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ORIGINAL ARTICLE

CDA and *MTHFR* polymorphisms are associated with clinical outcomes in gastroenteric cancer patients treated with capecitabine-based chemotherapy

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Abstract

Purpose The impact of pharmacogenetics on predicting survival in gastroenteric cancer remains unclear.

Methods We tested 322 consecutive patients treated with capecitabine-based chemotherapy for *CDA* and *MTHFR* polymorphisms.

Results Patients who carried the *CDA* 79 A>C (rs2072671) CC genotype showed significantly shorter progression-free survival (PFS) comparing with A-allele ($P=0.008$). A significant better PFS was found in the patients with 451 A>G (rs532545) G-allele ($P=0.002$) and 92 C>T (rs602950) T-allele ($P=0.002$). In addition, a shorter PFS was also observed in patients with *MTHFR* 1298 A>C (rs1801131) CC genotype than the patients with AC or AA genotype after capecitabine-based chemotherapy ($P=0.002$). Furthermore, the colon, female, or elder (> 65 years old) patients with *MTHFR* 1298 A>C CC genotype had poorer PFS than A-allele. Moreover, *CDA* 451 A>G was independent predictors of chemotherapy-induced toxicity in colon patients. Multivariate Cox regression analysis demonstrated that the *CDA* 79 A>C CC, 451 A>G AA, 92 C>T CC, and *MTHFR* 1298 A>C CC were predictive of shorter PFS in gastroenteric cancer patients.

Conclusions The results reminded us those gastroenteric cancer patients with *CDA* 79 A>C CC, 451 A>G AA, 92 C>T CC, or *MTHFR* 1298 A>C CC genotype are not likely to benefit from the therapy of capecitabine-based chemotherapy.

Keywords Gastroenteric cancer · Capecitabine · *CDA* · *MTHFR* · Polymorphism · Prognosis

Introduction

Colorectal and gastric cancers are the most common gastroenteric tumors, with a wide range of outcomes. Capecitabine is an oral pro-drug of the anti-metabolite 5-fluorouracil, and has been registered for treatment of advanced colorectal and gastric cancer. Although the introduction of capecitabine alone or in combination with other chemotherapeutic has improved prognosis and allowed some tailoring of therapy, a significant fraction of gastroenteric tumor patients still fail treatment and die [1–4].

Capecitabine is activated to 5-fluorouracil requiring carboxylesterase (CES), cytidine deaminase (CDA) and thymidine phosphorylase (TYMP), of which polymorphisms might be associated with efficacy and toxicity [5, 6]. Moreover, the catabolism of 5-fluorouracil is mainly controlled by DPD in the liver. *DPYD* (encoding DPD) variants were considerably among individuals with consequences for outcome, and dose reduction of fluoropyrimidines based on the presence of *DPYD* variants has already been recommended [7, 8]. CDA is the rate-limiting enzyme activating capecitabine to 5-fluorouracil, and high CDA activity in cancer cells has been associated with increased sensitivity to capecitabine [9, 10]. A potential role of CDA in capecitabine-induced severe life-threatening toxicity has been suggested [11]. Folate metabolism plays a key role in influencing the anticancer activity of fluoropyrimidines. 5-fluorouracil exerts its anticancer activity through misincorporation of its metabolites into RNA and DNA, and inhibition of thymidylate synthase (TS). The optimal inhibition of TS depends on elevated cellular concentrations of 5,10-methylenetetrahydrofolate

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(5,10-MTHF) [12]. Methylenetetrahydrofolate reductase (MTHFR) is an important enzyme in folate-metabolizing pathway though regulates the levels of 5,10-MTHF [13]. *CDA* and *MTHFR* polymorphisms are closely related to the efficacy of fluoropyrimidines (capecitabine and 5-fluorouracil) for the treatment of gastroenteric tumors. But the impact of genetic polymorphisms on drug activity/metabolism and subsequently treatment outcome is largely unclear.

Thus, we assessed the effects of the *CDA* and *MTHFR* polymorphisms on survival in a cohort of 322 Chinese colorectal and gastric patients treated with capecitabine-based chemotherapy to define that specific subgroups were more likely to benefit from the therapy.

Materials and methods

Patient and data collection

This cohort of patients had newly diagnosed, histopathologically confirmed, and untreated colorectal and gastric cancer who received first-line standard XELOX (intravenous oxaliplatin 130 mg/m^2 on day 1, followed by capecitabine 1000 mg/m^2 per twice daily on days 1–14 with cycles repeated every 21 days) or capecitabine only (1250 mg/m^2 twice daily from day 1–14 of 21-day cycle) from January 2015 until December 2017. The patients received chemotherapy regimen containing capecitabine at Harbin Medical University Cancer Hospital, and 322 patients were recruited and underwent genotype analysis. Some blood samples for the genotyping were not available for the patients who were recruited early in the study and these patients were not included in final analysis. Samples were collected in tubes containing EDTA and frozen immediately on reception at − 80 °C. Patients without follow-up and those receiving only palliative treatment were excluded. A detailed questionnaire, including requests for demographic information, was administered to all the subjects.

