

UGT1A1*28 Polymorphism Predicts Irinotecan-induced Severe Toxicities Without Affecting Treatment Outcome and Survival in Patients With Metastatic Colorectal Carcinoma

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Supported by a grant from Taiwan Clinical Oncology Research Foundation to Wang Wei-Shu.

We thank Mr. Chih-Tung Ho for excellent technical assistance.

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Received September 4, 2007; revision received October 26, 2007; accepted November 15, 2007.

BACKGROUND. It is known that the uridine-diphosphoglucuronosyl transferase 1A1 (UGT1A1)*28 polymorphism reduces UGT1A1 enzyme activity, which may lead to severe toxicities in patients who receive irinotecan. This study was conducted to assess the influence of this polymorphism on the efficacy and toxicity of irinotecan treatment in Chinese patients with metastatic colorectal carcinoma (CRC).

METHODS. In total, 128 patients with metastatic CRC who had received previous treatment with irinotecan plus 5-fluorouracil/leucovorin were analyzed retrospectively. Genomic DNA samples were obtained from patients' leukocytes, and genotypes were determined by analyzing the sequence of TATA boxes in the *UGT1A1* gene. The influence of the UGT1A1*28 polymorphism on toxicity and treatment outcome was analyzed.

RESULTS. Approximately 20% of patients were identified with the UGT1A1*28 polymorphism, including 15.6% (n = 20 patients) with the thymine-adenine (TA)6/TA7 genotype and 4.7% (n = 6 patients) with the TA7/TA7 genotype. The remaining 79.7% of patients (n = 102) had wild type TA6/TA6. Marked increases in grade 3 or 4 neutropenia (53.8% vs 4.9%; $P < .01$), neutropenic fever (38.5% vs 3.9%; $P < .01$), diarrhea (26.9% vs 5.9%; $P < .01$), and pretreatment bilirubin level (23.1% vs 8.8%; $P = .04$) were observed in patients who had the TA6/TA7 or TA7/TA7 genotypes. Patients' pretreatment bilirubin levels correlated well with irinotecan-induced neutropenia ($P < .01$). It was noted that, although the requirement for irinotecan dose reduction was significantly greater in patients who had this genetic variant (42.3% vs 12.7%; $P < .01$), it did not affect the response rate to irinotecan-based chemotherapy (42.3% vs 45.1%; $P = .80$), and it did not significantly affect progression-free survival (10 months vs 11 months; $P = .94$) or overall survival (19 months vs 18 months; $P = .84$).

CONCLUSIONS. The current data suggested that the UGT1A1*28 polymorphism may be a key determinant for predicting irinotecan-induced severe toxicities without affecting treatment outcome for patients with metastatic CRC. Further prospective studies are warranted for using this polymorphism to optimize irinotecan-based chemotherapy. *Cancer* 2008;112:1932-40. © 2008 American Cancer Society.

KEYWORDS: uridine-diphosphoglucuronosyl transferase 1A1, polymorphism, colorectal cancer, irinotecan, toxicity.

The quantification of enzymes that involve in the targeting and metabolism of specific chemotherapeutic drugs, as well as DNA repair, may effectively predict the sensitivity and outcome to

treatments in patients with metastatic colorectal carcinoma (CRC). In addition, the analysis of genomic polymorphisms for predicting the efficacy and toxicity of treatment also may be helpful in identifying the patients who may benefit from chemotherapy.¹ For example, the functional polymorphisms of genes involved in the targeting and metabolism of 5-fluorouracil (5-FU)²⁻⁴ and DNA repair during platinum-based treatment^{5,6} can effectively predict the response and prognosis of patients with metastatic CRC who are receiving 5-FU and oxaliplatin.

Irinotecan is a camptothecin analogue that has been used widely in a variety of malignancies, including CRC. By effectively inhibiting topoisomerase I, an enzyme that involves in DNA replication, irinotecan may induce apoptosis of tumor cells. Because irinotecan-based chemotherapy has been considered standard treatment in the management of metastatic CRC, interpatient variation of the enzymes involved in the metabolism of irinotecan has been investigated extensively.⁷⁻¹¹ The metabolism of irinotecan is a complex process that involves several enzymes. Initially, the ester bond of irinotecan is cleaved by carboxylesterases (CESs) to form an active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38). Although SN-38 is much more active in inhibiting topoisomerase I than irinotecan,¹² it has been associated with more toxicities, especially severe neutropenia and diarrhea.¹³ Later on, SN-38 is conjugated with β -glucuronic acid by uridine-diphosphoglucuronosyl transferase (UGT) to form an inactive metabolite, SN-38G.¹⁴ Several factors, including CES, UGT, cytochrome p450 isoforms, multidrug resistance-associated proteins ABCB1 and ABCC2, organic anion-transporting polypeptide SLCO1B1, and the adenosine triphosphate (ATP)-binding cassette transporters, were involved in the metabolic pathway of SN-38 that had influence on its serum levels.¹⁴⁻¹⁶ Of these factors, UGT is considered the most important for the interindividual variability that affects SN-38 serum levels.

