Research Article

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Methylene tetrahydrofolate reductase (*MTHFR*) gene rs1801133 C>T polymorphisms and response to 5-FU based chemotherapy in patients with colorectal cancer: a meta-analysis

<https://doi.org/10.1515/pteridines-2019-0015>

received December 20, 2018; accepted April 25, 2019.

Abstract: Background: Methylene tetrahydrofolate reductase (*MTHFR*) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a cosubstrate for homocysteine remethylation to methionine. Single nucleotide polymorphisms (SNP) of *MTHFR* rs1801133 C>T can influence susceptibility to colorectal cancer. However, an association between *MTHFR* rs1801133 C>T polymorphisms and response to 5-Fluorouracil (5-FU) based chemotherapy in patients with colorectal cancer was not clear. **Methods:** Studies relevant to *MTHFR* rs1801133 C>T polymorphisms and response to 5-FU based chemotherapy in patients with colorectal cancer were systematically searched in the electronic databases of PubMed, Web of Science, Embase, and China National Knowledge Infrastructure (CNKI). The genotypes of CC, CT, and TT were extracted from each included publication. The genotypes CC, CT, and TT distribution in 5-FU based chemotherapy response and resistance groups were calculated and pooled through random or fixed effect model by the effect size of odds ratio (OR) and 95% confidence interval (95% CI). The publication bias was evaluated through Begg's funnel plot and Egger's line regression test. **Results:** After searching the electronic databases, 16 studies related to *MTHFR* gene rs1801133 C>T polymorphisms and a response to 5-FU based chemotherapy in patients with colorectal cancer were included in the present meta-analysis. The pooled data showed no statistical difference in tumor response

rate between CT+TT and CC groups in the dominant genetic model CT+CC vs CC (OR=1.21, 95% CI: 0.93~1.59, $p>0.05$) and recessive model TT vs CT+CC (OR=1.37, 95% CI: 0.91~2.06, $p>0.05$). The grade 3-4 adverse reaction rate between CT+TT and CC groups also had no statistical difference in the dominant genetic model CT+CC vs CC (OR=0.90, 95% CI: 0.76~1.07, $p>0.05$) and recessive model TT vs CT+CC (OR=1.12, 95% CI: 0.84~1.50, $p>0.05$). The Begg's funnel plot and Egger's line regression test demonstrated no publication bias. **Conclusion:** The response and adverse reaction of 5-FU based chemotherapy in colorectal patients were not different in terms of *MTHFR* rs1801133 C>T polymorphisms.

Keywords: *MTHFR*; polymorphism; chemotherapy; 5-FU; adverse reaction; meta-analysis.

Introduction

Methylene tetrahydrofolate reductase (*MTHFR*) is a key enzyme in folate metabolism and methionine metabolism. The rs1801133 C>T polymorphism of the *MTHFR* gene leads to a decrease in enzyme activity, inhibits the folic acid metabolism cycle, affects the synthesis of pyrimidine or purine, and leads to the damage of DNA synthesis or repair, as well as the defect or canceration of cell growth [1]. In recent years, with the development of pharmacogenomics, researchers began to explore the relationship between *MTHFR* gene SNPs and the sensitivity to chemotherapy regimens. 5-FU based chemotherapy regimen is extensive and used long-term clinically for esophageal-gastric cancer [2] and colorectal carcinoma [3, 4]. However, most of the studies relevant to *MTHFR* rs1801133 C>T polymorphisms and response to 5-FU based chemotherapy in patients with colorectal cancer were retrospective studies with small sample size. Therefore, we performed this meta-analysis

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by pooling the open published studies of the relationship between *MTHFR* rs1801133 C>T polymorphisms and 5-FU based chemotherapy response in order to provide a more reliable evidence for the rational use of 5-FU chemotherapy regimen in clinical practice.

Material and methods

Publications searching and inclusion

The electronic databases of PubMed, Web of Science, Embase, and CNKI were systematic searched to identify relevant studies related to *MTHFR* gene rs1801133 C>T polymorphisms and a response to 5-FU based chemotherapy in patients with colorectal cancer (**Figure 1**). The searching text words were methylene tetrahydrofolate reductase/*MTHFR*, colorectal cancer/colon cancer/rectal cancer/colorectal carcinoma/ colon carcinoma/ rectal carcinoma/ OR CRC, and chemotherapy. The publication screening procedure was last performed in October 10, 2018. The study inclusion criteria was (1) the patients were diagnosed of colorectal cancer by pathology, (2) the chemotherapy regimen was 5-FU based, (3) genotype of CC, CT, and TT can be extracted from original publications, and (4) tumor response rate and adverse reaction can be calculated from original studies of different genotype.