At our institution, patients with colorectal and gastric cancer are typically followed and monitored during and after treatments with regularly scheduled clinical and radiographic examinations. Clinicopathological data were collected from medical records including age, gender, location of primary tumor, Ann Arbor stage, general classification of tumor, histological classification of tumor, tumor differentiation, chemotherapy regimen, and family history of malignant. During treatment, the following variables were evaluated: total number of courses per patient, date and type of response and progression, each course of chemotherapy, red blood cell and platelet transfusion and toxicity. Hematological toxicity (anemia, febrile neutropenia, neutrophil count decreased, platelet count decreased, and white blood cell decreased) and extra-hematological toxicity (hand-foot syndrome (HFS), diarrhea, vomiting, fatigue, stomatitis, and hepatotoxicity) were graded according to Common Terminology Criteria for Adverse Events of the National Cancer Institute (CTCAE 4.0). All adverse events at any time were monitored and reported.

In this study, follow-up visits were scheduled every 3 months for the first 2 years and every 6 months thereafter. The median follow-up time was 32 months (range 1–73 months) for all subjects. No patient was lost to follow-up. The response to therapy was evaluated at the end of the fourth course and at the end of treatment. Progression-free survival (PFS) is defined as the time interval from the start of first-line chemotherapy to first disease progression or death from any cause if disease progression does not occur. Alive patients without progression will be censored at the last follow-up.

The protocol of the study was approved by the institutional review board of Harbin Medical University Cancer Hospital. All participants provided written informed consents and all efforts had been made to protect patient privacy and anonymity. The study was conducted according to the Declaration of Helsinki.

DNA extraction and genotyping

SNPs were selected according to the following criteria: (1) SNPs known to be relevant for the prediction of outcome or toxicity; (2) SNPs affecting regulatory regions and predicting altered expression or function of the gene; and (3) SNPs with a minor allele frequency > 5% in the study population. Twelve polymorphisms from 3 genes were analyzed as follows: (1) *CDA* (79 A>C, 92 C>T, 451 A>G, 1172 G>A); (2) *MTHFR* 1298 A>C.

DNA samples were extracted from stored blood samples using QIAamp DNA Blood Midi or Maxi kit (Qiagen, Hamburg, Germany). SNP genotyping was carried out using the Multiplex SNaPshot method. Polymerase chain reactions (PCRs) contained 10–50 ng of DNA, 10 × HotStarTaq buffer, 3 mM $MgCl_2$, 300 µM of each dNTP, 0.08 µM of each primer, and one unit of HotStar Taq polymerase (Qiagen, Hamburg, Germany) in 15 µl reaction volume. The following touchdown PCR program was used: denaturation at 95 °C for 3 min followed by 35 cycles of 94 °C for 15 s, annealing at 55 °C for 15 s, and extension at 72 °C for 30 s, and a final extension at 72 °C for 3 min. The PCR products were purified by treatment with Exonuclease I (USB Corporation, Cleveland, OH, USA) and Shrimp Alkaline Phosphatase (USB Corporation, Cleveland, OH, USA) at 37 °C for 15 min followed by incubation at 80 °C for 15 min. The extension reaction contained 1 × ABI Prism SNaPshot Multiplex ready reaction mix (Applied Biosystems, Grand Island, NY, USA), 0.5 µM of each primer, and 3 µl of each PCR product and was carried out as recommended (Applied

Biosystems, Grand Island, NY, USA). The extension PCR products were purified using one unit of Shrimp Alkaline Phosphatase and then analyzed using an ABI 3730xl Genetic Analyzer. SNP calling was carried out using GENEMAPPERTM software v.4.0 (Applied Biosystems, Grand Island, NY, USA). For quality control, 15% of the assays were randomly selected for sequencing. The results of the quality control analysis confirmed 100% concordance.

PRIMER5 (http://frodo.wi.mit.edu/) was used to design primers for each SNP within a multiplex. Extension primers were chosen from the sequence up- or down-stream of each SNP immediately, and the primers are listed in Table 1.

Statistical analysis

Responses were scored according to the International Working Group criteria, and assessed by Fisher exact test and Armitage trend test. PFS was defined as time to disease progression, relapse, or death. For each SNP, dominant and recessive genetic models were selected for analysis and the model with highest statistical significance was considered to be the best-fitting model. Survival distributions were estimated with the Kaplan–Meier method and compared with the log-rank test. Multivariate analyses were done using Cox proportional hazard models to estimate hazard ratios and their 95% confidence interval (CI) for having an event. Analysis for toxicity was performed by binary logistic regression analysis with SNP genotypes as the explainable variables.

The statistical analyses were performed using the Statistical Package for Social Sciences (SPSS) version 16.0 statistical software. Statistical significance was set at $P < 0.05$ and all tests were two-sided.

Results

Subjects' characteristics

A total of 322 patients who received capecitabine-based chemotherapy for colorectal and gastric cancers were included in the current study. All of them were Chinese Han, including 206 males and 116 females with median age at diagnosis of 56.4 years (range 31–78). Clinical characteristics and treatment outcomes of the cohort are displayed in Table 2.

The correlation of clinical characteristics with variant genotypes

We detected the association between the variant genotypes and the clinicopathological data of the patients according to Ann Arbor stage (I, II vs. III, IV) and tumor differentiation (middle plus high vs. low). However, the various SNPs were not correlated with clinical characteristics.

