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ORIGINAL ARTICLE

UGT1A1 gene polymorphism is associated with toxicity and clinical efficacy of irinotecan-based chemotherapy in patients with advanced colorectal cancer

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Abstract
Objectives To investigate the relationship between *uridine diphosphate glucoronosyltransferase 1A1* (UGT1A1)*28/*6 and toxicity and clinical efficacy of irinotecan-based chemotherapy in patients with advanced colorectal cancer (CRC) in Xinjiang Uygur and Han population.
Methods A total of 183 patients (Uygur, 114; Han, 69) with advanced CRC who received the irinotecan-based chemotherapy were enrolled in this retrospective analysis. Polymerase chain reaction amplification and direct sequencing method were used for *UGT1A1**28 and *UGT1A1**6 polymorphism detection. The patients were followed up to analyze the relationship between different genotypes with adverse reactions and the clinical outcome of irinotecan-based chemotherapy.
Results Significant differences were found in genotype frequencies of *UGT1A1**28 and *UGT1A1**28/*6 between Uygur and Han ($P = 0.02$ and $P = 0.002$). Uygur and Han patients carrying wild *UGT1A1**28 and *6 genotypes appeared to have significantly lower diarrhea incidence (I/II and III/IV) than those carrying mutant genotypes (all $P < 0.05$). In Uygur patients, *UGT1A1**28 genotypes were related with objective response rate and disease control rate ($P < 0.05$). Compared with *1 allele *1/*1, *1 allele *1/*28*1/*28 mutant of *UGT1A1**28 was associated with shorter OS in both Uygur and Han ethnicities (all $P < 0.05$). Compared with double allele variants (DW), single allele variants (SV), and double allele variants (DV) of *UGT1A1**28/*6 were associated with shorter overall survival (OS) in Uygur and Han (all $P < 0.05$). Cox regression analysis revealed factors significantly influencing OS, including *UGT1A1**28, *UGT1A1**6, combined genotypes and chemotherapy line in Ugyur, and only combined genotypes in Han (all $P < 0.05$).
Conclusion *UGT1A1* gene polymorphism predicts irinotecan-related adverse reactions in advanced CRC patients of Xinjiang Uygur and Han nationality; *UGT1A1* gene polymorphism is correlated with efficacy and prognosis in Uygur nationality, but only related to prognosis in Han nationality in irinotecan-based chemotherapy.

Keywords Advanced colorectal cancer · Irinotecan-based chemotherapy · *UGT1A1* · Polymorphism · Uygur nationality · Han nationality · Toxicity · Clinical efficacy

Introduction

Colorectal cancer (CRC) is caused by uncontrolled cell proliferation in the epithelial cells present in the rectum, colon, or appendix. It is not only the third most commonly diagnosed cancer in males and the second in females, but also the fourth most common cause of cancer death [1]. In addition, CRC is a relative common cancer in China, especially for males in urban areas, since China had an estimated 310,244 new cases and an estimated 149,722 death cases in 2011 [2]. Two-thirds of the patients with CRC were diagnosed at a more advanced stage as the early-stage disease is mostly asymptomatic, and conventional chemotherapeutic regimens have been proven useful against advanced/

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metastatic CRC [3]. As an established cytotoxic regimen, irinotecan, 5-fluorouracil, and leucovorin (FOLFIRI) is an efficacious treatment for advanced CRC with approximately 2 years of overall survival [4]. However, resistance, toxicity, and side effects of chemotherapy remain great challenges in the long-term treatment of incurable metastatic diseases and may eventually contribute to death as tumors accumulate means of evading treatment [5]. Patients still show no benefit from FOLFIRI, and progress is made in order to find the differences in response of each individual on FOLFIRI, suggesting that genetic factors might have an important role in advanced CRC patients receiving irinotecan-based chemotherapy [6, 7].

Irinotecan (CPT-11), a main drug for the treatment of CRC, has limited clinical applications due to the presence of adverse reactions and obvious individual differences. The genetic polymorphism of drug metabolism is one of the main factors that cause the difference in adverse reactions among individuals [8]. Irinotecan is activated by hydrolysis to 7-ethyl-10-hydroxy-camptothecin (SN-38) by carboxylesterases in human tissues, and inhibition of topoisomerase I by active metabolite SN-38 results in inhibition of DNA replication and transcription, thereby producing cytotoxic effects [9, 10]. Study confirmed that uridine diphosphate glucoronosyltransferase 1A1 (UGT1A1) is a key enzyme in the metabolism and inactivation of SN-38 and UGT1A1 can transform SN-38 into glycosylated SN-38 (SN-38G), suggesting that the function and the gene polymorphism of UGT1A1 enzyme are closely related to irinotecan toxicity [11]. Lethal toxicity may cause failure of irinotecan-based chemotherapy in metastatic CRC patients, and clinical pharmacological evidence has revealed that irinotecan chemotherapy-induced toxicity is related to exposure to SN-38 and genetic polymorphisms in *UGT1A1* gene [12]. More than 50 kinds of alterations of *UGT1A1* gene loci have been reported in previous studies in which the relationship between *UGT1A1**28 and *UGT1A1**6 gene polymorphisms and adverse reactions of irinotecan is important, and *UGT1A1**6/*28 may be useful as markers to prevent severe adverse reactions of irinotecan administration [11, 13, 14]. Studies have shown that there are significant differences in the distribution of polymorphism of *UGT1A1* gene in different ethnic groups, which could lead to differences in the irinotecan-induced adverse reactions in different ethnic groups [15–18]. Specifically, *UGT1A1**28 has been suggested as a biomarker for the prediction of irinotecan-induced neutropenia and toxicities in CRC in Caucasians [19]. However, prevalence of *UGT1A1**28 mutations in Chinese Han population was significantly lower compared with American and European populations, and whether the *UGT1A1**28 mutations can be used as a predictor of irinotecan toxicity remains to be investigated in Chinese Han population [20–22]. In addition, incidence of *UGT1A1** 6 mutations (a variant in exon 1 of the *UGT1A1* gene) in the Asian population is as high as 20 %, mainly found in East Asians, and *UGT1A1** 6 is also an important risk factor associated with severe neutropenia and is important for the prediction of severe toxicity in patients receiving irinotecan treatment in Asian populations, while the potential role of *UGT1A1** 6 in Caucasians has not been well clarified concerning irinotecan treatment [23–25]. Xinjiang is a multiethnic region since ancient times with complex national integration and gene exchange. The Han and Uygur were the main body accounting for 85 % of the total population in Xinjiang. Therefore, we carried out the present study to further clarify the relationship between *UGT1A1**28/*6 and toxicity and clinical efficacy of irinotecan-based chemotherapy in patients with CRC in Uygur and Han population in Xinjiang, to explore the clinical significance of the detection of *UGT1A1* gene polymorphisms on individualized treatment regarding different ethics.