UGT belongs to a superfamily of enzymes that catalyze the glucuronidation of many exogenous and endogenous substrates, including SN-38 and bilirubin. By alternative splicing, the UGT1 gene expresses 9 functional UGT1A proteins, and UGT1A1 is the major protein that catalyzes the glucuronidation of SN-38 and bilirubin.¹⁷ Mutations of the *UGT1A1* gene result in the absence or reduction of UGT1A1 activity that may lead to unconjugated hyperbilirubinemia.¹⁸ In particular, the UGT1A1*28 polymorphism consists of a thymine-adenine (TA) dinucleotide insertion in the TATAA element of the promoter region that results in the mutant genotype of

A(TA)₇TAA as opposed to the wild type of A(TA)₆TAA.^{19,20} According to the number of TA repeats in 2 alleles, genotypes of the UGT1A1*28 polymorphism can be classified as TA6/TA6 (wild type), TA6/TA7 (heterozygous mutant), or TA7/TA7 (homozygous mutant). In addition, the TA6/TA7 or TA7/TA7 mutants have been associated with decreased enzyme activity and glucuronidation of SN-38, which may account for increased irinotecan-related toxicity. It is noteworthy that ethnic differences do exist in the UGT1A1*28 polymorphism. The prevalence of homozygous TA7/TA7 was significantly greater in African populations (12%–27%) and in Caucasian populations (5%–15%) but was much lower (1.2%–5%) in South-east Asian and Pacific populations, including Taiwan.²¹⁻²⁴

Neutropenia and diarrhea are the major toxicities associated with irinotecan treatment, and the frequency with which these toxicities occur depends on the effectiveness of SN-38 glucuronidation mediated by several hepatic and extrahepatic UGT1A glucuronosyl transferases. Among these, the UGT1A1 protein has the highest capacity to glucuronidate SN-38.²⁵⁻²⁷ In addition to UGT1A1, hepatic UGT1A9 and extrahepatic UGT1A7 also are important components in the glucuronidation of SN-38, and their genotypes also may serve as predictors of response and toxicity in patients with CRC who are treated with irinotecan. It is noteworthy that patients with genotypes conferring a lower UGT1A7 activity and the UGT1A9 (dT)(9/9) genotype may be particularly likely to exhibit greater antitumor response with little toxicity.^{26,27} In addition to UGT1A1, UGT1A7, and UGT1A9, a minor role has been suggested for UGT1A6, UGT1A8, and UGT1A10 in SN-38 glucuronidation.²⁷

In the current study, we retrospectively examined the prevalence of UGT1A1*28 polymorphisms in 128 patients with metastatic CRC who had received irinotecan plus 5-FU and leucovorin (LV) as first-line treatment. Characteristics that were correlated with this polymorphism and toxicity and treatment outcomes from irinotecan-based chemotherapy were analyzed.

MATERIALS AND METHODS

Patients

To examine the impact of UGT1A1*28 polymorphism on the toxicity and treatment outcome from irinotecan-based chemotherapy in patients with metastatic CRC, 152 consecutive patients with unresectable, metastatic CRC who had received irinotecan plus 5-FU and LV as first-line treatment from January 2003 to June 2006 were examined retrospectively. Among them, 128 patients were enrolled and analyzed

(patients' characteristics are shown in Table 1). The remaining patients were excluded because they lacked measurable lesions (n = 8 patients), or did not have a primary tumor removed to determine accurate tumor (T) and lymph node (N) classifications (n = 5), or died before blood sampling (n = 5), or were unwilling to participate (n = 4), or were lost to follow-up (n = 2). The chemotherapeutic regimen consisted of irinotecan (180 mg/m² as a 1-hour infusion on Day 1), LV (100 mg/m² as a 2-hour infusion on Days 1 and 2) before bolus 5-FU (400 mg/m² on Days 1 and 2), and infusional 5-FU (600 mg/m² as a 22-hour infusion immediately after bolus 5-FU on Days 1 and 2) administered every 2 weeks for 12 cycles. Patients with or without UGT1A1*28 poly-

morphism were followed similarly for a median duration of 18 months. The responses and treatment-related toxicities were evaluated on the basis of standard World Health Organization criteria. Patients who achieved a complete response, a partial response, or stable disease remained in the protocol until they developed progressive disease or unacceptable toxicity was documented. Treatment was delayed until recovery if grade 3/4 neutropenia or diarrhea occurred, and the doses of irinotecan were reduced in subsequent cycles. In patients with intolerable neutropenia or uncontrolled diarrhea and in patients who failed on a front-line irinotecan-based regimen, the treatment was discontinued, and oxaliplatin-based or fluoropyrimidine-only regimens were administered subsequently according to the physicians' decision. During treatment, all patients visited our outpatient clinic regularly for physical examinations and check-ups, which included complete blood counts, liver and renal functions, and serum carcinoembryonic antigen (CEA) levels. Chest x-rays, abdominal ultrasound images, or computed tomography scans were obtained every 2 months. An institutional review board approved this study, and informed consent was provided by all patients before their blood was tested for genotyping.

