



OPEN *MTHFR* polymorphism is associated with increased adverse events and poor clinical outcomes in gastric cancer patients with adjuvant S-1 chemotherapy

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S-1, an oral fluoropyrimidine composed of tegafur (a prodrug of 5-fluorouracil), gimeracil and oteracil, is widely used for gastric cancer. However, data on genetic variants influencing S-1 metabolism and their clinical impact remain limited. We enrolled 334 patients who underwent gastrectomy between July 2007 and May 2013, followed by adjuvant S-1 therapy. Genotyping of 46 SNPs in six genes (*MTHFR*, *DPD*, *OPRT*, *TS*, *CYP2A6*, *TP*) was performed to investigate their association with AEs and outcomes. *MTHFR* rs1801133 C>T was associated with higher hematologic AEs in recessive model (OR = 3.00, $p = 0.006$), and wild-type haplotype (ht2) was associated with fewer hematologic AEs in dominant model (OR = 0.34, $p = 0.003$). *TP* rs470119 G>A was associated with increased non-hematologic AEs in recessive model (OR = 3.42, $p = 0.045$). Overall AEs were reduced with ht2 of *MTHFR* in recessive model (OR = 0.43, $p = 0.042$). With a median follow-up of 93.2 months, *MTHFR* rs1801133 C>T and *CYP2A6* rs28399468 G>T correlated with shorter disease-free survival in dominant model (HR = 2.02, $p = 0.014$; HR = 1.93, $p = 0.046$; respectively). *MTHFR* rs1801133 C>T was associated with worse overall survival in dominant model (HR = 1.95, $p = 0.023$). Overall, *MTHFR* rs1801133 C>T was associated with increased hematologic AEs and poorer survival, suggesting its potential relevance as a candidate biomarker in the context of adjuvant S-1 therapy.

Keywords S-1, Gastric cancer, Adjuvant chemotherapy, *MTHFR*, Single nucleotide polymorphism

S-1 is an oral chemotherapeutic agent consisting of tegafur (a prodrug of 5-fluorouracil [5-FU]) along with two 5-FU-modulating substances—gimeracil, an inhibitor of dihydropyrimidine dehydrogenase (DPD), and oteracil, an inhibitor of 5-FU in the gastrointestinal tract—and has been widely used for gastric cancer (GC) in East Asian countries¹. The Adjuvant Chemotherapy Trial of S-1 for Gastric Cancer (ACTS-GC) demonstrated that 1 year of adjuvant S-1 treatment significantly reduces tumor recurrence and improves overall survival (OS) in curatively resected GC patients². At 5 years, the disease-free survival (DFS) rate was 65.4% in the S-1 group versus 53.1% in the surgery-only group (hazard ratio [HR], 0.653; 95% confidence interval [CI], 0.537–0.793) and the OS rate was 71.7% versus 61.1% (HR, 0.669; 95% CI, 0.540–0.828), respectively. Retrospective studies

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have suggested no significant differences in DFS and OS between 1-year S-1 treatment and 6-month capecitabine plus oxaliplatin, particularly in early-stage disease^{3,4}. These findings may support considering S-1 as a convenient oral option for selected patients.

Given that patients should take S-1 orally for a year, managing treatment-related adverse events (AEs) is important for ensuring good drug compliance, leading to positive clinical outcomes. Previous studies have suggested that single nucleotide polymorphisms (SNPs) in genes involved in 5-FU metabolism—such as methylenetetrahydrofolate reductase (*MTHFR*), dihydropyrimidine dehydrogenase (*DPD*), orotate phosphoribosyltransferase (*OPRT*), thymidylate synthase (*TS*), cytochrome P450 2A6 (*CYP2A6*), and thymidine phosphorylase (*TP*)—may affect both the toxicity and efficacy of 5-FU-based chemotherapy^{5–8}. Nonetheless, S-1 differs from infusional 5-FU in that it is a prodrug combined with gimeracil and oteracil, which can modify 5-FU metabolism and toxicity profiles⁹. Accordingly, pharmacogenomic associations may also differ between S-1 and intravenous 5-FU. However, pharmacogenomic studies for S-1 are sparse. Furthermore, most of the previous studies were conducted in heterogeneous populations across studies and focused on SNPs of a single gene. The results of these are inconsistent. Therefore, comprehensive studies investigating the relationship between SNPs in genes associated with 5-FU metabolism and their influence on both AEs and clinical outcomes in patients undergoing adjuvant S-1 chemotherapy after GC surgery are needed.

This study aimed to assess the correlation between the SNPs identified from blood samples and their impact on both the AE profiles and clinical outcomes in patients receiving adjuvant S-1 chemotherapy following GC surgery. Findings from this study are expected to aid in identifying genetic biomarkers that can help guide the appropriate management of patients undergoing adjuvant S-1 therapy after surgery.

Methods

Patients and sample collection

This study was based on prospective cohorts of GC patients maintained at Seoul National University Bundang Hospital^{10–13}. In these cohorts, all patients underwent curative gastrectomy followed by adjuvant S-1 chemotherapy. Eligibility criteria included histologically confirmed gastric or gastroesophageal junction adenocarcinoma; pathologic stage II–III according to the American Joint Committee on Cancer (AJCC) 6th or 7th edition at the time of diagnosis—with all cases subsequently reclassified using the 8th edition to ensure consistent staging for this study's analysis; Eastern Cooperative Oncology Group (ECOG) performance status of 0–2; and adequate bone marrow, renal, and hepatic function. Blood samples were prospectively collected for pharmacogenomic analyses, and comprehensive clinical data—including demographics, treatment details, outcomes, and toxicity profiles—were systematically recorded.

Using this prospectively maintained cohort database, we conducted a retrospective analysis. Among patients who underwent GC surgery between July 2007 and May 2013 and received adjuvant S-1 monotherapy, those with both SNP analysis results (derived from blood samples collected prior to the initiation of adjuvant therapy) and toxicity evaluations were included. The primary aim of our study was to identify key germline SNPs involved in S-1 metabolism that influence toxicity and survival in gastric cancer. Written informed consent was obtained from all participants for the use of archived blood samples and clinical data. The study was approved by the Institutional Review Board of Seoul National University Bundang Hospital (B-2504-966-110). All methods were performed in accordance with the relevant guidelines and regulations.

