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
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%. Although the incidence of CRC has decreased in recent years because of improvements in its early diagnosis and treatment, 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.

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.

The two most common loci for MTHFR single nucleotide polymorphisms (SNPs) are rs1801133 (C677T) and rs1801131 (A1298C). Both are associated with a deficiency in enzymatic activity. The MTHFR rs1801133 polymorphism is a point mutation at the position 677C>T, in which alanine is replaced with valine. The MTHFR rs1801131 polymorphism is a point mutation at position 1298A>C, in which glutamate is replaced with valine.

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. For example, two meta-analyses were performed to investigate the associations between MTHFR polymorphisms and the response of patients with CRC to chemotherapy. 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. 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. 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.
| 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.               | 2009                | Denmark  | 1996–2003 CRC | 331      | 166         | 61 (NR)         | II–IV                  | NR    | NR      | 5-FU + LV               | NR          | 120 (NR)                      |
| Budai et al.               | 2012                | Hungary  | 2006–2008 CRC | 85       | NR          | NR (NR)         | IV                     | NR    | NR      | 5-FU + LV + CPT-11 + BEV | NR          | NR (NR)                       |
| Castillo-Fernández et al. | 2010                | Mexico   | 1998–2004 mCRC | 29       | 11          | 55.9 (NR)      | IV                     | NR    | NR      | 5-FU + FA               | NR          | NR (NR)                       |
| Cecchin et al.             | 2015                | Italy    | 2003–2007 CRC | 112      | 62          | 65 (30–85)     | II, III                | All   | NR      | 5-FU/CAPE              | NR          | 80 (10–185)                   |
| Chua et al.                | 2009                | Britain  | 1999–2000 mCRC | 118      | 80          | 61 (31–75)     | IV                     | NR    | NR      | 5-FU + LV + OX          | NR          | NR (NR)                       |
| Custodio et al.            | 2014                | Spain    | 2004–2009 CRC | 202      | 115         | 63.8 (30–85)   | II, III                | All   | NR      | None                  | NR          | 51.4 (7–96)                   |
| Delgado-Plasencia et al.   | 2013                | Spain    | 1990–2003 CRC | 50       | 28          | NR (NR)        | NR                     | NR    | NR      | 5-FU-based             | NR          | NR (NR)                       |
| Dong et al.                | 2016                | China    | 2012–2013 CRC | 81       | 44          | 56.2 (27–76)   | NR                     | NR    | NR      | 5-FU + LV + OX         | NR          | 14 (5–20)                     |
| Etienne et al.             | 2004                | France   | NR mCRC       | 98       | 57          | 64 (40–82)     | IV                     | NR    | NR      | 5FU + FA               | NR          | NR (NR)                       |
| Fernández-Peralta et al.   | 2010                | Spain    | 1992–1996 CRC | 143      | 81          | 67.3 (NR)      | NR                     | All   | NR      | 5-FU-based             | NR          | 44.3 (NR)                     |
| Gusella et al.             | 2009                | Britain  | 1999–2008 CRC | 130      | 84          | 64.7 (34–84)   | B, C*                  | NR    | NR      | 5-FU + LV              | NR          | 45.6 (4.8–120.0)            |
| Huang et al.               | 2011                | China    | 2005–2009 mCRC | 157      | 85          | 62.5 (36–82)   | IV                     | NR    | NR      | 5-FU + LV + OX         | NR          | 35 (8–56)                     |
| Jang et al.                | 2014                | Korea    | 1996–2009 CRC | 372      | 215         | 62.1 (NR)      | I–IV                   | All   | NR      | 5-FU-based             | NR          | 34 (4–173)                    |
| Kim et al.                 | 2010                | Korea    | 1995–2004 CRC | 103      | 49          | 57.0 (NR)      | II–IV                  | All   | NR      | 5-FU/5-FU + OX         | NR          | 62.2 (18–121)                |
| Negandhi et al.            | 2013                | Canada   | 1999–2003 CRC | 784      | 327         | 61.4 (20.7–75.0| I–IV                   | All   | NR      | 5-FU (partial)         | NR          | 76.8 (4.8–130.8)            |
| Qiu et al.                 | 2013                | China    | 2004–2006 CRC | 76       | 48          | 57 (21–75)     | I–III                  | All   | NR      | 5-FU + OX + LV         | NR          | NR (37–67)                    |
| Ruzzo et al.               | 2007                | Italy    | NR mCRC       | 166      | 87          | 66 (NR)        | IV                     | NR    | NR      | 5-FU + LV + OX         | NR          | 24 (NR)                       |
| Ruzzo et al.               | 2008                | Italy    | NR mCRC       | 146      | 80          | 61 (38–75)     | IV                     | NR    | NR      | 5-FU + LV + CPT-11     | NR          | NR (NR)                       |
| Sharma et al.              | 2008                | Australia| 2002–2003 mCRC | 54       | 35          | 72 (42–86)     | IV                     | NR    | NR      | CAPE                  | NR          | NR (NR)                       |
| Suh et al.                 | 2006                | Korea    | NR mCRC       | 54       | 30          | 57.8 (35–39)   | II–IV                  | NR    | NR      | 5-FU + LV + OX         | NR          | 23.6 (6–35)                   |
| Taffini et al.             | 2011                | Sweden   | 1999–2006 CRC | 649      | 88          | 66 (32–82)     | III                    | All   | NR      | 5-FU + LV              | NR          | 70 (NR)                       |
| Ulrich et al.              | 2014                | America  | 1994–2000 Rectal cancer | 754      | 480         | 61 (19–86)     | II, III                | All   | NR      | 5-FU + LV              | All         | NR (NR)                       |
| Zhang et al.               | 2007                | America  | 1992–2003 mCRC | 318      | 177         | 58 (25–86)     | IV                     | NR    | NR      | FU + CPT-11/FU + OX    | NR          | 30 (NR)                       |
| Zhu et al.                 | 2013                | China    | 2004–2007 CRC | 411      | 245         | 60 (NR)        | I–IV                   | All   | NR      | 5-FU/5-FU + OX         | NR          | 64 (1–88)                     |