TABLE 1
Clinicopathologic Correlations of UGT1A1*28 Polymorphism in Patients With Metastatic Colorectal Cancer

Characteristic	No. of patients (%)		P
	TA6/TA6 (Wild type)	TA6/TA7 or TA7/TA7	
All patients	102 (100)	26 (100)	
Age, y			
<50	46 (45.1)	11 (42.3)	.80
≥50	56 (54.9)	15 (57.7)	
Sex			
Men	70 (68.6)	16 (61.5)	.49
Women	32 (31.4)	10 (38.5)	
Performance status			
0	61 (59.8)	16 (61.5)	.87
1, 2	41 (40.1)	10 (38.5)	
Primary tumor			
Colon	66 (64.7)	14 (53.8)	.31
Rectum	36 (35.3)	12 (46.2)	
Histologic differentiation			
Well/moderate	84 (82.4)	20 (76.9)	.53
Poorly/unknown	18 (17.6)	6 (23.1)	
Invasive extent*			
T1-T2	40 (39.2)	8 (30.8)	.43
T3-T4	62 (60.8)	18 (69.2)	
Lymph node involvement*			
N0	23 (22.5)	4 (15.4)	.42
N1-N3	79 (77.5)	22 (84.6)	
Serum CEA level, ng/mL			
≤6	21 (20.6)	3 (11.5)	.29
>6	81 (79.4)	23 (88.5)	
TSER 28-bp polymorphism			
2R/2R	1 (0.1)	0 (0)	.82
2R/3R	35 (34.3)	8 (30.7)	
3R/3R	66 (64.7)	18 (69.2)	

T indicates thymine; A, adenine; CEA, carcinoembryonic antigen; TSER 28-bp polymorphism, germline polymorphisms of the number of 28-base pair tandemly repeated sequences in the 5'-enhancer region of the thymidylate synthase gene.

* According to the American Joint Committee on Cancer/International Union Against Cancer tumor, lymph node, metastasis (TNM) staging system for colorectal carcinoma.

Examination of the UGT1A1*28 Polymorphism

Genomic DNA was extracted from patients' leukocytes, which were obtained from 0.5 mL of whole blood by using standard phenol-chloroform methods, and subjected to the genotyping procedure. Polymorphism of the *UGT1A1* gene was examined according to a method described previously.²⁸ To analyze TA repeat polymorphisms in the promoter of the *UGT1A1* gene, an amplification product was generated by polymerase chain reaction (PCR) for sequencing. The sequences of the forward and reverse primers were 5'-AAA TTC CAG CCA GTT CAA CTG TTG TT-3' and 5'-CTG CTG GAT GGC CCC AAG-3', respectively. Amplification was performed for 34 cycles and consisted of denaturation at 94°C for 45 seconds, annealing at 62°C for 45 seconds, and extension at 72°C for 60 seconds between the initial denaturation at 94°C for 2 minutes and a final extension at 72°C for 1 minute. Finally, the PCR products were sequenced to determine the number of TA repeats over the promoter of the *UGT1A1* gene.

Examination of the Number of 28-Base Pair Tandemly Repeated Sequences in the 5'-Enhancer Region of the Thymidylate Synthase Gene

Because 5-FU has been used in combination with irinotecan to treat these patients, and because germline

polymorphisms of the number of 28-base pair (bp) tandemly repeated sequence in the 5'-enhancer region of the thymidylate synthase gene (TSER) have a remarkable influence on the response and survival of patients with CRC who receive 5-FU,²⁹ we believed that the influence of this polymorphism on patients with or without UGT1A1*28 was of interest and deserved further study. To examine this polymorphism in the TSER, an amplification product was generated by PCR for analysis. Genomic DNA was prepared from patients' leukocytes accordingly and a set of primers for amplification of the TSER was used according to a method described previously.³⁰ The sequences of the forward and reverse primers were 5'-GTG GCT CCT GCG TTT CCC CC-3' and 5'-CCA AGC TTG GCT CCG AGC CGG CCA CAG GCA TGG CGC GG-3', respectively. Amplification was performed for 30 cycles and consisted of denaturation at 94°C for 1 minute, annealing at 60°C for 1 minute, and extension at 72°C for 2 minutes between the initial denaturation at 94°C for 2 minutes and a final extension at 72°C for 5 minutes. Finally, the amplified DNA fragments were analyzed by electrophoresis on a 4% agarose gel to determine the number of a 28-bp tandemly repeated sequence over the TSER. The terms 2R/2R, 3R/3R, and 2R/3R represent homozygous double-repeat, homozygous triple-repeat, and heterozygous TSER polymorphisms, respectively.