Treatment and toxicity assessment

S-1 was administered orally for 4 weeks, followed by a 2-week rest period. S-1 treatment was repeated every 6 weeks, and this interval was considered one cycle. The dosage of S-1, administered twice daily, was determined based on the patient's body surface area (BSA): 40 mg for BSA < 1.25 m², 50 mg for BSA between 1.25 and 1.49 m², and 60 mg for BSA ≥ 1.50 m². Dosage adjustment or schedule change (i.e., changing from a 6-week treatment interval to a 3-week interval) was made according to observed AEs, as previously documented^{11,12}. The planned duration of adjuvant S-1 treatment was one year, provided there was no tumor recurrence, unacceptable AEs, or patient refusal.

AEs were graded using the National Cancer Institute Common Toxicity Criteria (version 4.0). All AEs were prospectively recorded at each treatment visit, and the highest grade observed during S-1 therapy was used for analysis. Dose intensity (DI) was calculated as the total S-1 dose per BSA of the patient divided by the entire treatment duration. The relative dose intensity (RDI) was computed by dividing the received DI by the planned DI.

SNP genotyping

Germline DNA extracted from peripheral blood collected prior to initiation of adjuvant therapy was used for all genotyping analyses. We conducted the SNP array analysis to identify the genetic factors influencing the response to S-1 treatment. A total of 46 SNPs across six different genes (*MTHFR*, *DPD*, *OPRT*, *TS*, *CYP2A6*, and *TP*) were selected based on strong prior evidence from pharmacogenomic studies^{14–19}. Genotyping for all loci was performed using the Fluidigm high-throughput platform and Fluidigm EP1 SNP Genotyping 96.96 Dynamic Array (Fluidigm Corp., South San Francisco, CA). The discrete genotype data were analyzed using the BioMark SNP Genotyping analysis software (version 4.3.2). After genotyping, linkage disequilibrium (LD) among SNP pairs was evaluated using Haploview v4.2 software downloaded from the Broad Institute (<http://www.broadinstitute.org/mpg/haploview>) by examining Lewontin's D' ($|D'|$) and the LD coefficient r^2 between all pairs of bi-allelic loci²⁰. Highly correlated SNPs ($r^2 > 0.8$) within the same gene were grouped into haplotypes, whereas SNPs showing low LD were analyzed individually. Haplotypes were estimated using PHASE v2.1 software²¹, and those with a frequency ≥ 0.05 were included in subsequent analyses.

Statistical analysis

Each SNP was evaluated under co-dominant, dominant, and recessive genetic models. Logistic regression models were used to compare genotype distributions and to calculate odds ratios (ORs), 95% CIs, and corresponding *P*-values using IBM SPSS Statistics for Windows version 28.0 (IBM Corp., Armonk, NY, USA). In the co-dominant model, outcomes were evaluated by separately comparing the wild-type genotype (AA) with the heterozygous genotype (Aa) and the homozygous variant genotype (aa). Statistical significance in the co-dominant model was concluded if the overall *P*-value was significant and the ORs or HRs for both AA vs. Aa and AA vs. aa were in the same direction. In the dominant model, the heterozygous and homozygous variant genotypes (Aa + aa) were combined and compared against the wild-type genotype (AA). In the recessive model, the homozygous variant genotype (aa) was compared with the combined group of wild-type and heterozygous genotypes (AA + Aa). The Kaplan–Meier method was used to calculate the DFS and OS. DFS was defined as the time from gastrectomy to the recurrence of the tumor, any cause of death before relapse of disease, or censoring. OS was defined as the period between the time of gastrectomy and the date of death due to any cause or censoring. Cox proportional hazard models were used to analyze the influence of specified risk factors on survival outcomes in the multivariable analysis. *P*-values less than 0.05 were considered significant. The proportional hazards assumption was assessed using Schoenfeld residuals and was not violated in the reported Cox models. This study was reported in accordance with the REMARK²² recommendations, and a completed checklist is provided in the Supplementary File 1.

Results

Patient characteristics

Between July 2007 and May 2013, a total of 446 patients underwent gastrectomy for GC, and blood samples were collected for pharmacogenomic analysis. Among these patients, 379 received adjuvant S-1 chemotherapy, and a subset of 374 patients underwent prospective evaluation for S-1 toxicity. Finally, 334 patients who underwent toxicity evaluation and were able to undergo SNP genotyping with the collected blood samples were included in this study (Fig. 1. CONSORT diagram). The median age was 59 years (range, 20–82 years), and 209 patients (62.6%) were male. Tumor stages, reclassified according to the AJCC 8th edition, were distributed as follows: stage IB, 6 (1.8%); stage IIA, 99 (29.6%); stage IIB, 75 (22.5%); stage IIIA, 68 (20.4%); stage IIIB, 75 (22.5%); and stage IIIC, 11 (3.3%). Other detailed characteristics of the patients are presented in Table 1. Planned one-year treatment with S-1 was completed by 260 patients (77.8%), and the mean number of delivered S-1 chemotherapy cycles was 7.3 (95% CI, 7.0–7.6). Mean and median RDIs during all S-1 therapy cycles were 81.2% (95% CI, 78.3–84.2) and 93.8% (IQR, 77.8–100.0), respectively. Overall, S-1 treatment was well tolerated, with anemia, anorexia, nausea, and diarrhea being the most common AEs (Table 2). Neutropenia was the most common grade 3–4 AE (10.2%), followed by abdominal pain (8.1%) and diarrhea (7.2%). Among the patients with grade 4 neutropenia, one (0.3%) developed febrile neutropenia. Additionally, one patient (0.3%) died of hepatic failure. The median follow-up duration was 93.2 months.

Association between candidate SNPs and AEs

Table 3 shows minor allele frequencies of 46 SNPs across six different genes (*MTHFR*, *DPD*, *OPRT*, *TS*, *CYP2A6*, and *TP*) in our study cohort. Haplotypes (ht) and their frequencies are given in Supplementary Table 1. As germline polymorphisms, these SNPs are not biologically expected to be associated with baseline clinical characteristics, and no meaningful imbalances in baseline factors were observed across genotype groups in our cohort.