In order to further clarify the distribution of human *UGT1A1* gene polymorphism in Xinjiang Uygur and Han ethnicities, understand the association of *UGT1A1* gene polymorphism and toxic reactions and therapeutic effects of irinotecan, and explore the clinical significance of the detection of UGT1A1 gene polymorphism on individualized treatment, this study conducted UGT1A1 gene polymorphism (*UGT1A1**28 and *UGT1A1**6) detection on 183 advanced CRC patients receiving irinotecan-based chemotherapy, and follow-up analyses were carried out.

Materials and methods

Study subjects

A total of 183 advanced CRC patients who underwent chemotherapy with irinotecan were chosen from the Affiliated Tumor Hospital of Xinjiang Medical University during November, 2012 to November, 2015. There were 114 Uygur people including 78 males and 36 females (mean age, 43.61 ± 15.82) and 69 Han people including 46 males and 23 females (mean age, 44.48 ± 14.77). The Uygur and Han subjects were selected if three generations of the subjects lived in the Xinjiang area and their families had no history of intermarriage. Inclusion criteria: CRC patients in a relatively good state, confirmed by histology or cytology; performance score from Eastern Cooperative Oncology Group (ECOG): 0–1 [26]; expected survival time over 3 months; above 18 years old; absolute value of neutrophil granulocyte $\geq 1.5 \times 10^9$/L; platelet count $\geq 80 \times 10^9$/L; normal kidney, liver, and bone marrow functions ; total bilirubin level ≤ 19.2 μmol/L; serum creatinine $\leq 10^5$ μmol/L; no uncontrolled or severe internal diseases; no

active infection; no other primary malignant tumors. This study was approved by Ethics Committee of the Affiliated Tumor Hospital of Xinjiang Medical University, and all the study subjects and their families had given their consent. All the patients were treated with second- or third-line chemotherapy regimen (irinotecan), and complete blood count was performed after treatment or before the next consultation.

Chemotherapy regimens

The chemotherapy regimens included FOLFIRI and a combination of irinotecan and capecitabine. FOLFIRI: first day, intravenous (iv) injection with irinotecan 150 mg/m^2; first and second day, iv injection with tetrahydrofolic acid (THFC) 200 mg/m^2 and 5-FU 400 mg/m^2, and afterward with a continuous infusion pump with 5-FU 1200 mg/m^2 for 44 h; repeated every 2 weeks, four cycles of treatment. Combination of irinotecan and capecitabine: first day, iv drip with irinotecan 150 mg/m^2 for 90 min; second to the fifteenth day, iv drip with capecitabine 1000 mg/m^2 (twice a day); repeated every 3 weeks.

Evaluation criteria for drug toxicity

FOLFIRI and irinotecan-induced severe drug toxicity generally occurred at the first four cycles of treatment. The adverse reactions of patients in the first four cycles were detected in our experimental process. Adverse reactions after chemotherapy were evaluated by National Cancer Institute Common Toxicity Criteria (NCI-CTC) version 3.0 [27]. Adverse reactions included diarrhea and neutropenia. Adverse reactions were recorded in accordance with 0–V stages and III–IV mean severe diarrhea and neutropenia. According to the severity of adverse reactions, diarrhea, and neutropenia were divided into five stages. Diarrhea: Grade I, the number of cacation increased less than four times and the excretion increased slightly compared to the baseline values; Grade II, compared to the baseline values, the number of cacation increased to 4–6 times and the excretion moderately increased but not affect the normal life, and it is necessary to carry out intravenous infusion for less than 24 h; Grade III, compared to the baseline values, the number of cacation increased more than seven times and the excretion extremely increased and had impact on normal life, and patients need to be hospitalized and received intravenous infusion for more than 24 h; Grade IV, patients has hemodynamic disorders and other life-threatening symptoms; Grade V, death. Neutropenia: Grade I, neutropenia (1.5–2.0) $\times$ 10^9 L; Grade II, neutropenia (1.0–1.5) $\times$ 10^9 L; Grad III, neutropenia (0.5–1.0) $\times$ 10^9 L; Grade IV, neutropenia (0.5 $\times$ 10^9 L); and Grade V, death.