Ethical approval: The conducted research is not related to either human or animals use.

Data extraction from original publication

After reviewing the included full text paper, the general information and useful data was extracted by two reviewers independently and checked by a third reviewer as described according to the Cochrane Handbook for systematic reviews. The general information included the name of the authors, the manuscript publication year, region, chemotherapy regimen, and chemotherapy adverse reaction and response. The useful data of sample size, distribution of CC, CT, TT genotype in tumor response and resistance groups were carefully extracted from the original publications.

Statistical methods

STATA/SE 11.0 StataCorp LP, <http://www.stata.com> software were used for the data analysis. The odds of CC, CT and TT genotypes in chemotherapy sensitivity groups

Figure 1: Literature screening process and results.

versus chemotherapy resistance groups, or grade 3-4 adverse reaction groups versus non-grade 3-4 adverse reaction groups were expressed by odds ratio (OR) and 95% confidence intervals (95% CI). The statistical heterogeneity across the included 16 publications in each effect size was assessed by I^2 test [5]. The OR was pooled by random or fixed effect models according to statistical heterogeneity. The publication bias was assessed by Begg's funnel plot and Egger's line regression tests [6].

Results

General information of the included publications

After searching the electronic databases of PubMed, Web of Science, Embase, and CNKI, 16 studies [7-22] related to *MTHFR* gene rs1801133 C>T polymorphisms and response to 5-FU based chemotherapy in patients with colorectal cancer were included in the present meta-analysis. The general information of the included 16 publications is shown in **Table 1**.

Tumor response rate in dominant mutation model [(CT+TT) vs CC]

For dominant genetic model [(CT+TT) vs CC], there was no significant statistical heterogeneity ($I^2=42.2\%$, $P=0.07$). Therefore, the data was pooled under a fixed effect model. The combined results demonstrated there was no statistical difference in tumor response rate between CT+TT and CC groups (OR=1.21, 95% CI: 0.93~1.59, $p>0.05$), **Figure 2**.

Table 1: General characteristics of the included 16 studies.

First author	Publication (year)	Region	Sample size	Genotype			Chemotherapy	Results
				CC	CT	TT		
Cohen	2003	Canada	64	17	42	5	5FU/ Capecitabine	TR
Etienne	2004	France	97	52	27	18	5-FU/LV	TR
Jakobsen	2005	Denmark	79	32	38	9	5-FU/LV	TR
Marcuello	2006	Spanish	94	32	50	12	FOLFOX/FOLFIRI	TR
Capitain	2007	France	67	28	31	8	5-FU/LV	TR
Ruzzo	2008	Italia	146	40	73	33	FOLFIRI	TR
Suh	2008	Korea	50	16	28	6	FOLFOX	TR
Sharma	2008	Australia	54	27	20	7	FLFOX/FOLFIRI	AR
Braun	2009	England	1601	734	719	148	FU/FLFOX/FOLFIRI	AR
Chua	2009	Australia	116	43	54	19	FOLFOX	AE
Etienne-Grimaldi	2010	France	116	44	58	14	FOLFOX	TR
Glimelius	2011	Sweden, Norway	140	68	63	9	FLIRI/FOLIRI	AR
van Huis-Tanja	2013	Netherlands	264	106	131	27	Capecitabine /XELIRI	AR
Kumamoto	2013	Japan	62	26	30	6	mFOLFOX6	AR TR
Shen	2015	China	127	51	60	16	FOLFOX	TR
Dong	2016	China	72	39	30	3	FOLFOX6	TR

TR: tumor response; AR: adverse reaction

Figure 2: Forest plot of MTHFR gene rs1801133 C>T polymorphisms and chemosensitivity of colorectal cancer in dominant genetic model [(CT+TT) vs CC].

Figure 3: Forest plot of MTHFR gene rs1801133 C>T polymorphisms and chemosensitivity of colorectal cancer in recessive genetic model [(TT vs (CT+CC))].

Figure 4: Forest plot of MTHFR gene rs1801133 C>T polymorphisms and grade 3-4 adverse reaction of colorectal cancer in recessive genetic model [(CT+TT) vs CC].

Tumor response rate in recessive mutation model [TT vs (CT+CC)]

For recessive genetic model [TT vs (CT+CC)], the pooled OR for tumor response rate between TT and CT+CC groups was (OR=1.37, 95% CI: 0.91~2.06, $p>0.05$) in a fixed effect model without statistical difference, **Figure 3**.