Logistic regression analysis was conducted to identify factors contributing to AEs associated with S-1 treatment, adjusting for age and sex based on previous literature^{23,24}. In the analysis of hematologic adverse events (Table 4), rs1801133 C > T and haplotype containing only this variant (ht1) of the *MTHFR* gene showed

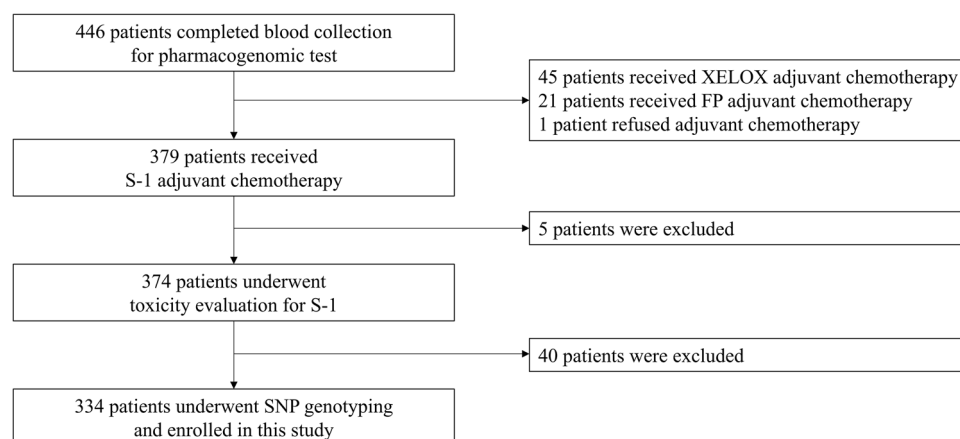


Fig. 1. CONSORT diagram. Flowchart of patient selection from initial enrollment to final inclusion in the study cohort for SNP analysis.

		N(%)
Age (years)	< 70	267 (79.9)
	≥ 70	67 (20.1)
Sex	Male	209 (62.6)
	Female	125 (37.4)
p Stage (AJCC 8th edition)*	IB	6 (1.8)
	IIA	99 (29.6)
	IIB	75 (22.5)
	IIIA	68 (20.4)
	IIIB	75 (22.5)
	IIIC	11 (3.3)
Operation	Distal gastrectomy	223 (66.8)
	Total gastrectomy	103 (30.8)
	Proximal gastrectomy	7 (2.1)
	Segmental resection	1 (0.3)
WHO classification	Papillary adenocarcinoma	3 (0.9)
	Well differentiated tubular adenocarcinoma	3 (0.9)
	Moderately differentiated tubular adenocarcinoma	110 (32.9)
	Poorly differentiated tubular adenocarcinoma	125 (37.4)
	Mucinous adenocarcinoma	14 (4.2)
	Poorly cohesive carcinoma	58 (17.4)
	Undifferentiated carcinoma	4 (1.2)
	Gastric carcinoma with lymphoid stroma	3 (0.9)
	Mixed adenocarcinoma	14 (4.2)
Lauren classification	Intestinal	125 (37.4)
	Diffuse	179 (53.6)
	Mixed	28 (8.4)
	Unknown†	2 (0.6)
Ming classification	Infiltrative	279 (83.5)
	Expanding	53 (15.9)
	Unknown†	2 (0.6)
Lymphatic invasion	Absent	108 (32.3)
	Present	224 (67.1)
	Unknown†	2 (0.6)
Vascular invasion	Absent	282 (84.4)
	Present	50 (15)
	Unknown†	2 (0.6)
Perineural invasion	Absent	282 (84.4)
	Present	50 (15)
	Unknown†	2 (0.6)

Table 1. Patients' characteristics. *Initially staged as pathologic stage II–III according to AJCC 6th or 7th edition at diagnosis; all cases were subsequently reclassified using the 8th edition for consistency in this study. †Unknown values reflect missing data from patients referred after surgery at external hospitals.

the association with higher rate of AEs in the recessive model (OR = 3.00, P -value = 0.006; OR = 3.13, P -value = 0.004; respectively). In contrast, wild-type haplotype (ht2) of the *MTHFR* gene showed association with a lower rate of AEs in the dominant model (OR = 0.34, P -value = 0.003). In the analysis of non-hematologic AEs (Supplementary Table 2), rs470119 G > A of the *TP* gene linked to a higher rate of AEs in the recessive model (OR = 3.42, P -value = 0.045). In the analysis of total AEs (Supplementary Table 3), ht2 of the *MTHFR* gene had an association with a lower rate of AEs in the recessive model (OR = 0.43, P -value = 0.042).

Association between candidate SNPs and clinical outcome

Significant associations were found between selected SNPs and clinical outcomes of S-1 treatment. Cox proportional hazards regression analysis was used to identify factors contributing to clinical outcomes, adjusting for stage and age. In DFS analysis (Supplementary Table 4), rs1801133 C > T of the *MTHFR* gene was associated with shorter DFS in the dominant model (HR = 2.02, P -value = 0.014) (Fig. 2A). Similarly, ht1 of the *MTHFR* gene (HR = 1.86, P -value = 0.026) (Fig. 2B) and rs28399468 G > T of the *CYP2A6* gene (HR = 1.93, P -value = 0.046) (Supplementary Fig. 1) also showed significant associations with shorter DFS in the dominant model. In OS

	G1	G2	G3	G4	G5	Any grade
Hematologic AEs						
Anemia	188 (56.3)	68 (20.4)	2 (0.6)	-	-	258 (77.3)
Neutropenia	49 (14.7)	67 (20.1)	32 (9.6)	2 (0.6)	-	150 (44.9)
Thrombocytopenia	40 (12)	12 (3.6)	1 (0.3)	-	-	53 (15.9)
Neutropenic fever	-	-	1 (0.3)	-	-	1 (0.3)
Non-hematologic AEs						
Bilirubin	122 (36.5)	15 (4.5)	1 (0.3)	-	1 (0.3)	139 (41.6)
AST/ALT	81 (24.3)	9 (2.7)	4 (1.2)	-	1 (0.3)	91 (28.4)
Anorexia	139 (41.6)	114 (34.1)	12 (3.6)	-	-	265 (79.3)
Nausea	143 (42.8)	34 (10.2)	7 (2.1)	-	-	184 (55.1)
Vomiting	47 (14.1)	6 (1.8)	3 (0.9)	-	-	56 (16.8)
Constipation	14 (4.2)	4 (1.2)	-	-	-	18 (5.4)
Stomatitis	49 (14.7)	27 (8.1)	3 (0.9)	-	-	79 (23.7)
Diarrhea	136 (40.7)	50 (15)	23 (6.9)	1 (0.3)	-	210 (62.9)
Abdomen pain	67 (20.1)	36 (10.8)	27 (8.1)	-	-	130 (38.9)
Fatigue	157 (47)	67 (20.1)	3 (0.9)	-	-	227 (68.0)
Paresthesia	27 (8.1)	-	-	-	-	27 (8.1)
Hand food syndrome	48 (14.4)	19 (5.7)	3 (0.9)	-	-	70 (21.0)
Skin rash	40 (12)	5 (1.5)	2 (0.6)	-	-	47 (14.1)

Table 2. Adverse events. For patients who experienced the same adverse event at multiple grades, only the highest grade was included in the count.

analysis (Supplementary Table 5), rs1801133 C > T of the *MTHFR* gene showed association with shorter OS in the dominant model (HR = 1.95, *P*-value = 0.023) (Fig. 2C), as was ht1 of the *MTHFR* gene (HR 2.05, *P*-value 0.015) (Fig. 2D).