Response to therapy and outcome

For *CDA* variants, we observed differences in the survival curve for patients. The median PFS was 24.8 months (95% CI 0.4–49.1) for patients with CC genotype of 79 A>C, and were 55.6 months (95% CI 46.8–64.4) and 51.6 months (95% CI 48.2–55.0) in the AC and AA genotypes, respectively (log-rank; $P = 0.008$; Fig. 1a). In addition, patients with 92 C>T CC had shorter PFS than patients with CT or TT genotype (log-rank test, $P = 0.002$; Fig. 1b). Meanwhile, there was a significant better PFS for the patients with 451 A>G AG or GG genotype than AA genotype (log-rank test, $P = 0.002$; Fig. 1c). Moreover, we have undertaken a combined analysis comparing favorable with unfavorable alleles. And we found that patients with 79 A>C A-allele had better PFS than patients with non-A-allele (log-rank

Table 1 Genotyping primers of *CDA* and *MTHFR* SNPs

SNP	Allele	PCR primer	SNaPshot primer
CDA rs2072671 (79 A>C)	A/C	F: CTGAAGCCTGAGTGTGTCCA R: GTGCCCACCTTTACCTTTGAA	ctgactgactgactgactgaCTGTAGGGGCAGTAGGCTGACT
CDA rs602950 (92 C>T)	C/T	F: CAGAGCAGCGGGAAACAG R: TGCGTACCTGAGAGCCTG	TCTGGCTGCAGGGACACACCCA
CDA rs532545 (451 A>G)	A/G	F: GAAGGGCTGAGGCTGAAAG R: TGGGCTAGGGCAAAGAGAA	ctgactgactgactCTGCAGCTTGTTCATGCCTCCTGCCT
CDA rs6690069 (1172 G>A)	A/G	F: TGAGGACACTGAGGCTTAGG R: GAGACTGTAGAAGGGCTCCA	ctgacTATGACATTGGGAAGGTCACACA
MTHFR rs1801131 (1298 A>C)	A/C	F: TTCTACCTGAAGAGCAAGTCC R: CACTCCAGCATCACTCACTT	ctgactgactgaATGTGGGGGGAGGAGCTGACCAGTGAAG

PCR polymerase chain reaction, *SNP* single-nucleotide polymorphism

Table 2 Clinical characteristics of the gastroenteric tumor cohort at diagnosis

Clinical characteristics	Patients ($n=322$)
Age (years)	56.4 (31–78)
Age > 65 years	64
Male to female	206:116
Location of primary tumor	
Colon	132
Rectum	110
Stomach	80
Ann Arbor stage	
I–II	92
III–IV	230
General classification of tumor	
Protrude type	120
Invasive and ulcerative type	202
Histological classification of tumor	
Adenocarcinoma	280
Mucinous adenocarcinoma	42
Tumor differentiation	
High	17
Middle	202
Low	103
Chemotherapy	
XELOX[a]	287
Capecitabine as single chemotherapy	13
Others	22
Family history of malignant	
Yes	36
No	286

[a]XELOX contained capecitabine plus oxaliplatin

test, $P=0.002$; Fig. 2a). Meanwhile, there was a significant better PFS for the patients with 92 C>T T-allele (log-rank test, $P=0.001$; Fig. 2b) or 451 A>G G-allele (log-rank test, $P=0.001$; Fig. 2c) than non-T-allele or non-G-allele, respectively.

Patients who carried the *MTHFR* 1298 A>C CC genotype showed significantly shorter PFS comparing with A-allele (log-rank test, $P=0.002$; Fig. 3a). The estimated median PFS for patients who had the AA, AC or CC genotype of 1298 A>C was 58.2 months (95% CI 50.0–66.5), 34.4 months (95% CI 29.5–39.3) and 18.0 (95% CI 10.9–25.0), respectively. In addition, the patients who carried the *MTHFR* 1298 A>C non-A-allele showed significantly shorter PFS comparing with the patients with A-allele (log-rank test, $P<0.001$; Fig. 3b). Furthermore, potential implications including location of primary tumor/age/gender were investigated. We found that colon patients with 1298 A>C CC genotype had shorter PFS comparing with AA or AC genotype (log-rank test, $P=0.004$; Fig. 4a). Female patients with 1298 A>C CC genotype also had shorter PFS comparing with A-allele (log-rank test, $P=0.021$; Fig. 5a). Meanwhile, elder patients (> 65 years old) with CC genotype of 1298 A>C displayed a poor PFS (log-rank test, $P=0.005$; Fig. 6a). However, this trend was not found in rectal patients (log-rank test, $P=0.519$; Fig. 4b), male (log-rank test, $P=0.085$; Fig. 6b), and younger patients, respectively (log-rank test, $P=0.069$; Fig. 5b).

With respect to the occurrence of side effects, chemotherapy-induced grade 3 or 4 hepatotoxicity was less frequent in *CDA* 451 A>G AA genotype compared with AG or GG genotype ($P=0.035$; OR = 0.200, 95% CI 0.045–0.899). In addition, we found that 451 A>G AA was also an independent predictor of grade 3–4 hematologic toxicity ($P=0.039$; OR = 0.205, 95% CI 0.045–0.927) (Table 3). However, other various SNPs were not correlated with the occurrence of side effects.

Multivariate analysis

To estimate the independent impact of each variable on PFS, a descriptive Cox proportional hazard model was established adjusting for age and gender. The results of the multivariate analysis are shown in Table 4. Multivariate Cox regression analysis showed that a shorter PFS was found in the patients with *CDA* 79 A>C non-A-allele than the patients with A-allele ($P=0.001$; HR = 8.671, 95% CI 2.358–31.887). In addition, the patients who carried the 92 C>T T-allele ($P<0.001$) or 451 A>G G-allele ($P<0.001$) both had better PFS compared with the patients with non-T-allele or non-G-allele genotype, respectively. In addition, compared to *MTHFR* 1298 A>C A-allele, the relative risk of non-A-allele was 4.900 for PFS ($P<0.001$; HR = 4.900, 95% CI 1.879–12.783).