Statistical Analysis and Plotting Survival Curves

According to the number of TA repeats in 2 alleles, genotypes of the UGT1A1*28 polymorphism were classified as TA6/TA6 (wild type), TA6/TA7 (heterozygous mutant), or TA7/TA7 (homozygous mutant). To analyze survival and clinicopathologic characteristics, patients were divided into 2 groups: those with TA6/TA6 (wild type) and those with TA6/TA7 or TA7/TA7 (mutant type). Progression-free and overall survival curves were plotted by using the Kaplan-Meier product limit method, and the statistical differences in survival between subgroups were compared by using the log-rank test. The correlations of age, sex, performance status, primary tumor site, histologic grade, TNM classification, serum CEA levels, germ-line polymorphisms of the number of a 28-bp tandemly repeated sequence in the TSER, response rate, and toxicity to irinotecan-based treatment were analyzed separately according to the UGT1A1 genotypes. The statistical differences of these correlations were determined by using chi-square tests and were considered statistically different when P values were $<.05$. All statistical analyses were performed using the SPSS software system (SPSS for Windows, version 10.0, Chicago, Ill).

RESULTS

UGT1A1*28 Polymorphism Is Associated With Increased Irinotecan-induced Toxicities, Hospitalization Because of Toxicities, and Treatment-related Mortality

An example of the PCR products that were analyzed by agarose gel electrophoresis is shown in Figure 1. Approximately 20% of patients were identified with UGT1A1*28 polymorphism, and the frequencies of the TA6/TA6, TA6/TA7, and TA7/TA7 genotypes were 79.7% ($n = 102$ patients), 15.6% ($n = 20$ patients), and 4.7% ($n = 6$ patients), respectively. There was no significant between-group difference in clinicopathologic features, including TSER 28-bp polymorphisms, among patients with or without this polymorphism (Table 1). To no surprise, in the current study, marked increases in grade 3 or 4 neutropenia (53.8% vs 4.9%; $P < .01$) and diarrhea (26.9% vs 5.9%; $P < .01$) were observed in patients with the TA6/TA7 or TA7/TA7 genotypes (Table 2). In total, 19 patients with or without the UGT1A1*28 allele experienced grade 3 or 4 neutropenia, and the timing varied between the first cycle ($n = 3$ patients), the second cycle ($n = 4$ patients), the third cycle ($n = 7$ patients), and the fourth cycle ($n = 5$ patients) of treatment.

Concordantly, dramatic increases in neutropenic fever (38.5% vs 3.9%; $P < .01$), hospitalization for managing neutropenic fever or grade 3 or 4 diarrhea (50% vs 8.8%; $P < .01$), and treatment-related mortality (11.5% vs 2%; $P < .01$) also were observed in patients with the TA6/TA7 or TA7/TA7 genotypes (Table 2). Three patients (11.5%) with TA6/TA7 or TA7/TA7 genotypes died of treatment-related neutropenia and bacteremia (*Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, respectively), and

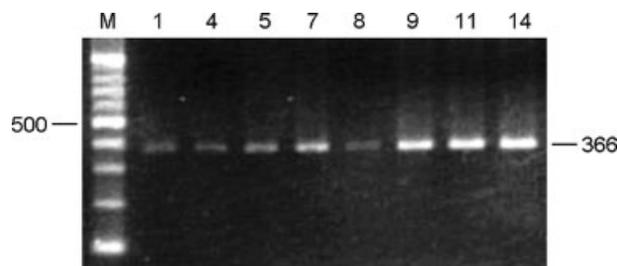


FIGURE 1. Representative patterns of the polymerase chain reaction (PCR) products from patients' blood samples for analyzing thymine-adenine (TA) repeat polymorphisms in the promoter of the uridine-diphosphoglucuronosyl transferase 1A1 (UGT1A1) gene. Genomic DNA obtained from patients' leukocytes was subjected to PCR amplification using 5'-AAA TTC CAG CCA GTT CAA CTG TTG TT-3' and 5'-CTG CTG GAT GGC CCC AAG-3' as forward and reverse primers, respectively. PCR products were revealed by agarose gel electrophoresis. Lane M indicates PCR marker.