Association between candidate gene polymorphisms and relative dose intensity

The relationship between candidate SNPs and RDI was investigated (Supplementary Table 6). The *MTHFR* rs1801133 C > T polymorphism, a key SNP in our study, was associated with increased hematologic AEs and worse DFS and OS. However, no significant differences in RDI were observed among the different genotypes. Likewise, most of the other SNPs and genes analyzed showed no significant association with RDI. While *MTHFR* rs1801131 A > C showed a statistically significant association with higher mean RDI in the recessive model (*P*-value = 0.002), the small number of patients with the CC genotype (*n* = 9) limits the clinical relevance of this result.

Discussion

In this study, we analyzed 46 SNPs across six genes related to 5-FU metabolism (*MTHFR*, *DPD*, *OPRT*, *TS*, *CYP2A6*, and *TP*) to investigate factors influencing toxicity and prognosis in patients undergoing adjuvant S-1 chemotherapy after GC surgery. Our findings indicate that the rs1801133 C > T SNP (C677T, Ala222Val) of *MTHFR* gene was associated with a higher incidence of hematologic AEs, as well as worse DFS and OS.

The *MTHFR* enzyme regulates folate homeostasis by catalyzing the conversion of 5,10-methylenetetrahydrofolate (5,10-methylene-THF)—a folate form critical for thymidylate synthesis—into 5-methyltetrahydrofolate (5-methyl-THF), which is required for methionine biosynthesis and DNA methylation. The *MTHFR* gene polymorphism rs1801133 C > T results in an alanine-to-valine substitution at position 222 in the enzyme's catalytic domain, which is vital for its function in folate metabolism. Previous studies have shown that the T allele in the rs1801133 C > T polymorphism reduces *MTHFR* enzyme activity. Individuals with the homozygous variant TT have approximately 70% lower enzyme activity, and heterozygous variant CT carriers have about 35% lower enzyme activity compared to those with the wild-type CC genotype^{25–27}. This enzymatic reduction can lead to increased levels of 5,10-methylene-THF, potentially enhancing 5-FU-mediated TS inhibition. Such metabolic alterations may contribute to both heightened toxicity and altered clinical outcomes in patients receiving adjuvant S-1 chemotherapy. Importantly, in the context of S-1 therapy, gimeracil-mediated inhibition of dihydropyrimidine dehydrogenase results in sustained systemic exposure to 5-FU, which may further amplify the downstream effects of folate-pathway perturbations caused by reduced *MTHFR* activity, particularly in rapidly proliferating hematopoietic cells. In our study, the *MTHFR* rs1801133 TT genotype was associated with increased hematologic side effects, as well as worse DFS and OS. Similar associations between *MTHFR* rs1801133 C > T and fluoropyrimidine-related hematologic toxicity have been reported in Asian colorectal cancer cohorts²⁸. Furthermore, previous research has shown that patients harboring this variant experience poorer prognosis when treated with adjuvant fluoropyrimidine-based chemotherapy after colorectal cancer surgery²⁹. Taken together, these convergent data highlight the clinical relevance of *MTHFR* rs1801133 C > T as a key folate-pathway variant that may influence both toxicity and outcomes under fluoropyrimidine-based therapy.