Discussion

In this study, we document that: (1) significantly shortened PFS after capecitabine-based chemotherapy was observed in the patients with *CDA* 79 A>C TT, 451 A>G AA, or 92 C>T CC genotype; (2) the patients with *MTHFR* 1298 A>C CC genotype had poorer PFS than AA or AC genotype; and (3) chemotherapy-induced grade 3 or 4 hepatotoxicity and hematologic toxicity were less frequent in *CDA* 451 A>G AA genotype compared with AG or GG genotype.

Capecitabine is activated to 5-fluorouracil through an enzymatic process requiring CDA, which is a ubiquitous enzyme mainly expressed in the liver. High expression of CDA in cancer cells is associated with increased sensitivity to capecitabine. It is of note that while CDA as a major detoxifying enzyme for other anti-metabolites is involved in the activation of capecitabine [9–11]. Mostly investigated

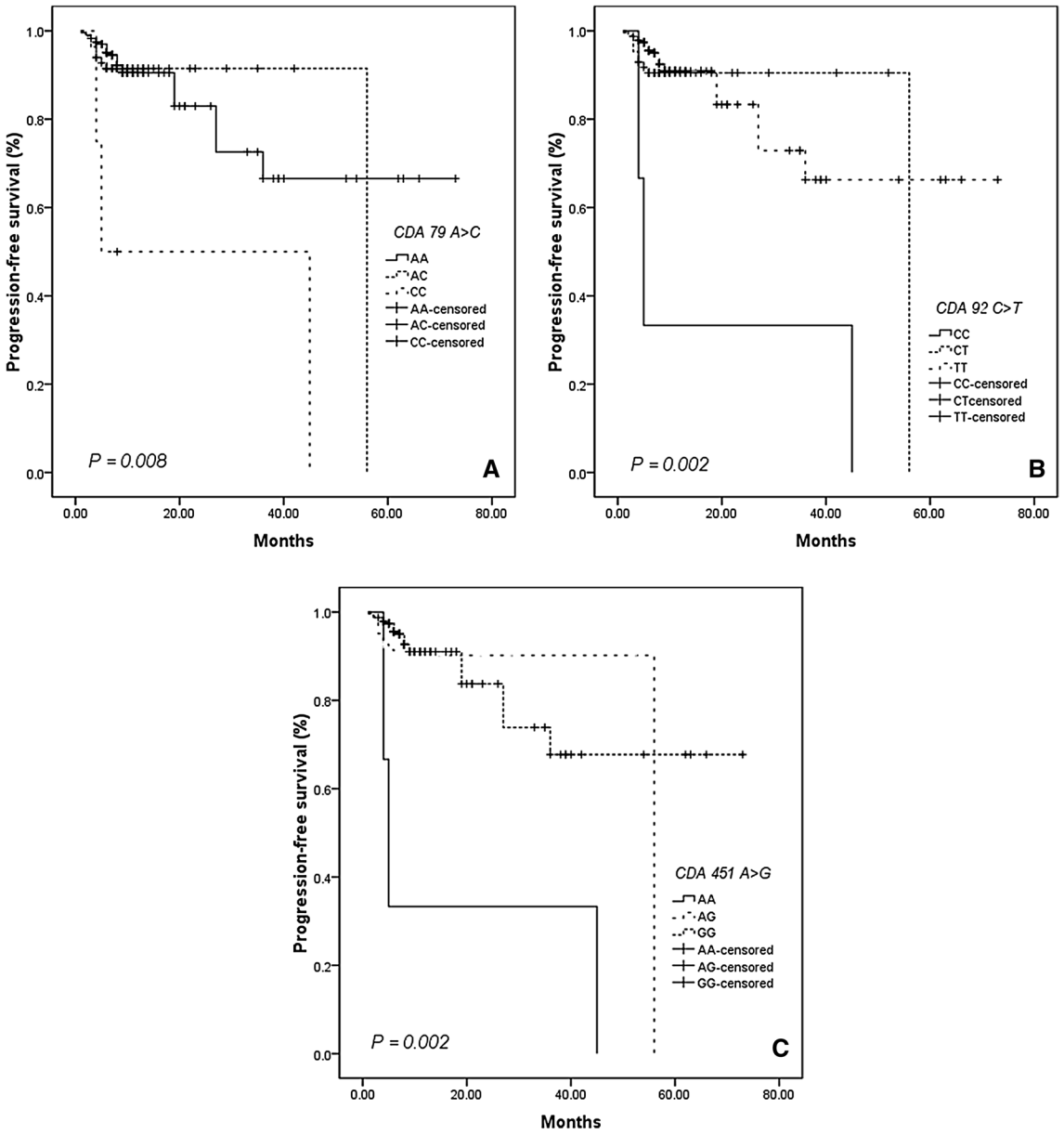

Fig. 1 Kaplan–Meier curves illustrate progression-free survival of patients with capecitabine-based chemotherapy according to the variant genotype of *CDA* 79 A>C (**a**), *CDA* 92 C>T (**b**), and *CDA* 451 A>G (**c**)

CDA 79 A>C had shown to be associated with altered enzyme activity or to affect exposure to CDA-metabolized drugs. 79 A>C A-allele had a significantly higher tumor response compared with C allele in metastatic colorectal cancer patients [14]. In addition, C-containing allele of 79A>C had significantly shorter OS (9.0 vs. 12.6 months, $P=0.036$) and a higher occurrence rate of metastatic disease (100% vs. 79%, $P=0.005$) compared with non-CC genotypes for advanced gastric cancer [15]. In addition, Marta et al. found the patients with 79 A>C AA genotype had a lower risk of overall toxicity (OR = 0.500; 95% CI 0.301–0.831, $P=0.007$) [16]. Homozygous AA patients presented a higher risk of overall toxicity than the CRC patients with AC or CC genotype in Spain (OR = 1.84; 95% CI 1.06–3.18, $P=0.029$) [17]. Grade 2–3 HFS was associated with *CDA* 451 A>G (OR = 2.02, $P=0.039$) [18], 79 A>C (OR = 0.275, $P=0.008$) [16], and 1172 G>A (OR = 3.50, 95% CI 1.13–10.9, $P=0.030$) [19]. Moreover, carriers of the 451C>T and 92A>G were associated with increased risk of 2–4 diarrhea, respectively [19, 20]. But no associations were found in other two studies [21, 22].