TABLE 2
Correlations of UGT1A1*28 Polymorphisms and Treatment Outcome of Irinotecan-based Chemotherapy in Patients With Metastatic Colorectal Cancer*

Characteristic	No. of patients (%)		P
	TA6/TA6 (Wild type)	TA6/TA7 or TA7/TA7	
All patients	102 (100)	26 (100)	
Neutropenia			
Grade 0-2	97 (95.1)	12 (46.2)	<.01
Grade 3, 4	5 (4.9)	14 (53.8)	
Neutropenic fever			
Presence	4 (3.9)	10 (38.5)	<.01
Absence	98 (96.1)	16 (61.5)	
Thrombocytopenia			
Grade 0-2	95 (93.1)	22 (84.6)	.17
Grade 3, 4	7 (6.9)	4 (15.4)	
Diarrhea			
Grade 0-2	96 (94.1)	19 (73.1)	<.01
Grade 3, 4	6 (5.9)	7 (26.9)	
Hospitalized because of toxicity			
Required	9 (8.8)	13 (50)	<.01
Not required	93 (91.2)	13 (50)	
Treatment-related mortality			
Presence	2 (2.0)	3 (11.5)	<.01
Absence	100 (98)	23 (88.5)	
Pretreatment total bilirubin			
Elevated, >1.5 mg/dL	9 (8.8)	6 (23.1)	.04
Normal	93 (91.2)	20 (76.9)	
Irinotecan dose-reduction			
Needed	13 (12.7)	11 (42.3)	<.01
Not needed	89 (87.3)	15 (57.7)	
Switch to oxaliplatin by toxicity			
Needed	4 (3.9)	7 (26.9)	<.01
Not needed	98 (96.1)	19 (73.1)	
Response rate			
CR and PR	46 (45.1)	11 (42.3)	.80
SD and PD	56 (54.9)	15 (57.7)	

T indicates thymine; A, adenine; CR, complete remission; PR, partial remission; SD, stable disease; PD, progressive disease.

* According to World Health Organization criteria for response and toxicity evaluation.

2 patients (2%) with the TA6/TA6 genotype died of treatment-related neutropenia and bacteremia (*Pseudomonas aeruginosa*; $P < .01$). Although severe toxicities did occur in patients with the UGT1A1*28 allele, the initial dose of irinotecan was not adjusted (or reduced) according to the patients' genotype, because this was a retrospective study, and the majority of patients were not assessed for UGT1A1*28 before treatment. Furthermore, the "optimal dose" of irinotecan for patients with the UGT1A1*28 allele remains unclear.

Because 5-FU was used in combination with irinotecan, the differences in the numbers of 28-bp tandemly repeated sequences in the TSER of patients with or without the UGT1A1*28 polymorphism were

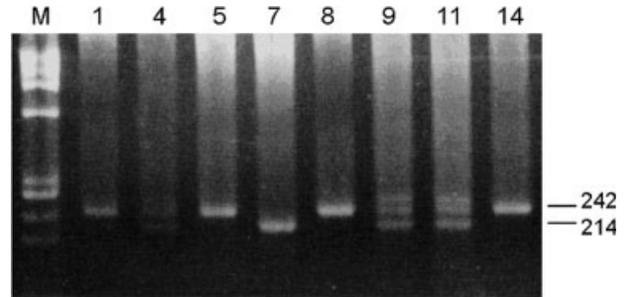


FIGURE 2. Representative patterns of polymorphisms of the 5'-enhancer region of the thymidylate synthase gene (TSER). Genomic DNA obtained from patients' leukocytes was subjected to PCR amplification using 5'-GTG GCT CCT GCG TTT CCC CC-3' and 5'-CCA AGC TTG GCT CCG AGC CGG CCA CAG GCA TGG CGC GG-3' as forward and reverse primers, respectively. Polymerase chain reaction products were revealed by agarose gel electrophoresis for analyzing germ-line polymorphisms of the number of a 28-base pair tandemly repeated sequence in the TSER. Lanes 7, 8, and 9 represent TSER 2R/2R, 3R/3R, and 2R/3R, respectively. Lane M indicates PCR marker.

analyzed. Table 1 shows that the percentages of patients with TSER 2R/2R, 2R/3R, and 3R/3R were 0.1%, 34.3%, and 64.7%, respectively, among those who had the TA6/TA6 genotype. These rates were very similar to the rates of 0%, 30.7%, and 69.2%, respectively, in patients with the TA6/TA7 or TA7/TA7 genotypes ($P = .82$). Examples of the PCR products that were analyzed by agarose gel electrophoresis for the presence of 28-bp tandemly repeated sequence polymorphisms of the TSER are shown in Figure 2.

UGT1A1*28 Is Associated With Increased Pretreatment Bilirubin Levels, and Pretreatment Bilirubin Levels Also May Predict Irinotecan-induced Toxicities

Because UGT1A1 is the major enzyme that catalyzes the glucuronidation of bilirubin, and mutations of this gene result in the absence or reduction of UGT1A1 activity, which may lead to unconjugated hyperbilirubinemia,^{17,18} it was proposed that patients who had the UGT1A1*28 polymorphism would have a greater prevalence of elevated pretreatment serum bilirubin levels. Table 2 shows that a dramatic increase in the prevalence of elevated pretreatment bilirubin levels (23.1% vs 8.8%; $P = .04$) indeed was observed in patients with the TA6/TA7 or TA7/TA7 genotypes.