Evaluation criterion for curative effects

The curative effects were evaluated based on Response Evaluation Criteria in Solid Tumors (RECIST) version 1.1 [28] every 6 weeks through CT. The curative effects were divided into complete remission (CR): The target lesions disappeared completely, and the lymph nodes were all shorter than 10 mm in minor diameter, and no new lesions were found; partial remission (PR): the sum of major diameter of the patient's baseline lesions were reduced by more than 30 %, and no new lesions were found; stable disease (SD): the sum of major diameter of the patient's baseline lesions were reduced but did not reach PR or increased but did not up to PD, and no new lesions were found; progression of disease (PD): The sum of major diameter of the patient's baseline lesions were increased by 20 % or more, the absolute value increased by 5 mm or more, or new lesions were found. Objective response rate (ORR) was calculated based on CR and PR; disease control rate (DCR) was calculated based on CR and PR and SD; overall survival (OS) was followed up.

Gene detection of *UGT1A1*

A total of 3 ml peripheral venous blood samples were collected before treatment and were placed in the ethylenediaminetetraacetic acid (EDTA) anticoagulant tube for anticoagulation. Genomic DNA was extracted according to QIAamp Blood kit (Qiagen, Hilden, Germany). After the purity of DNA was confirmed, the primer was designed with the following sequencing: forward 5-CCTGCTAC CTTTGTGGACTGAC-3 and reverse 5-TGCCCGAGAC TAACAAAAGACT-3. DNA fragment included *UGT1A1**6 (211 G>A) and *UGT1A1**28 (−53 *1 allele >*28) which were amplificated by polymerase chain reaction (PCR). PCR system (25 μL): 5-ng template DNA, 10 $\times$ KOD plus buffer, 1 mmol/L dNTPs, 0.4 mmol/L $MgSO_4$, 0.5 U KOD plus enzyme (TOYOBO CO., LTD., Osaka, Japan), and 0.25 gmol/L forward and reverse primers. Reaction conditions: denaturation at 95 °C for 5 min, then another 15 s at 95 °C, 25 s at 60 °C and 30 s at 72 °C which was cycled for 40 times, and then being extended for 10 min at 72 °C. The amplification products were kept at −20 °C and analyzed using ABI-3730 through two-way analysis. Polyphred 5.04 was used to detect and analyze SNP and perform manual reading of genotype of *UGTlA1**28 and *UGTlA1**6.

Statistical methods

SPSS 21.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Measurement data were expressed as mean ± SD ($\bar{x}$ ± s). Comparison between two groups was performed using an independent sample *t* test. The

Table 1 Baseline characteristics in Ugyur and Han patients

Characteristics	Ugyur	Han	*P* value
Age (year)			0.573
≥60	41 (35.96)	22 (31.88)	
18–60	73 (64.04)	47 (68.12)	
Sex			0.806
Male	78 (68.42)	46 (66.67)	
Female	36 (31.58)	23 (33.33)	
ECOG			0.092
0	86 (75.44)	44 (63.77)	
1	28 (24.56)	25 (36.23)	
Location of primary tumor			0.757
Colon cancer	42 (36.84)	27 (39.13)	
Rectal cancer	72 (63.16)	42 (60.87)	
Degree of differentiation			0.634
High differentiation	64 (56.14)	35 (50.72)	
Low differentiation	50 (43.86)	34 (49.28)	
CEA level			0.741
≥10	80 (70.18)	50 (72.46)	
<10	34 (29.82)	19 (27.54)	
Chemotherapy line			0.600
Second line	85 (74.56)	49 (71.01)	
Third line	29 (25.44)	20 (28.99)	
Chemotherapy regimen			0.841
FOLFIRI	76 (66.67)	45 (65.22)	
Iri- Plus Cape-	38 (33.33)	24 (34.78)	

ECOG Eastern Cooperative Oncology Group, *CEA* carcinoembryonic antigen, *Iri-* irinotecan, *Cape-* capecitabine, *FOLFIRI* irinotecan, 5-fluorouracil, and leucovorin

representativity of the samples was analyzed by Hardy–Weinberg disequilibrium test. Clinical features, differences between adverse reactions of irinotecan and curative effects were analyzed through Chi-square test. Fitting curves were analyzed using Kaplan–Meier survival analysis, and influence factors were analyzed by Cox regression analysis. A *P* value of less than 0.05 was regarded as significant.

Results

Baseline characteristics

Baseline characteristics of the patients can be seen in Table 1. For the 114 Uygur patients, we can see that they were aged from 18 to 72 (average: 43.61 ± 15.82 years), and there were 78 males and 36 females, while the 69 Han patients were aged from 18 to 71 (average: 44.48 ± 14.77 years), with 46 males and 23 females. There was no difference in age, male/female (M/F) ratio, ECOG score, location of primary tumor, degree of differentiation, carcinoembryonic antigen (CEA) levels, chemotherapy line, and regimen between the two nationalities (all $P > 0.05$).