Methylenetetrahydrofolate reductase (MTHFR) plays a key role in the metabolism of folates. MTHFR carries out a central reaction by catalyzing the conversion

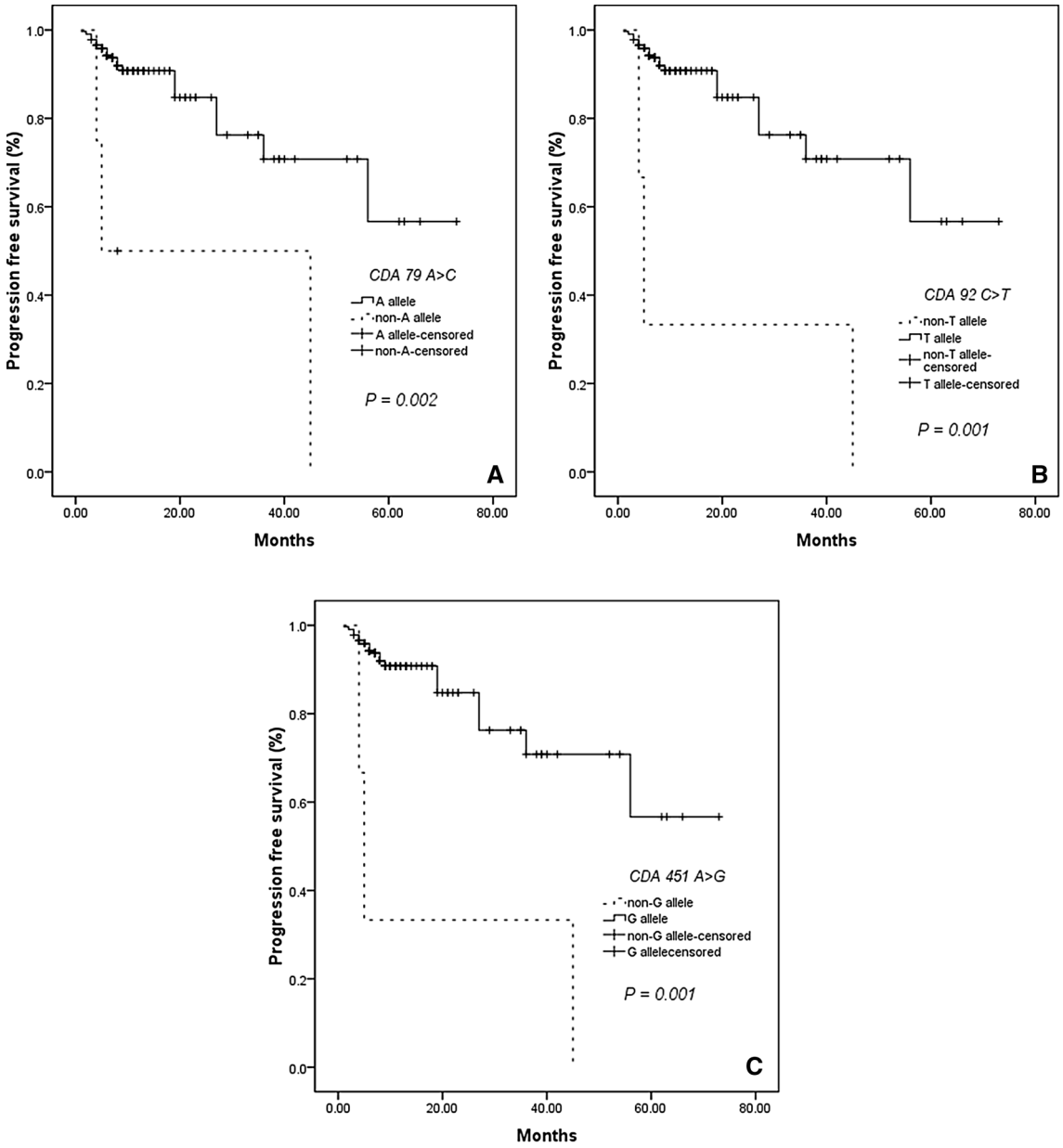

Fig. 2 Kaplan–Meier curves illustrate progression-free survival of patients with capecitabine-based chemotherapy according to the comparison of favorable with unfavorable alleles of *CDA* 79 A>C (**a**), *CDA* 92 C>T (**b**), and *CDA* 451 A>G (**c**)

of 5,10-MTHF to 5-methyltetrahydrofolate, which serves as a methyl group for DNA methylation reactions [13]. Low MTHFR activity might theoretically enhance cytotoxicity of 5-fluorouracil. Two common SNPs related to MTHFR activity are *MTHFR* − 677C>T (rs1801133) and − 1298C>A. Zhong et al. conducted a meta-analysis to estimate the correlation of *MTHFR* polymorphisms with the clinical response to fluoropyrimidine-based chemotherapy in CRC patients [23]. The publication concluded five Asian studies. Of these, four studies investigated the association between *MTHFR* C677T polymorphism and response to fluoropyrimidine-based chemotherapy in CRC patients. There was no significant correlation of *MTHFR* C677T SNP with drug response [24–27]. Only one study detected the association between *MTHFR* A1298C polymorphism and response in the south of China [28], but the association was unclear in northern China. For gastric cancer (GC), Cheng Tang et al. performed a systematic literature search exploring *MTHFR* polymorphisms C677T's relationship with the clinical outcomes of GC patients treated with 5-fluorouracil-based chemotherapy [29].