5-FU, 5-fluorouracil; BEV, bevacizumab; CAPE, capecitabine; CPT-11, irinotecan; CRC, colorectal cancer; FA, folic 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.\cite{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.\cite{17}

Linkage disequilibrium among the variants can vary across populations.\cite{18,19} For example, Haerian and Haerian\cite{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).

### 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

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

$^*$, Not available;  
$^\dagger$, for both rs1801133 and rs1801131;  
$^\ddagger$, 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 Mexico33 and the remaining articles were conducted in European or North American countries. All but two eligible articles targeted CRC or mCRC: one addressed rectal cancer22 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-fluouracil (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 CC genotype. Table 3 shows that the HR of the TT versus CC genotype for patients from Asian regions was 1.22 (95% CI = 0.99–1.50) 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

| Subgroup   | Number of articles | Number of patients | HR (95% CI) | Heterogeneity (I², 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         | 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 (CT + CC) genotype; **CT/CC:** CT genotype versus CC genotype; **CT/CC:** TT genotype versus CC genotype; **CT/CC:** CT genotype versus CC genotype; **CT/CC:** CT genotype versus CC genotype; **CT/CC:** CT genotype versus CC genotype; **CT/CC:** CT genotype versus CC genotype.

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 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 4 Results for the subgroup analysis of the MTHFR rs1801131 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) |
| 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    | –                  | –                  | –           |

**, 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).

### 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 677C\(^{49}\). Therefore, the reduction in

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

|          | Number of articles | Number of patients | HR (95% CI) | Heterogeneity (I², 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.

### 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) |

*Results from the random-effects model.

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. (iii) 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 rs1801131 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 rs1801131 and rs1801133 polymorphisms.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflicts of interest.

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Additional supporting information may be found online in the Supporting Information section at the end of the article.

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