In addition, because patients with UGT1A1*28 polymorphism have higher treatment-related toxicities and pretreatment bilirubin levels, a greater prevalence of toxicity was proposed in patients who had elevated pretreatment bilirubin levels. Indeed, grade 3 or 4 neutropenia did occur in patients who had higher pretreatment bilirubin levels (>1.5 mg/dL).

TABLE 3
Response to Irinotecan-based Chemotherapy in Patients With or Without UGT1A1*28 Polymorphism*

Response	No. of patients (%)		<i>P</i> [‡]
	TA6/TA6 (Wild type)	TA6/TA7 or TA7/TA7	
All patients enrolled	102 (100)	26 (100)	
CR	5 (4.9)	2 (7.7)	.80
PR	41 (40.2)	9 (34.6)	
OR	46 (45.1)	11 (42.3)	
SD	39 (38.2)	10 (38.5)	
PD	17 (16.7)	5 (19.2)	

CR indicates complete remission; PR, partial remission; OR, overall response; SD, stable disease; PD, progressive disease.

* The response rate was assessed based on World Health Organization criteria. In this table, the assessment was made after 4 cycles of treatment.

[‡] *P* values define the difference in overall response rate between 2 groups of patients.

In the current study, elevated bilirubin levels were identified in 15 patients, whereas the remaining patients (n = 113) had normal bilirubin levels (Table 2). Ten patients (66.7%) who had higher pretreatment bilirubin levels developed grade 3 or 4 neutropenia, whereas only 9 patients (8%) who had normal pretreatment bilirubin levels developed grade 3 or 4 neutropenia (*P* < .01). Therefore, the pretreatment bilirubin level also may serve as a marker for predicting irinotecan-induced toxicities.

The UGT1A1*28 Polymorphism Is Associated With Increased Requirement for Irinotecan Dose Reduction Without Affecting Response Rate, Progression-free Survival, or Overall Survival

Because the UGT1A1*28 polymorphism was associated with increased irinotecan-induced severe neutropenia, neutropenic fever, and diarrhea, an increased requirement for irinotecan dose reduction in patients with this polymorphism was proposed. Table 2 shows that the percentage of patients who required an irinotecan dose reduction indeed was significantly greater in patients with this polymorphism. In total, 24 patients required dose reductions for irinotecan, including 11 patients (42.3%) with TA6/TA7 or TA7/TA7 genotypes and 13 patients (12.7%) with TA6/TA6 genotypes (*P* < .01). These patients developed neutropenic fever (n = 10), intolerable diarrhea (n = 10), or both (n = 4). The doses of irinotecan were reduced by approximately 30% in subsequent cycles, and irinotecan was administered at 120 mg/m² (n = 15) or at 130 mg/m² (n = 9), whereas the doses of 5-FU and LV remained unchanged. Eighteen patients (75%) tolerated the

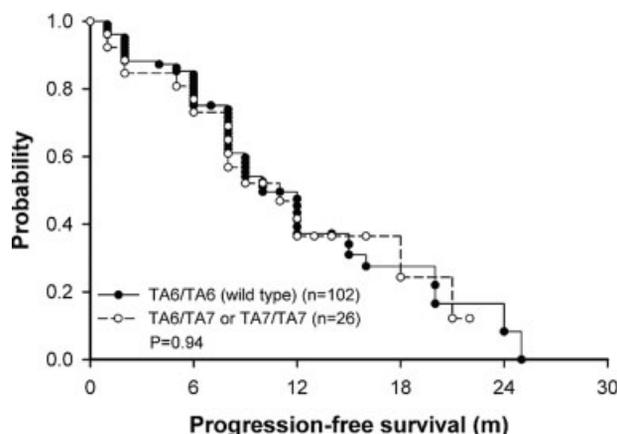


FIGURE 3. Progression-free survival curves of patients with (open circles) or without (solid circles) uridine-diphosphoglucuronosyl transferase 1A1 (UGT1A1)*28 polymorphism who received irinotecan-based chemotherapy plotted by the Kaplan-Meier method (*P* = .94; log-rank test). TA indicates thymine-adenine.

modified doses well without requiring further dose reductions of irinotecan. However, intolerable toxicities still occurred in 6 patients (25%), and subsequent treatments were switched to oxaliplatin-based regimens immediately in these patients.