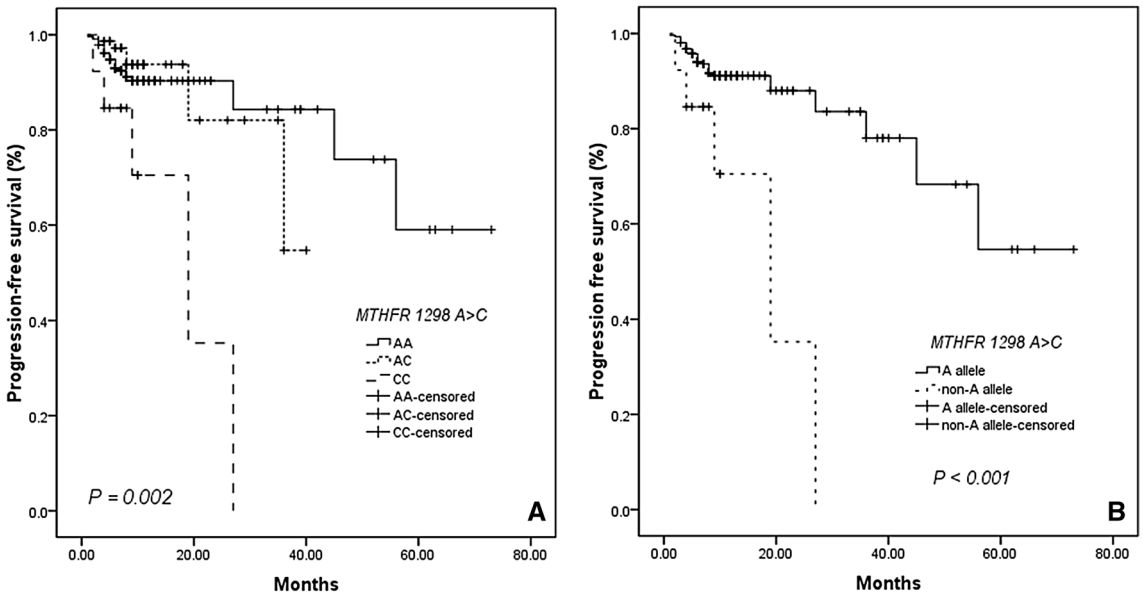

Fig. 3 Kaplan–Meier curves illustrate progression-free survival of patients with capecitabine-based chemotherapy according to the variant genotype (**a**) and the comparison of favorable with unfavorable alleles (**b**) of *MTHFR* 1298 A>C

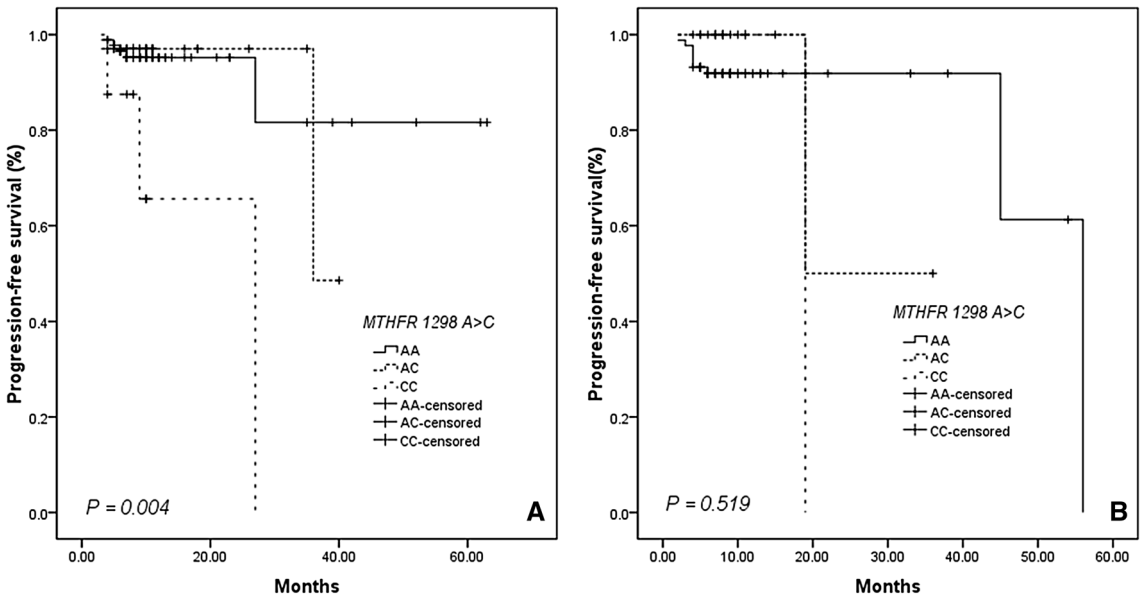

Fig. 4 Kaplan–Meier curves illustrate progression-free survival of colon patients (**a**) and rectal patients (**b**) with capecitabine-based chemotherapy according to the variant genotype of *MTHFR* 1298 A>C