Grade 3-4 adverse reaction in dominant mutation model[(CT+TT) vs CC]

For dominant genetic model [(CT+TT) vs CC], there was no significant statistical heterogeneity ($I^2=17.8\%$, $p=0.29$). The pooled data indicated no statistical difference of grade 3-4 adverse reaction in (CT+TT) and CC group with the (OR=0.90, 95% CI: 0.76~1.07, $p>0.05$), **Figure 4**.

Figure 5: Forest plot of MTHFR gene rs1801133 C>T polymorphisms and grade 3-4 adverse reaction of colorectal cancer in recessive mutation model [TT vs(CT+CC)].

Table 2: The publication bias evaluation by Egger's line regression test.

Model	Coef	t	p	95% CI
TR_dominant	-1.64	-0.54	0.60	-8.49~5.20
TR_recessive	3.86	1.52	0.17	-2.15~9.87
AE_dominant	-0.25	-0.41	0.70	-1.82~1.32
TR_recessive	0.51	0.50	0.65	-2.75~3.78

Grade 3-4 adverse reaction in recessive mutation model [TT vs (CT+CC)]

Without statistical heterogeneity, the data was pooled under a fixed effect model. The combined results indicated no statistical grade 3-4 adverse reaction between TT and CT+CC groups (OR=1.12, 95% CI: 0.84~1.50, $p>0.05$), **Figure 5**.

Publication bias analysis

The publication bias of the included 16 studies was assessed by Begg's funnel plot (**Figure 6**) and Egger's line regression test. The funnel plots were general symmetrical which indicating no obvious publication bias. And the Egger's line regression test also demonstrated no significant publication bias ($p_{all}>0.05$), **Table 2**.

Discussion

Colorectal cancer (CRC) is one of the most diagnosed malignant tumor of the digestive system [23]. It was the fourth most diagnosed cancer in the United States with an estimated 143,460 new cases in the year 2012 [24]. The general prognosis of the early stage CRC was good in patients who received surgery. However, the 5-year survival rate was low even under the treatment of chemoradiotherapy for advanced stages of the disease. The most used chemotherapy regimen was fluorouracil (5-FU) based chemotherapy regimen for colorectal cancer patients [4, 25].

Folic acid is involved in the synthesis and transformation of nucleotides. Methylene tetrahydrofolate reductase (*MTHFR*), a key enzyme in folate metabolism, catalyzes the irreversible conversion of 5,10-methylene tetrahydrofolate (5,10-MTHF) to 5-methyltetrahydrofolate, which converts homocysteine to methionine and then to S-adenosylmethionine (SAM). The most common SNP site of *MTHFR* gene is rs1801133 C>T, where cytosine C mutates to thymine T (677C>T), resulting in alanine being replaced by valine, which reduces the activity of *MTHFR* [26-29]. 5-FU is transformed into fluorouracil deoxynucleotide (FdUMP) in cells with thymidylate synthase (TS) and 5,10-MTHF, inhibiting the function of thymidylate synthase, preventing the conversion of deoxyuridine (dUMP) to deoxythymidine (dTTP), affecting DNA synthesis[30, 31]. Therefore, *MTHFR* gene rs1801133 C>T polymorphisms can affect 5-FU base chemotherapy in patients with colorectal cancer.

Figure 6: The funnel plot for publication bias evaluation.

Recently, several studies [16, 18, 20] have discussed the correlation between *MTHFR* gene rs1801133 C>T polymorphisms and response to 5-FU based chemotherapy in patients with CRC. However, because of small sample size and weak statistical power, clinical evidence was weak. Therefore, we performed this meta-analysis in order to further evaluate the relationship between *MTHFR* rs1801133 C>T polymorphism and 5-FU based chemotherapy response in order to provide a more reliable evidence for the rational use of 5-FU chemotherapy regimens in clinic. We found the response and adverse reaction of 5-FU based chemotherapy in colorectal patients was not different in terms of *MTHFR* rs1801133 C>T polymorphisms.

Although we did not find any correlation between *MTHFR* rs1801133 C>T polymorphism and 5-FU based chemotherapy response or adverse reactions, the results demonstrated a trend that patients with TT/CT genotype were more prone to response to 5-FU based chemotherapy response and had less grade 3-4 adverse reactions.

Conclusion

Base on the present evidence, the response and adverse reactions of 5-FU based chemotherapy in colorectal patients were not different in terms of *MTHFR* rs1801133 C>T polymorphisms. However, only 16 publications were included with a relative small sample size. More studies are needed to further discuss this correlation.

Conflict of interest: Authors state no conflict of interest

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