Because the UGT1A1*28 polymorphism leads to a marked alteration in pharmacokinetics as well as the need for irinotecan dose reduction, the influence of this polymorphism on treatment outcomes has become of extreme interest. In the current study, the response rate was assessed every 4 cycles of treatment, and 17 patients (16.7%) with the TA6/TA6 genotype and 5 patients (19.2%) with the TA6/TA7 or TA6/TA7 genotypes had progressive disease after 4 cycles of treatment (Table 3). It is noteworthy that, although the requirement for irinotecan dose reduction was significantly greater in patients with the TA6/TA7 or TA7/TA7 genotypes (42.3% vs 12.7%; *P* < .01), there were no significant between-group differences in the response rate (42.3% vs 45.1%; *P* = .80) (Table 3), in progression-free survival (10 months vs 11 months; *P* = .94) (Fig. 3), or in overall survival (19 months vs 18 months; *P* = .84) (Fig. 4). Therefore, the UGT1A1*28 polymorphism does not seem to affect treatment outcomes with irinotecan-based chemotherapy in patients with metastatic CRC.

DISCUSSION

The identification of genetic variants that predispose patients to severe toxicities from chemotherapeutic agents is a critical issue. Our current data indicate that the UGT1A1*28 polymorphism, through an extra TA repeat insertion in the promoter of the UGT1A1

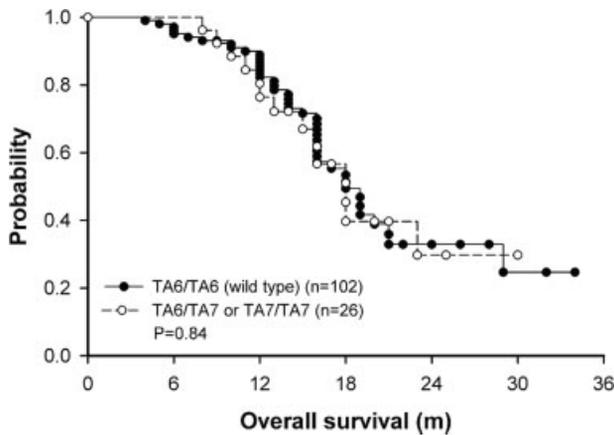


FIGURE 4. Overall survival curves of patients with (open circles) or without (solid circles) uridine-diphosphoglucuronosyl transferase 1A1 (UGT1A1)*28 polymorphisms who received irinotecan-based chemotherapy plotted by the Kaplan-Meier method ($P = .84$; log-rank test). TA indicates thymine-adenine.

gene, is associated with a marked increase in irinotecan-induced grade 3 or 4 neutropenia (53.8% vs 4.9%; $P < .01$), neutropenic fever (38.5% vs 3.9%; $P < .01$), treatment-related death (11.5% vs 2%; $P < .01$), and diarrhea (26.9% vs 5.9%; $P < .01$), findings that are compatible with previous reports.^{9,31} It is worth noting that, although the percentage of patients who required an irinotecan dose reduction was significantly greater in patients who had this genetic variant (42.3% vs 12.7%; $P < .01$), it did not affect the response rate to irinotecan-based chemotherapy (42.3% vs 45.1%; $P = .80$), progression-free survival (10 months vs 11 months; $P = .94$), or overall survival (19 months vs 18 months; $P = .84$). This leads us to consider the possibility of using the UGT1A1*28 polymorphism to optimize (or reduce) irinotecan dosage without affecting its efficacy in the treatment of patients with metastatic CRC.

Ethnic differences do exist in enzymes that involve in the targeting and metabolism of specific chemotherapeutic drugs, and these differences may affect the sensitivity and toxicity to treatments in cancer patients. For example, germ-line polymorphism of a 28-bp tandem repeat sequence in the TSER significantly affected gene expression levels, response, and survival in patients with metastatic CRC who received fluoropyrimidine treatment.^{29,30} The homozygous triple-repeat polymorphism (3R/3R) in TSER was twice as prevalent in Chinese individuals (67%) as it was in Caucasian individuals (38%), a finding that may account for impaired treatment outcomes with fluoropyrimidine regimens.³² Table 1 shows that approximately 66% of patients had a

homozygous triple-repeat polymorphism (3R/3R) in the TSER, which is compatible with previous reports.³² Although 5-FU was used in combination with irinotecan in this study, the percentages of TSER 2R/2R, 2R/3R, and 3R/3R were very similar in patients with and without the UGT1A1*28 polymorphism ($P = .82$). Therefore, the influence of 5-FU on treatment toxicity, response rates, and survival in patients with or without the UGT1A1*28 polymorphism may be negligible.