Stratifying by patients' ethnicities, the pooled HRs of GC patients with genotype CT plus TT versus those with genotype CC were 0.92 (95% CI 0.66–1.28) for studies conducted in Asian (China) [30–33]. Up to date, only three studies detected the association between *MTHFR* A1298C polymorphism and response in Asia [30, 33, 34]. Liu R et al. did not find that *MTHFR* A1298C polymorphism was significantly associated with GC patients' survival [30]. One study suggested that *MTHFR* 1298CC genotype showed protective effect for all patients. However, 284 of all the patients were treated with 5-fluorouracil-based chemotherapy, and the association between these patients and *MTHFR* A1298C polymorphism was not shown [33]. Meanwhile, another study only recruited nine GC patients in Japan [34]. To sum up, the association of *MTHFR* A1298C remained unclear. Thus, the present study was performed to provide a comprehensive estimate in this account.

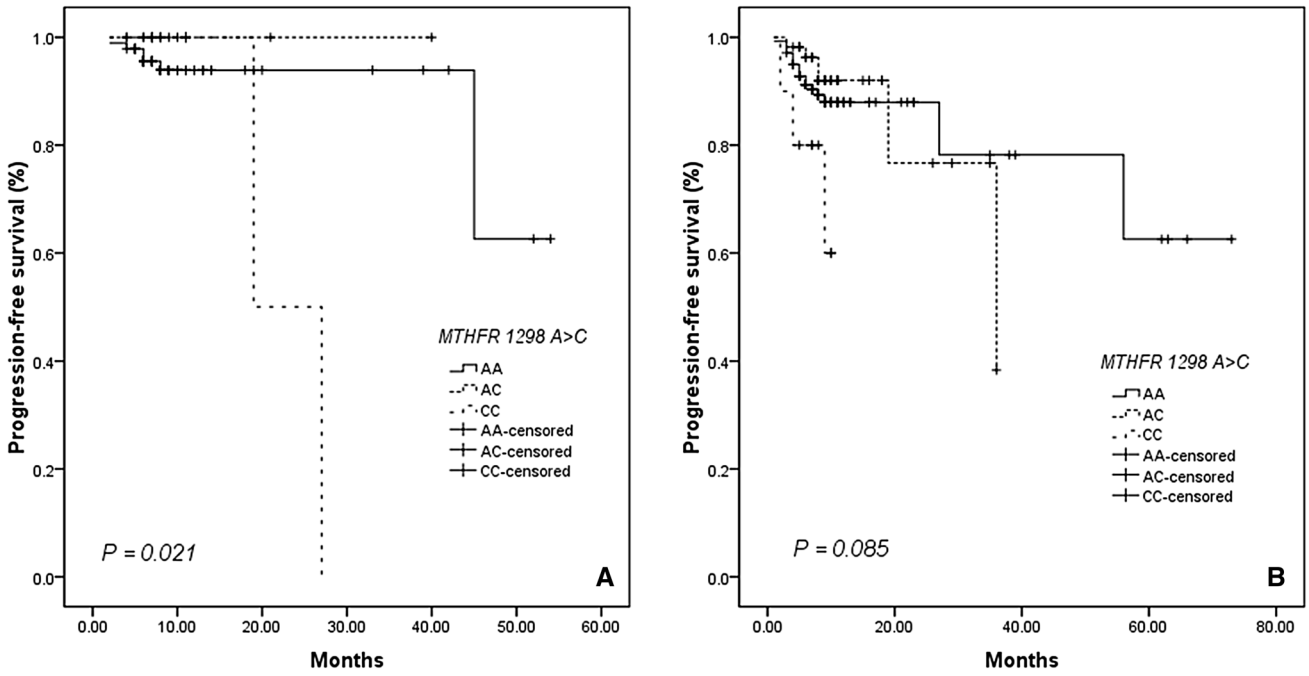

Fig. 5 Kaplan–Meier curves illustrate progression-free survival of female patients (**a**) and male patients (**b**) with capecitabine-based chemotherapy according to the variant genotype of *MTHFR* 1298 A>C