Table 2 Distribution of genotype and allele frequencies of *UGT1A1**28/*6 in Ugyur and Han patients

	Ugyur *n* (%)	Han *n* (%)	*P* value
*UGT1A1**28			
*1/*1	75 (65.78)	46 (66.67)	0.020
*1/*28	28 (24.62)	23 (33.33)	
*28*28	11 (9.6)	0 (0)	
*1	178 (78.07)	115 (83.33)	0.280
*28	50 (21.93)	23 (16.67)	
*UGT1A1**6			
G/G	79 (69.30)	50 (72.46)	0.461
G/A	34 (29.82)	17 (24.64)	
A/A	1 (0.88)	2 (2.9)	
G	192 (84.21)	117 (84.78)	1.000
A	36 (15.79)	21 (15.22)	
*UGT1A1**28/*6			
DW	55 (48.24)	30 (43.48)	0.002
SV	32 (20.07)	34 (49.28)	
DV	27 (23.69)	5 (7.25)	

DW double wild type, *SV* single variant, *DV* double variant

Distribution of genotype and allele frequencies

The distribution of genotype and allele frequencies of *UGT1A1**28/*6 were consistent with the Hardy–Weinberg disequilibrium (all $P > 0.05$). In the 114 Uygur patients, the variants of *UGT1A1**28 and *UGT1A1**6 were divided into three types: double wild type (DW) (*1/*1 and G/G, $n = 55$, 48.24 %), single variant (SV) (G/A and *1/*1 or G/G and *1 allele *1/*28, $n = 32$, 20.07 %), and double variant (DV) (G/G and *28/*28 or A/A and *1/*1 or G/A and *1/*28, $n = 27$, 23.69 %). In the 69 Han patients: DW (*1/*1 and G/G, $n = 30$, 43.48 %), SV (G/A and *1/*1 or G/G and *1/*28, $n = 34$, 49.28 %), and DV (G/A and *1/*28 or A/A and *1/*1, $n = 5$, 7.25 %). Obvious differences in the distribution frequencies of *1/*1, *1/*28, and *28/*28 of *UGT1A1**28 and DW, SV, and DV of *UGT1A1**28/*6 were found between Uygur and Han patients ($P = 0.02$ and $P = 0.002$). There was no difference in distribution frequencies of *UGT1A1**28 allele and *UGT1A1**6 genotype and allele between the two ethnicities ($P > 0.05$) (Table 2).

Gene polymorphism and clinical features

There was no difference in age, sex, ECOG score, location of primary tumor, degree of differentiation, CEA level,

chemotherapy line and regimen among Ugyur patients with DW, SV, and DV genotypes (all $P > 0.05$); there was also no difference found in age, sex, ECOG score, location of primary tumor, degree of differentiation, CEA level, chemotherapy line, and regimen among Han patients with DW, SV, and DV genotypes (all $P > 0.05$) (Table 3).

*UGT1A1**28/*6 polymorphisms and adverse reactions of irinotecan

Among the 114 Uygur patients, 27 (23.69 %) suffered from severe delayed diarrhea and 14 (12.28 %) had severe neutropenia; among the 69 Han patients, 16 (23.19 %) had severe delayed diarrhea and 11 (15.94 %) had severe neutropenia (Table 4). Uygur patients carrying *UGT1A1**28 mutant *1/*28 and *28/*28 genotypes were more likely to suffer from diarrhea (I/II and III/IV) compared with those with wild-type *1/*1; compared with mutant G/A and A/A in *UGT1A1**6, wild-type G/G genotype significantly decreased the risk of diarrhea (I/II and III/IV) (all $P < 0.05$). Han patients carrying mutant *UGT1A1**28 *1/*28 and *28/*28 genotypes were more likely to suffer from diarrhea (I/II and III/IV) compared with those with wild-type *1/*1; in *UGT1A1**28, incidence of diarrhea (I/II and III/IV) was lower in patients with wild-type G/G than those with mutant G/A and A/A (all $P < 0.05$). In addition,

Table 4 Distribution of diarrhea and neutropenia stages of Uygur and Han patients with advanced colorectal cancer

Symptoms	0	I/II	III–IV
Ugyur			
Diarrhea			
n (%)	52 (45.61)	35 (30.70)	27 (23.69)
Neutropenia			
n (%)	66 (57.89)	34 (29.83)	14 (12.28)
Han			
Diarrhea			
n (%)	33 (47.83)	20 (28.98)	16 (23.19)
Neutropenia			
n (%)	31 (44.93)	27 (39.13)	11 (15.94)

Table 3 Association of gene polymorphisms and clinical features in Uygur and Han patients

Characteristics	Ugyur			*P* value	Han			*P* value
	DW	SV	DV		DW	SV	DV	
Age (year)				0.639				0.757
≥60	22	11	8		9	12	1	
18–60	33	21	19		21	22	4	
Sex				0.560				0.785
Male	35	23	20		19	24	3	
Female	20	9	7		11	10	2	
ECOG				0.062				0.905
0	38	29	19		20	21	3	
1	17	3	8		10	13	2	
Location of primary tumor			0.842					0.933
Colon cancer	20	13	9		11	14	2	
Rectal cancer	35	19	18		19	20	3	
Degree of differentiation				0.192				0.552
High differentiation	32	14	18		17	15	3	
Low differentiation	23	18	9		13	19	2	
CEA level				0.284				0.686
≥10	39	25	16		21	26	3	
<10	16	7	11		9	8	2	
Chemotherapy line				0.064				0.969
Second line	32	26	20		26	28	4	
Third line	23	6	7		4	6	1	
Chemotherapy regimen				0.952				0.497
FOLFIRI	36	22	18		21	20	4	
Iri- Plus Cape-	19	10	9		9	14	1	

DW double wild type, *SV* single variant, *DV* double variant, *ECOG* Eastern Cooperative Oncology Group, *CEA* carcinoembryonic antigen, *Iri-* irinotecan, *Cape-* capecitabine, *FOLFIRI* irinotecan, 5-fluorouracil, and leucovorin

there was no difference in incidence of neutropenia (I/II and III/IV) among Uygur and Han patients with different *UGT1A1**28 and *UGT1A1**6 genotypes (all $P > 0.05$). Compared with DW genes, there were significant differences in incidence of neutropenia (I/II and III/IV) in Uygur and Han patients with heterozygous and homozygous type ($P < 0.05$) (Table 5).