Gene	SNP name	Allele	Genotype	Co-dominant	Dominant	Recessive	MAF
<i>MTHFR</i>	rs201095365	C > A	CC/AC/AA	333/0/0	333/0	333/0	0.000
	rs2274976	G > A	GG/AG/AA	272/58/2	272/60	330/2	0.093
	rs4846051	A > G	AA/AG/GG	333/0/0	333/0	333/0	0.000
	rs1801131	A > C	AA/AC/CC	216/108/9	216/117	324/9	0.189
	rs1801133	C > T	CC/CT/TT	112/163/53	112/216	275/53	0.410
	rs138189536	G > A	GG/AG/AA	330/0/0	330/0	330/0	0.000
	MTHFR_ht1	other > ht1	other-other/other-ht1/ht1ht1	113/168/53	113/221	281/53	0.410
	MTHFR_ht2	other > ht2	other-other/other-ht2/ht2ht2	116/170/48	116/218	286/48	0.398
	MTHFR_ht3	other > ht3	other-other/other-ht3/ht3ht3	269/65/0	269/65	334/0	0.097
	MTHFR_ht4	other > ht4	other-other/other-ht4/ht4ht4	273/60/1	273/61	331/1	0.093
<i>DPD(DPYD)</i>	rs67376798	T > A	TT/AT/AA	334/0/0	334/0	334/0	0.000
	rs1801160	G > A	GG/AG/AA	324/10/0	324/10	334/0	0.015
	rs3918290	G > A	GG/AG/AA	334/0/0	334/0	334/0	0.000
	rs55886062	A > T	AA/AT/TT	333/0/0	333/0	333/0	0.000
	rs1801159	A > G	AA/AG/GG	197/116/21	197/137	313/21	0.237
	rs78060119	C > A	CC/AC/AA	334/0/0	334/0	334/0	0.000
	rs75017182	G > C	GG/CG/CC	294/39/0	294/39	333/0	0.059
	rs72549306	C > A	CC/AC/AA	334/0/0	334/0	334/0	0.000
	rs2297595	T > C	TT/CT/CC	323/11/0	323/11	334/0	0.017
	rs1801265	T > C	TT/CT/CC	305/29/0	303/29	334/0	0.043
	rs80081766	C > T	CC/CT/TT	296/31/1	296/32	327/1	0.050
	rs72549310	G > A	GG/AG/AA	329/0/0	329/0	329/0	0.000
	DPYDM182K	T > A	TT/AT/AA	329/0/0	329/0	329/0	0.000
	DPYDA777S	G > T	GG/GT/TT	334/0/0	334/0	334/0	0.000
	DPD_ht1	A > G	AA/AG/GG	141/146/47	141/193	287/47	0.359
	DPD_ht2	A > G	AA/AG/GG	211/108/15	211/123	319/15	0.207
<i>OPRT(UMPS)</i>	rs121917890	A > G	AA/AG/GG	333/0/0	333/0	333/0	0.000
	rs121917892	T > G	TT/GT/GG	334/0/0	334/0	334/0	0.000
	rs1801019	G > C	GG/CG/CC	194/119/21	194/140	313/21	0.241
	rs2291078	T > A	TT/AT/AA	290/42/2	290/44	332/2	0.069
	rs144643225	A > G	AA/AG/GG	334/0/0	334/0	334/0	0.000
	rs3772809	A > G	AA/AG/GG	310/24/0	310/24	334/0	0.036
	rs3772810	A > G	AA/AG/GG	216/111/5	216/116	327/5	0.182
<i>TS(TYMS)</i>	rs151264360	TTAAAG	DD/DI/II	166/140/27	166/167	306/27	0.291
	rs34743033	3R > 2R	3R3R/2R3R/2R2R	230/85/16	230/101	315/16	0.177
<i>CYP2A6</i>	rs28399468	G > T	GG/GT/TT	290/32/0	290/32	322/0	0.050
	rs5031016	T > C	TT/CT/CC	243/84/0	243/84	327/0	0.128
	rs1809810	A > T	AA/AT/TT	330/0/0	330/0	330/0	0.000
	rs28399454	G > A	GG > AG > AA	334/0/0	334/0	334/0	0.000
	rs111033610	T > C	TT/CT/CC	325/0/0	325/0	325/0	0.000
	rs150091942	A > C	AA/AC/CC	329/0/0	329/0	329/0	0.000
	rs8192726	G > T	GG/GT/TT	227/75/18	227/93	302/18	0.173
	rs1801272	T > A	TT/AT/AA	324/2/0	324/2	326/0	0.003
	rs28399434	G > A	GG > AG > AA	331/0/0	331/0	331/0	0.000
	rs28399433	T > G	TT/GT/GG	185/135/0	185/135	320/0	0.211
<i>TP(TYMP)</i>	rs470119	G > A	GG > AG > AA	185/134/12	185/146	319/12	0.239

Table 3. SNP summary. MAF = minor allele frequency, D = deletion, I = insertion, ht = haplotype, 2R = double repeats of sequence, 3R = triple repeats of sequence. See Supplementary Table 1 for detailed information of haplotypes. For each SNP, the effective number of patients analyzed corresponds to the sum of the reported genotype counts in that row. Genotyping success rates were high overall, but a small number of patients (< 2% per SNP) had missing genotype calls, resulting in slight variation in N across SNPs. Analyses were conducted using complete-case data for each SNP.

Gene	SNPs	Co-dominant		Dominant		Recessive	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
<i>MTHFR</i>	rs2274976		0.160	0.92 (0.36–2.35)	0.867	14.93 (0.85–261.88)	0.064
		0.77 (0.28–2.11)	0.614				
		14.27 (0.81–251.22)	0.069				
	rs1801131		0.686	1.36 (0.67–2.76)	0.395	1.03 (0.12–8.63)	0.973
		1.38 (0.67–2.84)	0.385				
		1.15 (0.13–9.85)	0.900				
	rs1801133		0.022	1.41 (0.65–3.04)	0.385	3.00 (1.38–6.51)	0.006
		0.99 (0.42–2.30)	0.976				
		2.97 (1.18–7.51)	0.021				
	MTHFR_ht1		0.016	1.20 (0.57–2.54)	0.637	3.13 (1.44–6.79)	0.004
		0.80 (0.35–1.84)	0.595				
		2.69 (1.08–6.66)	0.033				
	MTHFR_ht2		0.011	0.34 (0.17–0.68)	0.003	0.50 (0.15–1.71)	0.268
		0.35 (0.17–0.74)	0.006				
		0.30 (0.09–1.09)	0.067				
	MTHFR_ht3		-	1.74 (0.79–3.84)	0.172	-	-
		1.70 (0.77–3.77)	0.189				
		-	-				
	MTHFR_ht4		-	0.85 (0.34–2.15)	0.728	-	-
		0.88 (0.35–2.24)	0.789				
		-	-				
<i>DPD (DPYD)</i>	rs1801160		-	1.90 (0.38–9.53)	0.436	-	-
		1.90 (0.38–9.53)	0.436				
		-	-				
	rs1801159		0.615	1.05 (0.52–2.14)	0.895	0.40 (0.05–3.08)	0.378
		1.18 (0.57–2.44)	0.663				
		0.42 (0.05–3.34)	0.414				
	rs75017182		-	1.07 (0.35–3.26)	0.906	-	-
		1.07 (0.35–3.26)	0.906				
		-	-				
	rs2297595		-	1.78 (0.36–8.75)	0.480	-	-
		1.78 (0.36–8.75)	0.480				
		-	-				
	rs1801265		-	1.22 (0.39–3.78)	0.730	-	-
		1.22 (0.39–3.78)	0.730				
		-	-				
	rs80081766		-	0.79 (0.23–2.79)	0.717	-	-
		0.84 (0.24–2.95)	0.962				
		-	-				
	DPD_ht1		0.429	1.31 (0.64–2.69)	0.458	-	-
		1.54 (0.73–3.26)	0.258				
		0.89 (0.28–2.88)	0.844				
	DPD_ht2		0.751	1.07 (0.52–2.18)	0.858	-	-
		1.21 (0.58–2.54)	0.612				
		0.59 (0.07–4.76)	0.620				
Continued							