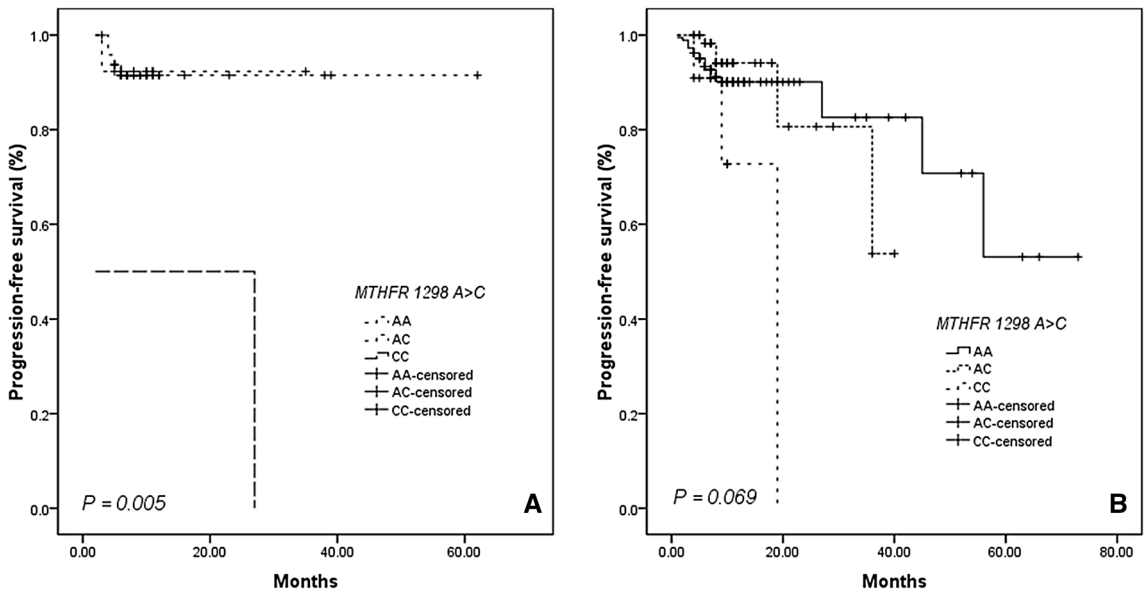

Fig. 6 Kaplan–Meier curves illustrate progression-free survival of elder patients (age > 65 years) (**a**) and younger patients (age ≤ 65 years) (**b**) with capecitabine-based chemotherapy according to the variant genotype of *MTHFR* 1298 A>C

Oral capecitabine monotherapy have been shown to be as effective as intravenous 5-fluorouracil as first-line treatment for gastroenteric cancer, and are generally associated with an improved safety profile. Capecitabine could induce hematological toxicity [35, 36], and the frequency of grade 3–4 hematological toxicity could be increased when capecitabine was combined with oxaliplatin which induce hematological toxicity. Peripheral neuropathy was the most common dose-limiting side effect of oxaliplatin which was known to cause acute and chronic neuropathy. Acute neuropathy was mainly cold-triggered, occurs in approximately 90% of patients and reversed characteristically within a week. In addition chronic

Table 3 Association between *CDA* 451 A>G and grade 3–4 toxicity in colon patients

Toxicity	Genotypes	P^a	OR[b]	95% CI
Hematological	AA	0.039	0.205	0.045–0.927
	AG/GG	–	–	–
Hepatotoxicity	AA	0.035	0.200	0.045–0.895
	AG/GG	–	–	–

[a]Comparison of genotype frequencies using Pearson's χ^2

[b]OR and 95% CI values were calculated by logistic regression

Table 4 Cox regression analysis of potential factors for PFS survival in gastroenteric cancer patients

Variable	Allele	P^a	Hazard ratio[a]	95% CI
***CDA* 79 A>C**	**A/C**	**0.001**	**8.671**	**2.358–31.887**
***CDA* 92 C>T**	**C/T**	**< 0.001**	**0.095**	**0.026–0.356**
***CDA* 451 A>G**	**A/G**	**< 0.001**	**0.095**	**0.026–0.356**
CDA 1172 G>A	A/G	0.653	1.317	0.397–4.369
***MTHFR* 1298 A>C**	**A/C**	**0.001**	**4.900**	**1.879–12.783**

Bold means significant differences ($P < 0.05$)

[a]*P*, HR, and 95% CI were assessed using multivariate Cox regression analysis adjusting for age and gender

cumulative peripheral neuropathy persisted between and after treatment, and severe oxaliplatin-induced peripheral neuropathy resolved in approximately 13 weeks after treatment [37, 38]. The present study did not find SNPs were correlated with the occurrence of severe peripheral neuropathy. In addition, hand-foot syndrome was a cutaneous adverse effect also referred to as palmar-plantar erythrodysesthesia or chemotherapy-induced acral erythema. It was the most common adverse effect associated with capecitabine [39]. However, no SNPs were found to be associated with grade 2–3 HFS toxicity in the present study. It might be due to the use of celecoxib which was a prophylactic and symptomatic treatment of grade 1 HFS could reduce the occurrence of grade 2–3 HFS toxicity to some extent [40].

We also recognize the potential limitations of this study. First, possible explanations for divergent findings in clinical studies should also consider the different chemotherapy regimens used, i.e., capecitabine monotherapy or capecitabine-combined chemotherapy (capecitabine/oxaliplatin). An ideal model to establish the relationship between polymorphisms and survival would be the study of patients treated with a single drug, but this situation was extremely uncommon in clinical practice, where combined chemotherapy regimens were mainly used. In the present study, chemotherapy regime of subjects was capecitabine only (30/322), and combined with anticancer drug oxaliplatin (292/322) as a first-line regime. Both capecitabine and oxaliplatin played key roles in patients' efficacy and side effects. Second, the number of patients limited the statistical analysis to demonstrate the relationship between polymorphisms and patient response to chemotherapy. We observed only two male patients with 79 A>C CC genotype, and their PFS was much shorter than the patients with AC or AA genotypes ($P < 0.001$). However, there was no significant difference in the female group ($P = 0.819$). In the further studies, we will focus on the detailed tracking medical information and find the association between gene polymorphisms and the clinical evaluation of cancer therapeutics. Larger sample sizes and in vivo functional studies are needed to confirm our results and to clearly characterize the underlying mechanisms.

In conclusion, our study suggests that SNPs involved in the *CDA* and *MTHFR* pharmacogenetics of capecitabine-based chemotherapy may have a significant role in predicting the failure of therapy in patients with colorectal and gastric cancer in the Chinese population. Our findings will help develop a highly cost effective treatment based on these predictive and prognostic bio-marker tests.

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Compliance with ethical standards

Conflict of interest The authors declare no competing financial interests.

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