*UGT1A1**28/*6 polymorphisms and chemotherapy efficacy

Chemotherapy effects of the 114 Ugyur patients treated with irinotecan were evaluated: CR ($n = 0$), PR ($n = 20$), SD ($n = 48$), and PD ($n = 46$). In *UGT1A1**28, there were differences in clinical responses (ORR and DCR) to *1/*1, *1/*28, and *28/*28 ($P = 0.001$ and $P = 0.004$) polymorphisms but no difference was found to *UGT1A1**6 polymorphism ($P > 0.05$). The therapeutic efficacy of the 69 Han patients was assessed: CR ($n = 0$), PR ($n = 33$), SD ($n = 14$), and PD ($n = 22$). There was no difference in clinical responses (ORR and DCR) to *UGT1A1**28 ($P = 0.618$ and $P = 1.00$) and *UGT1A1**6 ($P = 0.821$ and $P = 0.946$) polymorphisms (Table 6).

*UGT1A1**28/*6 polymorphisms and OS

The median survival time was 25 months in 114 Ugyur patients, there were differences in OS in patients with *UGT1A1**28 *1/*28, *28/*28, and *1/*1 ($P < 0.05$); differences were also found in patients with *UGT1A1**6 AA and GG ($P < 0.05$); compared with double wild-type genes, single-point mutant and double-point mutant genes had different influences on OS ($P < 0.05$) (Fig. 1). The median survival time was 24 months in 69 Han patients, there were differences in OS in patients with *UGT1A1**28 *1/*28, and *1/*1 ($P < 0.05$); differences were also revealed in *UGT1A1**6 AA and GG ($P < 0.05$); compared with double wild-type genes, single-point mutant and double-point

Table 5 Association of genotypes of *UGT1A1**28/*6 and adverse reactions of irinotecan in Uygur and Han patients

Genotypes	I/II diarrhea *n* (%)	*P* value	III/IV diarrhea *n* (%)	*P* value	I/II neutropenia *n* (%)	*P* value	III/IV neutropenia *n* (%)	*P* value
Ugyur								
*UGT1A1**28		0.002		0.002		0.881		0.726
*1/*1 ($n = 75$)	13 (16.46)		12 (16)		22 (29.33)		8 (10.67)	
1/*28($n = 28$)	18 (64.29)		8 (28.57)		8 (28.57)		4 (14.29)	
*28/*28 ($n = 11$)	4 (36.36)		7 (63.64)		4 (36.36)		2 (18.18)	
*UGT1A1**6		0.007		0.004		0.824		0.924
G/G ($n = 79$)	18 (22.78)		12 (15.19)		23 (29.11)		10 (12.66)	
G/A ($n = 34$)	17 (50)		15 (44.12)		11 (32.35)		4 (11.76)	
A/A ($n = 1$)	0 (0)		0 (0)		0 (0)		0 (0)	
Numbers of mutational alleles		<0.001		<0.001		0.003		0.027
DW ($n = 55$)	2 (3.64)		5 (9.09)		12 (21.82)		8 (14.55)	
SV ($n = 32$)	23 (71.88)		7 (21.88)		17 (53.13)		0 (0)	
DV ($n = 27$)	10 (37.04)		15 (55.56)		5 (18.52)		6 (22.22)	
Han								
*UGT1A1**28		0.024		0.001		0.601		1.000
*1/*1 ($n = 46$)	9 (19.57)		5 (10.87)		17 (36.96)		7 (15.22)	
*1/*28 ($n = 23$)	11 (47.83)		11 (47.83)		10 (43.48)		4 (17.39)	
*28/*28 ($n = 0$)	0 (0)		0 (0)		0 (0)		0 (0)	
*UGT1A1**6		0.028		0.014		0.366		0.812
G/G ($n = 50$)	10 (20)		7 (14)		17 (34)		8 (16)	
G/A ($n = 17$)	9 (53.06)		8 (47.06)		9 (52.94)		3 (17.64)	
A/A ($n = 2$)	1 (50)		1 (50)		1 (50)		0 (0)	
Numbers of mutational alleles		<0.001		<0.001		0.032		0.002
DW ($n = 30$)	0 (0)		0 (0)		7 (23.33)		7 (23.33)	
SV ($n = 34$)	18 (52.94)		12 (35.29)		19 (55.88)		1 (2.94)	
DV ($n = 5$)	2 (40)		4 (80)		1 (20)		3 (60)	

DW double wild type, *SV* single variant, *DV* double variant

Table 6 *UGT1A1**28/*6 polymorphisms and chemotherapy efficacy in Uygur and Han patients

Genotypes	CR and PR	*P* value	CR and PR and SD	*P* value
	n (%)		*n* (%)	
Ugyur				
*UGT1A1**28		0.001		0.004
*1/*1 ($n = 75$)	9 (12)		51 (68)	
*1/*28 ($n = 28$)	22 (78.57)		23 (82.14)	
28/*28 ($n = 11$)	2 (18.18)		3 (27.27)	
*UGT1A1**6		0.724		0.162
G/G ($n = 79$)	22 (27.85)		51 (64.56)	
G/A ($n = 34$)	11 (32.35)		26 (76.47)	
A/A ($n = 1$)	0 (0)		0 (0)	
Han				
*UGT1A1**28		1.000		0.587
*1/*1 ($n = 46$)	22 (47.83)		30 (65.22)	
*1/*28 ($n = 23$)	11 (47.83)		17 (73.91)	
*28/*28 ($n = 0$)	0 (0)		0 (0)	
*UGT1A1**6		0.491		0.839
G/G ($n = 50$)	26 (52)		34 (68)	
G/A ($n = 17$)	6 (35.29)		12 (70.59)	
A/A ($n = 2$)	1 (50)		1 (50)	