Gene	SNPs	Co-dominant		Dominant		Recessive	
		OR (95% CI)	P	OR (95% CI)	P	OR (95% CI)	P
OPRT (UMPS)	rs1801019		0.911	1.09 (0.54–2.18)	0.816	0.81 (0.18–3.66)	0.780
		1.13 (0.55–2.32)	0.743				
		0.85 (0.18–3.97)	0.834				
	rs2291078		-	0.50 (0.14–1.72)	0.27	-	
		0.53 (0.15–1.82)	0.311				
		-	-				
	rs3772809		-	0.63 (0.14–2.82)	0.541	-	-
		0.63 (0.14–2.82)	0.541				
		-	-				
	rs3772810		0.39	0.77 (0.37–1.64)	0.501	3.58 (0.36–35.55)	0.276
		0.72 (0.33–1.55)	0.398				
		3.23 (0.32–32.37)	0.319				
TS (TYMS)	rs151264360		0.555	1.01 (0.51–2.02)	0.974	0.34 (0.05–2.64)	0.304
		1.13 (0.56–2.29)	0.732				
		0.36 (0.05–2.87)	0.337				
	rs34743033		0.453	0.59 (0.26–1.35)	0.214	0.52 (0.07–4.13)	0.536
		0.62 (0.26–1.48)	0.277				
		0.47 (0.06–3.73)	0.472				
CYP2A6	rs28399468		-	1.11 (0.36–3.41)	0.854	-	-
		1.11 (0.36–3.41)	0.854				
		-	-				
	rs5031016		-	0.77 (0.34–1.78)	0.543	-	-
		0.77 (0.34–1.78)	0.543				
		-	-				
	rs8192726		0.480	1.54 (0.74–3.22)	0.253	1.73 (0.46–6.46)	0.414
		1.45 (0.65–3.25)	0.363				
		1.91 (0.50–7.28)	0.343				
	rs28399433		-	1.19 (0.58–2.44)	0.628	-	-
		1.19 (0.58–2.44)	0.628				
		-	-				
TP (TYMP)	rs470119		0.156	2.00 (0.99–4.03)	0.055	1.35 (0.28–6.58)	0.708
		2.02 (0.98–4.15)	0.058				
		1.83 (0.36–9.28)	0.467				

Table 4. Logistic regression analysis for predicting hematologic adverse events. OR, Odds ratio; CI, confidence interval. Patients were categorized based on the presence or absence of hematologic adverse events of any grade. Logistic regression analyses under co-dominant, dominant, and recessive models were performed to calculate ORs and corresponding *P*-values for each SNP, adjusting for sex and age 70 (categorized as <70 vs. ≥70 years) as covariates. Logistic regression was performed using available data for each SNP; the total number of patients included in each analysis may vary due to missing genotype data. Significant associations are shown in boldface.

One patient died from hepatic failure, and notably, this patient carried the *MTHFR* rs1801133 C>T SNP, which is the key polymorphism highlighted in this study due to its significant association with increased hematologic AEs and reduced DFS and OS. This SNP has a relatively high MAF of 0.410 (Table 3), indicating that even common variants can contribute to severe toxicity and should not be overlooked. In addition, one patient experienced grade 4 diarrhea, which resolved after discontinuation of S-1, and two patients developed grade 4 neutropenia, including one case of febrile neutropenia. These patients did not carry the *MTHFR* rs1801133 variant but harbored other SNPs, suggesting that multiple genetic factors may contribute to the risk of severe toxicities. Together, these findings underscore the potential utility of integrating pharmacogenetic data into clinical decision-making to better anticipate and manage adverse events during adjuvant S-1 chemotherapy.

While previous studies have explored the association between SNPs in genes involved in 5-FU metabolism and chemotherapy-related toxicity or outcomes, relatively few have focused specifically on S-1. Although S-1, capecitabine, and intravenous 5-FU share a common mechanism of action as fluoropyrimidines, differences in formulation and metabolic pathways may lead to variations in clinical efficacy and toxicity profiles. In particular, orally administered agents such as S-1 and capecitabine are subject to interindividual variability in bioavailability, largely due to differences in drug absorption. In addition, although both S-1 and capecitabine are prodrugs that require enzymatic activation, their metabolic pathways differ³⁰. S-1 is primarily converted