The UGT1A1*28 polymorphism is relatively rare in Asian populations.²¹⁻²³ The prevalence of homozygous TA7/TA7 genotype of the *UGT1A1* gene was significantly greater in Africans (12%–27%) and in Caucasians (5%–15%), but it was much lower in South-east Asian and Pacific populations (1.2%–5%).²¹⁻²³ In a study that was conducted by Huang et al., UGT1A1*28 polymorphisms were examined in a total of 290 healthy Taiwanese adults, and the prevalence of the TA6/TA7 and TA7/TA7 genotypes was 21.4% and 0%, respectively.²⁴ In our study, the percentage of TA6/TA7 and TA7/TA7 genotypes was 15.6% ($n = 20$) and 4.7% ($n = 6$), respectively. A significantly lower prevalence of the homozygous TA7/TA7 allele in our study correlates well with the observations of Huang et al. and others. In contrast to the rarity of UGT1A1*28, it was demonstrated that the UGT1A1*6 polymorphism was very frequent in Asian populations.^{33,34} A recent, noteworthy study indicated that the UGT1A1*6 (c.211G > A) allele was more predictive of neutropenia in Korean cancer patients who were receiving irinotecan.³³ In addition, the presence of the homozygous UGT1A1*6 allele was associated with a significantly higher exposure levels to SN-38, lower relative extent of glucuronidation, and an approximately 3-fold increased risk of developing grade 4 neutropenia in Asian cancer patients, particularly among Chinese patients, who were receiving irinotecan.³⁴

In the current study, a dramatic increase in the prevalence of elevated pretreatment bilirubin levels (23.1% vs 8.8%; $P = .04$) was observed in patients with the TA6/TA7 or TA7/TA7 genotypes (Table 2). Because UGT1A1 is the major enzyme that catalyzes the glucuronidation of bilirubin, and because mutations of this gene may lead to unconjugated hyperbilirubinemia,^{17,18} elevated pretreatment bilirubin levels may occur more often in patients who have the UGT1A1*28 polymorphism. In addition to UGT1A1 genotype, Innocenti et al. demonstrated that the pretreatment bilirubin level was a useful predictor of severe neutropenia in patients who were receiving irinotecan.⁹ In that study, both bilirubin and UGT1A1 genotype were statistically significant

for predicting which patients were at risk for developing severe toxicities, and the authors suggested that total bilirubin may replace genotyping information when the latter is not available. In our study, patients with higher pretreatment bilirubin levels developed significantly more grade 3 or 4 neutropenia than patients who had normal bilirubin levels (66.7% vs 8%; $P < .01$). Therefore, patients' pretreatment bilirubin level may also serve as a useful marker for predicting irinotecan-induced toxicities.

In addition to UGT1A1, several factors, including CES, cytochrome p450 isoforms, multidrug resistance-associated proteins ABCB1 and ABCC2, organic anion-transporting polypeptide SLCO1B1, and the ATP-binding cassette transporters, also are involved in the metabolic pathway of SN-38.¹⁴⁻¹⁶ Therefore, the clinical application of routinely screening for the UGT1A1*28 polymorphism to predict irinotecan-induced toxicity deserves further prospective studies. However, for patients who have experienced early and severe (grade 3 or 4) toxicities after irinotecan-based chemotherapy, it may be worthwhile to examine for the presence of the UGT1A1*28 polymorphism to optimize subsequent irinotecan-based treatment.

In the current study, both bolus and infusional 5-FU were administered with irinotecan as front-line treatment to patients with metastatic CRC. Greater than 80% of 5-FU is metabolized by the enzyme dihydropyrimidine dehydrogenase (DPD) in the liver, and mutations in the DPD gene (*DPYD*) have been associated with a lowered DPD activity, which may lead to severe 5-FU-associated toxicities, mainly neutropenia and diarrhea. In a study conducted by Hsiao et al., the frequency of 11 known mutations of the *DPYD* gene and the correlations between these mutations and DPD levels were analyzed in Taiwanese populations.³⁵ In that study, DPD protein levels were not altered (or reduced) significantly by these mutations. In this regard, because the incidence of severe neutropenia and diarrhea induced by irinotecan was significantly greater than that with 5-FU, and because the influence of *DPYD* mutations on 5-FU metabolism was relatively trivial in Taiwanese populations, 5-FU-associated toxicities may be negligible.

In the current study, 24 patients required dose reductions of irinotecan, and approximately 30% of irinotecan doses were reduced in subsequent cycles. Among them, 18 patients (75%) tolerated the modified doses well without requiring further dose reduction of irinotecan. Therefore, we proposed that irinotecan at doses of 120 mg/m² or 130 mg/m² every 2 weeks may be feasible and safe for patients

who have experienced grade 3 or 4 toxicities or who are at risk for developing life-threatening toxicities from irinotecan-based treatment, such as UGT1A1 polymorphisms.

In summary, the UGT1A1*28 polymorphism may be a key determinant for predicting irinotecan-induced severe toxicities in Chinese patients with metastatic CRC without affecting treatment outcome or survival. Further prospective studies are warranted for using this polymorphism to optimize irinotecan-based chemotherapy and to avoid life-threatening toxicity.

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