which is an important mediator of methylation reactions.[47,48] Regarding rs1801131, its mutation is linked to reduced MTHFR enzyme activity, although the decrease is less pronounced than the change induced by 677CNT.[49] Therefore, the reduction in

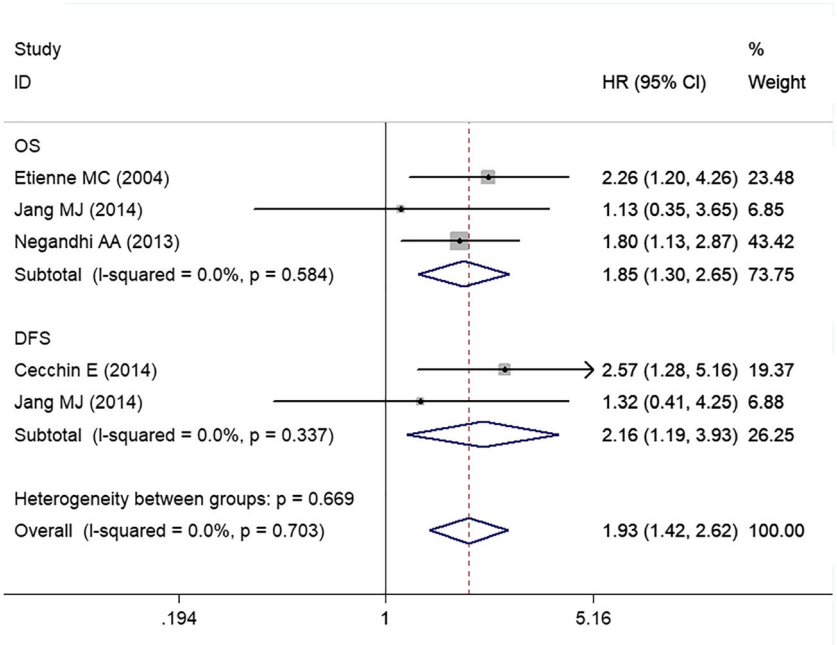

FIGURE 2 Meta-analysis plots of the HRs for survival in the comparison of patients with the CC genotype and patients with the AA + CA genotypes of rs1801131. OS, overall survival; PFS, progression-free survival; DFS, disease-free survival

TABLE 6 Results from the subgroup analysis of the *MTHFR* rs1801131 polymorphism in different geographic regions

	Subgroup	Number of articles	Number of patients	HR (95% CI)
CC/AA				
OS	Asian	2	783	0.81 (0.51–1.29)
	Western	5	2,084	1.25 (0.82–1.91)*
PFS	Asian	–	–	–
	Western	2	203	0.89 (0.58–1.37)
DFS	Asian	1	372	1.20 (0.37–3.89)
	Western	2	884	0.74 (0.50–1.10)
CC/CA + AA				
OS	Asian	1	372	1.13 (0.35–3.65)
	Western	2	882	1.95 (1.34–2.84)
PFS	Asian	–	–	–
	Western	–	–	–
DFS	Asian	1	372	1.32 (0.41–4.25)
	Western	1	112	2.57 (1.28–5.16)
CC + CA/AA				
OS	Asian	1	372	0.70 (0.41–1.19)
	Western	4	1,576	1.21 (0.94–1.56)*
PFS	Asian	1	81	0.69 (0.39–1.23)
	Western	1	331	0.87 (0.54–1.41)
DFS	Asian	1	372	0.92 (0.55–1.54)
	Western	–	–	–
CA/AA				
OS	Asian	2	783	0.94 (0.68–1.28)
	Western	4	1,766	0.98 (0.83–1.16)
PFS	Asian	–	–	–
	Western	2	312	0.97 (0.60–1.57)
DFS	Asian	1	372	0.89 (0.52–1.53)
	Western	2	884	0.88 (0.72–1.09)

–, Not available.

*Results from the random-effects model.

MTHFR enzyme activity as a result of the rs1801131 polymorphism may lead to a higher level of the precursor 5,10-MTHF and a correspondingly lower level of 5-MTHF, given the relatively low catalytic activity of the enzyme. The accumulation of 5,10-MTHF would provide a greater pool of nucleotides for DNA synthesis, thus prompting tumour cell proliferation, which requires an abundant supply of nucleic acids. Once CRC has developed, folate supplementation might enhance its growth and progression,[42,48,50,51] presumably by providing large amounts of nucleotide precursors for tumour growth.[42,48,51] Folate supplementation is associated with a higher risk of CRC.[52] These findings indicate a negative effect of high levels of MTHFR on patients with CRC. Therefore, the association between *MTHFR* polymorphisms and a worse prognosis of CRC may be ascribed to decreased MTHFR activity.

In the present study, data heterogeneity was not observed; therefore, all of the data were analysed using fixed-effects models. Subgroup analysis was performed according to the patient's nationality and revealed that rs1801133 and rs1801131 exerted the same effects on patients from Asian regions and patients from Western regions.

The *MTHFR* rs1801131 polymorphism may be associated with the prognosis of patients with CRC. In the future, additional high-quality prospective studies should be conducted to obtain a better understanding of the prognostic value of the *MTHFR* polymorphisms. The *MTHFR* rs1801131 polymorphism may be regarded as a target for drugs that are widely used to treat cancer and inflammatory diseases.[12] This polymorphism may better predict the prognosis of patients with CRC and facilitate the administration of individualized treatments.

Some limitations exist in this meta-analysis. (i) The eligible articles included in our meta-analysis were restricted to studies published in English and Chinese, which likely caused selection bias. Articles published in other languages were excluded, which might cause selection bias as a result of low reporting qualities. (ii) The therapy method substantially affected the survival of patients with CRC. Although all of the included patients with CRC were treated with 5-FU chemotherapy, the use of specific therapies differed among the included articles. Thus, the confounding effects of different therapies remain unclear. (ii) HRs calculated from the data or extracted from survival curves may be less reliable than HRs directly calculated with an analysis of variance.

In summary, the *MTHFR* rs1801133 polymorphism was not associated with the OS, PFS or DFS of patients with CRC. However, the *MTHFR* rs1801131 polymorphism was associated with a shorter OS and DFS in patients with CRC (CC + CA versus AA), although the other genotypes of *MTHFR* rs1801131 did not produce significant differences. Both rs1801133 and rs1801131 produced similar results among patients with CRC from Asian regions and Western regions. These results might provide guidance and prognostic predictive power for physicians during the clinical treatment of patients with CRC who are undergoing 5-FU chemotherapy. Well-designed prospective studies are necessary to further investigate the precise prognostic value of the *MTHFR* rs1801133 and rs1801131 polymorphisms.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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