CR complete remission, *PR* partial remission, *SD* stable disease

mutant genes had significantly different influences on OS (both $P < 0.05$) (Fig. 2). Multivariate survival analysis was performed among Ugyur and Han patients using Cox regression model to analyze influencing factors on OS included age, M/F ratio, ECOG score, location of primary tumor, degree of differentiation, CEA level, number of mutant integrated allele, chemotherapy regimen, and line. The results showed that for Ugyur patients, *UGT1A1**28, *UGT1A1**6, the number of mutant integrated alleles and chemotherapy line had significantly different influences on OS (all $P < 0.05$), but for Han patients, only the number of mutant integrated alleles had influenced OS ($P < 0.05$) (Table 7).

Discussion

Currently, irinotecan is widely used as a clinical topoisomerase I inhibitor, especially in the treatment of gastric cancer, CRC, small cell lung cancer, and other solid tumors, and clinical trials have demonstrated that irinotecan can significantly prolong the patient's life [13, 29, 30]. However, clinical application of irinotecan is limited due to its important dose dependent toxicity, and many scholars are working on evaluating predictors of irinotecan-induced toxicity in order to improve safety and efficacy of irinotecan application, especially in advanced and/or metastatic CRC [31, 32]. Several important clinical trials suggest that genetic factors may play important roles in drug metabolism, distribution, and toxicity of irinotecan. UGTIA1 is currently known as the main isozyme involved in irinotecan metabolism [33, 34]: UGTIA1 gene polymorphism influences the UGTIA1 enzyme activity, and pharmacogenetic studies on irinotecan have revealed the close relationship between *UGT1A1* polymorphisms and adverse reactions of irinotecan and therapeutic effects in patients with advanced CRC receiving irinotecan-based chemotherapy [25, 35].

Results in our study suggested significant differences in distribution of *1 allele*1/*1, *1 allele *1/*28*1/*28, and *28 allele*28/*28 of *UGT1A1**28, and DW, SV, and DV of *UGT1A1**28/*6 gene polymorphisms were found in the Uygur ethnicity when compared to the Han ethnicity, while the allele frequency distribution of *UGT1A1**28 and genotype and allele frequency distribution of *UGT1A1**6 were not statistically significant between the two ethnic groups. Anthropological studies have shown that the Uygur population contains Caucasian origins and has a unique genetic background. East Asians have less frequent *UGT1A1**28 and more frequent *UGT1A1**6 compared to that in Caucasians [15]. Intra-ethnic differences in genetic variation of *UGT1A1* gene have been analyzed previously in three ethnic groups of Chinese populations, and heterogeneity among different ethnic populations could be a result of microevolution [22]. In our study, the frequency of UGT1A1*28 (*1/*1) in Han was slightly higher than in Ugyur (66.7 vs. 65.78 %), as previously reported, and Chinese patients showed a higher frequency of wild-type UGT1A1*28 (*1/*1) compared with Caucasian population (69.9 vs. 45.2 %) in CRC patients receiving irinotecan [36]. Similarly, the associations between *UGT1A1* *28 and *6 polymorphisms and irinotecan toxicity were determined in Thai patients with CRC. Atasilp C, et al. found that the frequencies of *1/*28 and *28/*28 in *UGT1A1**28 polymorphism were 20.5 and 2.3 % in Thai, 24. 62 and 9.6 % in Ugyur, and 33.33 % and 0 in Han, respectively, and the frequencies of G/A in *UGT1A1**6 polymorphism were 15.9 % in Thai, 29.82 % in Ugyur and 24.64 % in Han, respectively, showing a certain difference. Consistent with our results, the study of Atasilp C, et al. suggested that *UGT1A1* *28 and *6 in combination may have an increased risk of irinotecan-induced neutropenia in Thai CRC patients [37]. In addition, Sukasem et al. [38] found