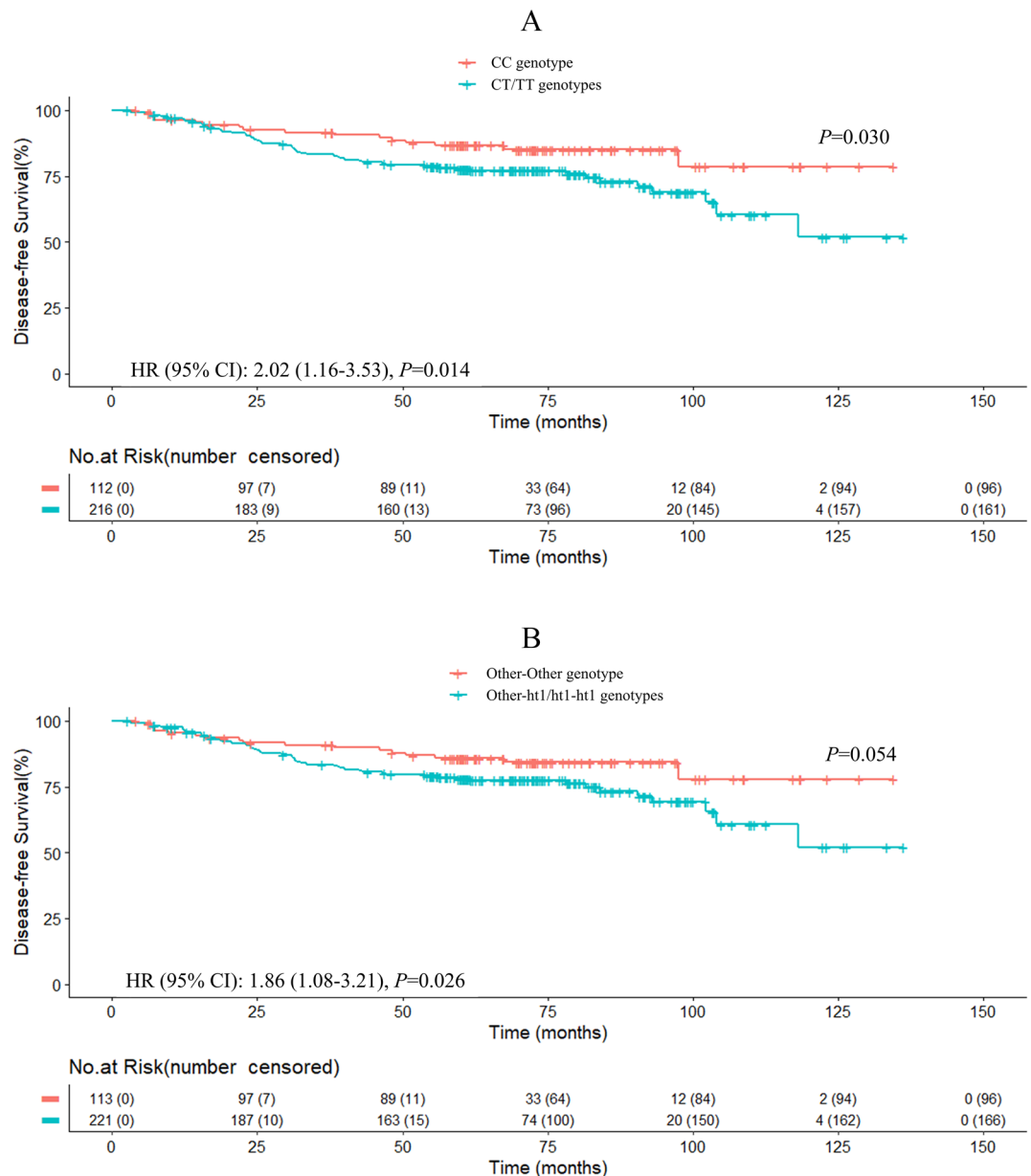


Fig. 2. Kaplan–Meier survival curves according to *MTHFR* SNP. **(A)** DFS according to the *MTHFR* rs1801133 C>T dominant model. Patients with CT/TT genotypes had significantly worse DFS than those with CC genotype. **(B)** DFS according to the *MTHFR* ht1 (haplotype containing only rs1801133 C>T) dominant model. The presence of ht1 was associated with significantly poorer DFS compared to other haplotypes. **(C)** OS according to the *MTHFR* rs1801133 C>T dominant model. CT/TT genotypes were associated with significantly worse OS compared to the CC genotype. **(D)** OS according to the *MTHFR* ht1 (haplotype containing only rs1801133 C>T) dominant model. The presence of ht1 was associated with poorer OS compared to other haplotypes.

to 5-FU in the liver, where gimeracil enhances systemic 5-FU exposure by strongly inhibiting DPD enzyme, and oteracil reduces gastrointestinal toxicity by suppressing 5-FU phosphorylation in the gastrointestinal tract. In contrast, capecitabine undergoes a three-step enzymatic activation process. It is first metabolized in the liver by carboxylesterase, then further processed by cytidine deaminase, which is highly expressed in the liver and tumor tissues, and finally converted to 5-FU by thymidine phosphorylase, which is more active in tumor tissue. This tumor-selective activation contributes to reduced gastrointestinal and bone marrow toxicity. These pharmacokinetic and pharmacogenomic distinctions between S-1 and capecitabine may influence how genetic variants affect drug metabolism and clinical outcomes. Therefore, specific pharmacogenomic study addressing S-1 are needed. In this study, we identified *MTHFR* rs1801133 C>T as a clinically relevant SNP associated with increased toxicity and poorer outcomes after S-1 treatment, which has not been emphasized in prior studies. While previous research has suggested that variants in *TS* or *CYP2A6* may affect S-1-related toxicity

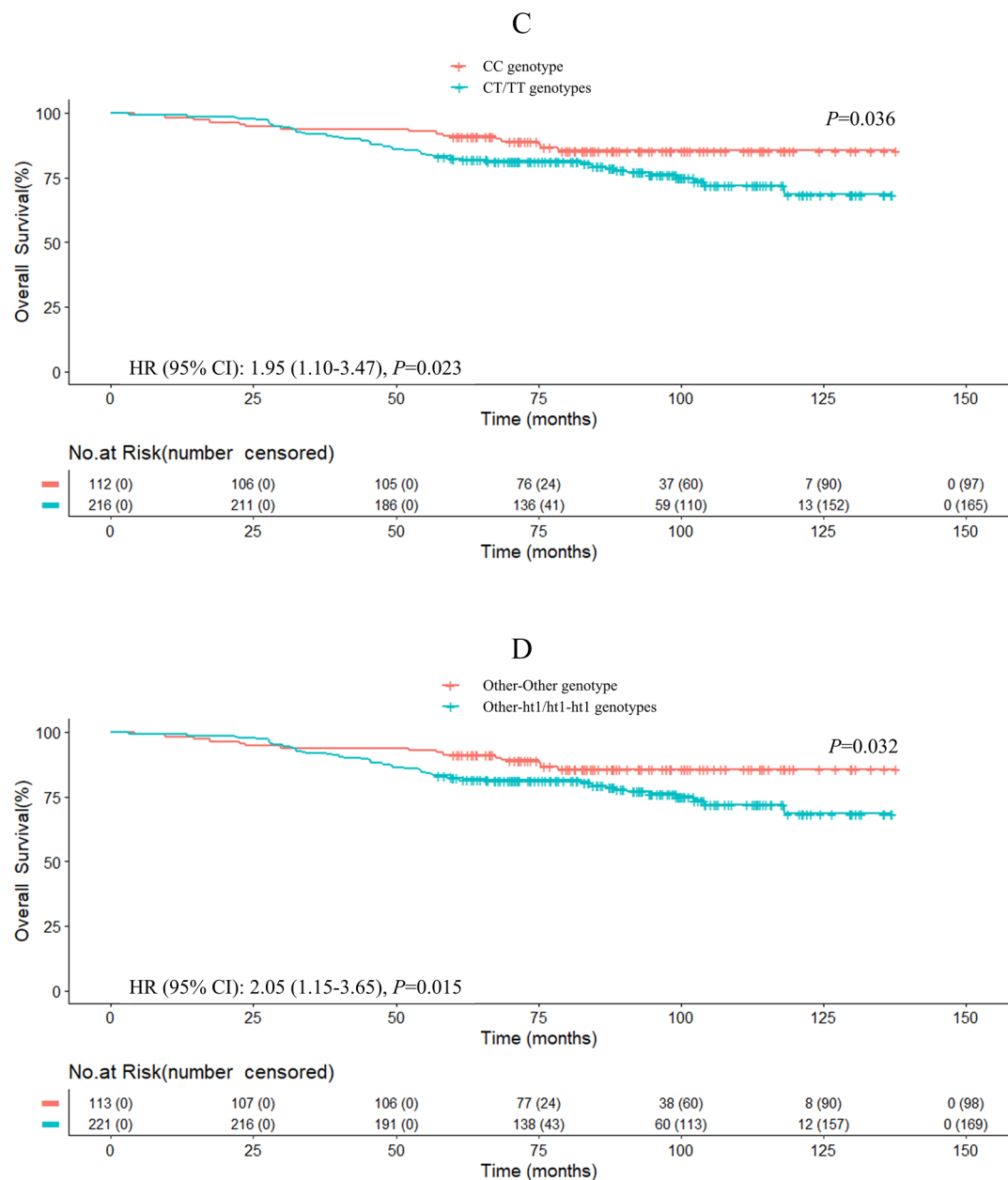


Fig. 2. (continued)

and efficacy, the results have been inconsistent^{31–33}. In our analysis, the *CYP2A6* rs28399468 G > T variant was associated with shorter DFS in the dominant model, but this finding could not be meaningfully connected to other toxicity parameters or OS, and therefore should be viewed as exploratory and subject to confirmation in larger, pharmacokinetically annotated cohorts.