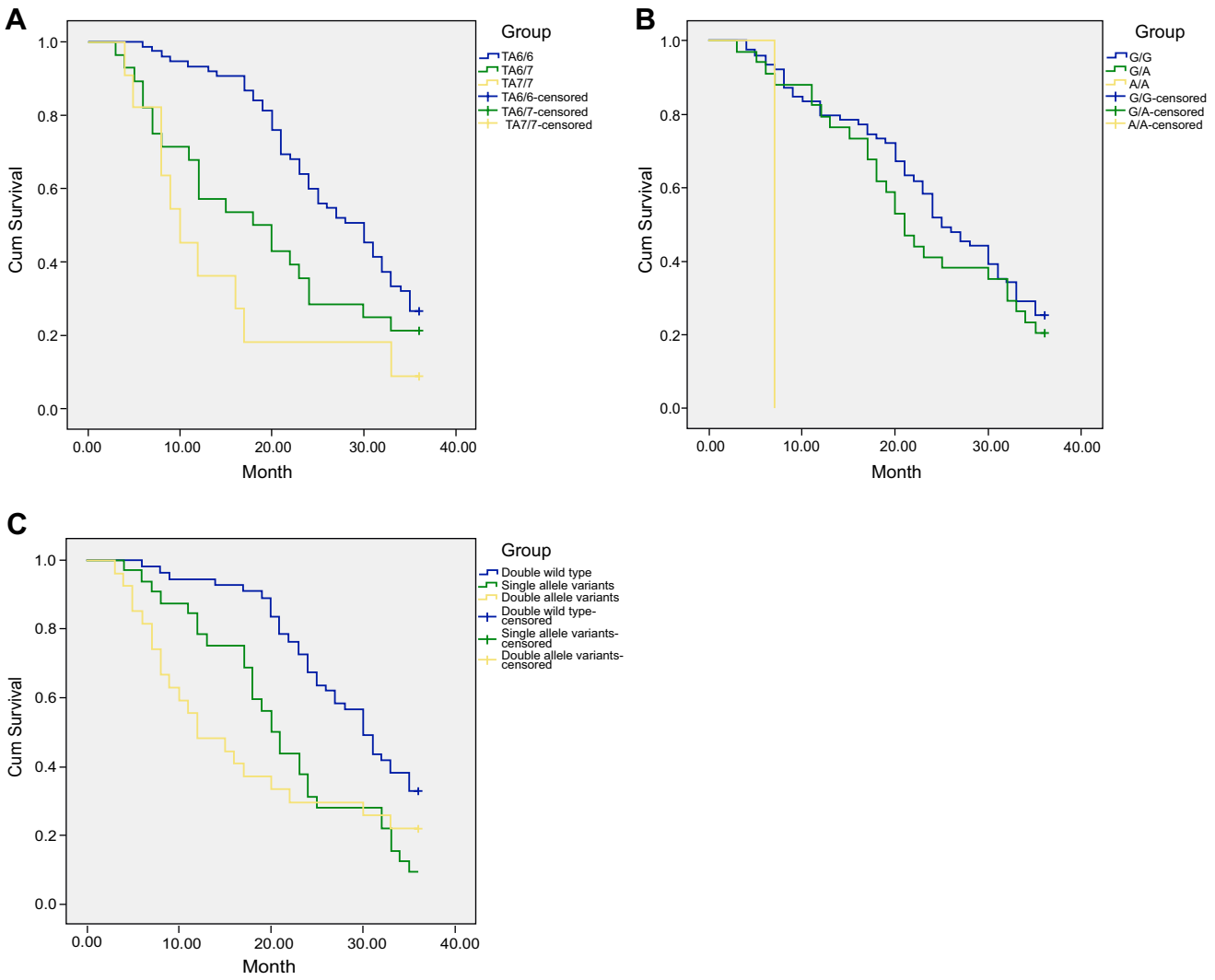

Fig. 1 Comparison of survival curves of *UGT1A1**28, *UGT1A1**6, and *UGT1A1**28/*6 among Ugyur patients. **a** Survival curve of *UGT1A1**28 in Ugyur patients; **b** survival curve of *UGT1A1**6 in Ugyur patients; **c** survival curve of *UGT1A1**28/*6 in Ugyur patients

that the allele frequencies for *UGT1A1* genetic polymorphisms were *1/*1 (54.95 %), *1/*6 (13.19 %), *1/*28 (25.27 %), *28/*6 (4.40 %), and *28/*28 (2.20 %), with no homozygous mutation *UGT1A1**6, suggesting that the UGT1A1*28 and *UGT1A1**6 alleles were similar in the Asian populations. Genotypes of *UGT1A1* *28 and *6 were analyzed by pyrosequencing technique in studies conducted by Atasilp et al. [37] and Sukasem et al. [38]. In our study, the methods for detection of *UGT1A1* were PCR amplification and the direct sequencing since PCR amplification and the direct sequencing had the characteristics of strong specificity, high sensitivity, simple operation and were time saving and easy to be standardized and automated [39].

In addition, neutropenia and diarrhea are the dose-limiting toxicities. Our findings revealed that advanced CRC patients carrying wild genotypes of *UGT1A1**28 and *UGT1A1**6 have significantly lower incidence of diarrhea in I/II stage and III/IV stage than those of mutant genotypes in irinotecan-based chemotherapy in the Uygur ethnicity and Han ethnicity. But this is without distinct difference in neutropenia incidence in I/II stage and III/IV stage, suggesting that *UGT1A1**6/*28 could be used as markers to prevent induction by irinotecan administration [13]. The polymorphism of *UGT1A1* gene is closely related to the function of the enzyme, and *UGT1A1* gene polymorphism may cause decreased or absence of UGT1A1 enzyme activity, and influencing the effects of irinotecan metabolism in human body, resulting in the accumulation of bioactive metabolite SN-38 in the body, thereby producing related adverse reactions [9, 40, 41]. Consistently with our findings, the *UGT1A1**28 and *UGT1A1**6 could be considered as predictors for severe delayed diarrhea associated