In our cohort, carriers of the *MTHFR* rs1801133 C > T variant tended to have a shorter duration of adjuvant S-1 treatment (7.2 vs. 7.6 cycles, CT/TT vs. CC) and a higher proportion of grade 3/4 hematologic adverse events (12.5% vs. 8.9%, CT/TT vs. CC), although these differences were not statistically significant. At the cohort level, this did not result in a significant reduction in RDI. This likely reflects the fact that most adverse events during adjuvant S-1 monotherapy were low grade, allowing dose intensity to be largely maintained despite increased toxicity. Importantly, the lack of a measurable difference in RDI does not diminish the clinical relevance of this variant. Our cohort included a fatal case of hepatic failure in a patient carrying rs1801133, and the variant was consistently associated with both increased toxicity and poorer survival outcomes. These findings suggest that *MTHFR* rs1801133 C > T may help identify patients at higher risk of toxicity during adjuvant S-1 therapy. However, its clinical utility remains to be established and should be validated in prospective studies designed to assess genotype-guided management strategies. If confirmed, early identification of this variant may support risk stratification and more personalized monitoring or toxicity management.

A recent study demonstrated that personalized dose adjustments of fluoropyrimidines based on *DPD* gene variants did not compromise prognosis, suggesting that dose modification may enhance safety in patients genetically predisposed to severe AEs³⁴. In line with this, the U.S. Food and Drug Administration (FDA) recently emphasized the necessity of screening for *DPD* deficiency before initiating fluoropyrimidine-based chemotherapy to minimize severe toxicity risks (January 24, 2025, FDA Safety Announcement), reinforcing the importance of pharmacogenetic evaluation in clinical practice. While *DPD* polymorphisms are a well-recognized determinant of fluoropyrimidine-related toxicity, their prevalence varies across ethnic groups³⁵. Previously reported high-risk variants, such as rs3918290 G > A in the *DPD* gene³⁶, were not detected in our cohort (Table 3). Instead, in our study population, *MTHFR* rs1801133 C > T was significantly associated with increased toxicity and poorer prognosis following S-1 chemotherapy, highlighting the potential relevance of ethnicity-specific pharmacogenetic markers. These findings underscore the importance of tailoring genetic screening strategies based on population-specific genetic risk factors to optimize the safety and efficacy of adjuvant S-1 therapy in gastric cancer patients.

When analyzing multiple SNPs, there is an inherent risk of false-positive findings due to multiple comparisons. While more stringent statistical thresholds are typically warranted in such cases, we consider that our study design justifies the use of a conventional significance threshold ($P < 0.05$) for several reasons. First, we calculated LD between all SNP pairs and grouped highly correlated SNPs into haplotypes. This approach reduced redundancy and minimized the number of effectively independent comparisons, thereby addressing the issue of non-independence. Second, haplotype analysis complemented individual SNP analysis by capturing combined effects of linked variants, which may better reflect biological relevance. Third, all genes and SNPs analyzed in this study were selected based on prior evidence implicating their relevance to 5-FU metabolism, efficacy, or toxicity. Taken together, the use of LD-based haplotype grouping and a hypothesis-driven candidate gene approach supports the application of a P -value threshold ($P < 0.05$) without additional correction for multiple testing. Similar analytical strategies have been adopted in previous studies that evaluated predefined SNPs using a conventional significance threshold without multiple-testing correction^{37,38}.

This study has several limitations. First, the lack of pharmacokinetic data for S-1 prevented us from directly assessing the relationship between polymorphisms and S-1 metabolism. Second, data on concomitant medications were not available, which could have influenced CYP activity and required dose adjustments. However, in clinical practice, physicians typically manage significant drug-drug interactions, making it unlikely that this limitation had a major impact on our findings. Third, although germline variants are unlikely to be systematically confounded by baseline clinical factors, our simplified adjustment strategy may not fully capture the influence of all treatment-related variables. Despite these constraints, this study offers a relatively comprehensive pharmacogenetic analysis by concurrently evaluating multiple SNPs across key 5-FU metabolism-related genes, considering their potential interactions. Although the analysis was retrospective, it was based on a prospectively collected cohort with systematically recorded toxicity data and clinical outcomes. The inclusion of 334 patients with a median follow-up of 93.2 months adds to the robustness and potential clinical relevance of our findings. To fully establish the clinical utility of *MTHFR* rs1801133 C > T, future prospective studies incorporating genotype-based treatment algorithms and predictive modeling will be essential.

Conclusions

In conclusion, this study indicates that the rs1801133 C > T polymorphism in *MTHFR* gene is associated with an elevated risk of hematologic toxicities and shorter DFS and OS in patients undergoing S-1 adjuvant chemotherapy. The findings of this study suggest that the *MTHFR* rs1801133 C > T polymorphism may represent a potential genetic biomarker to inform treatment decisions and optimize adjuvant S-1 therapy in patients with GC following curative surgery.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files. De-identified individual-level data may be made available from the corresponding author upon reasonable request and in accordance with institutional data-sharing regulations.

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Author contributions

M.K. contributed to data curation, formal analysis, validation, investigation, visualization, and methodology, and wrote the original draft. J.W.K. and K.-W.L. contributed to conceptualization, validation, supervision, funding acquisition, methodology, project administration, and writing of both the original draft and the revisions. J.H.L. contributed to formal analysis, visualization, and methodology. L.H.K. contributed to methodology and formal analysis. All authors provided research resources and participated in reviewing and editing the manuscript.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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