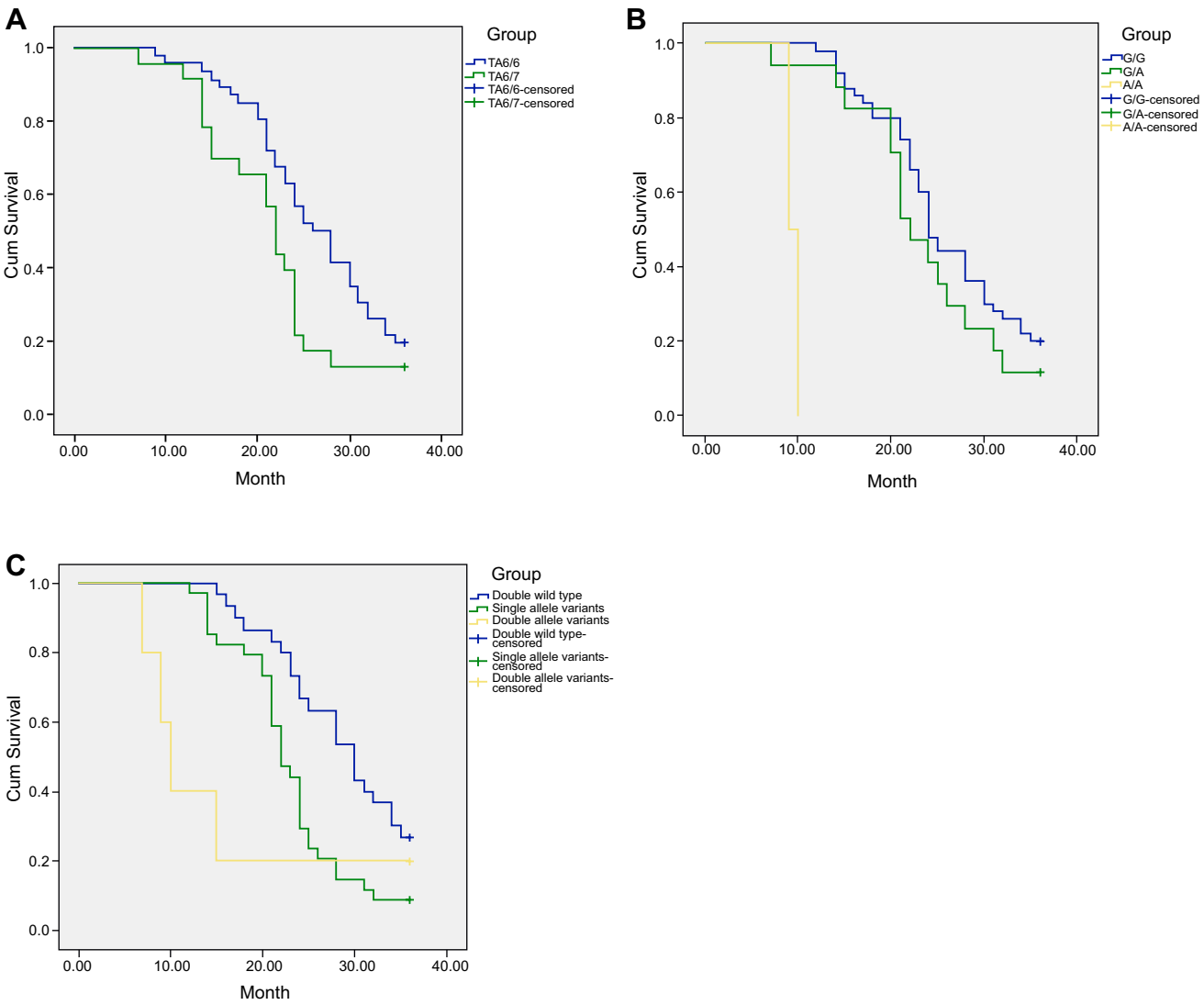

Fig. 2 Comparison of survival curves of *UGT1A1**28, *UGT1A1**6, and *UGT1A1**28/*6 among Han patients. **a** Survival curve of *UGT1A1**28 in Han patients; **b** survival curve of *UGT1A1**6 in Han patients; **c** survival curve of *UGT1A1**28/*6 in Han patients

with irinotecan, but without association with severe neutropenia in irinotecan-based regimens in CRC [42]. The homozygous variant of UGT1A1*6 served as a risk factor for severe diarrhea, and *UGT1A1**6 polymorphisms can be used as potential biomarkers for predicting irinotecan-induced severe toxicity in patients from Asia [35]. The change of TATA box of thymine and adenine (TA) repeat sequences may influence the activity of *UGT1A1* gene in the drug metabolism and its (TA) 7 repeats (*UGT1A1**28 gene polymorphism) can significantly increase the risk of severe granulocyte reduction and diarrhea in CRC patients treated with irinotecan-based chemotherapy in Caucasians [43]. Moreover, a combined test of *UGT1A1**6 and *UGT1A1**28 might be a potential biomarker of irinotecan-induced neutropenia in Asians, which still needs to be confirmed by additional research [20].

With respect to association between *UGT1A1**28/*6 gene polymorphisms and efficacy and survival of irinotecan-based chemotherapy, genotypes of *UGT1A1**28 are associated with ORR and DCR in Uygur patients. Compared with *1 allele *1/*1 wild-type genotype,*1 allele *1/*28*1/*28 mutant genotype of *UGT1A1**28 is associated with shorter OS in Uygur and Han nationality. Compared with wild-type DW, SV and DV mutant genotypes of *UGT1A1**28/*6 are associated with shorter OS in Uygur and Han nationality. Cox regression model analysis further confirmed that *UGT1A1**28, *UGT1A1**6, combined genotypes and chemotherapy line are factors

Table 7 Cox regression model to analyze the factors affecting overall survival (OS) in Uygur and Han patients

Variables	Exp (B)	Overall survival 95 %CI	*P* value
Ugyur			
Sex	0.646	0.174–2.398	0.514
Age	1.401	0.357–5.506	0.629
ECOG	1.271	0.419–3.850	0.672
Location of primary tumor	1.95	0.486–7.829	0.346
Degree of differentiation	1.094	0.469–2.551	0.836
CEA level	0.471	0.179–1.237	0.126
*UGT1A1**28	2.16	1.546–3.018	0.001
*UGT1A1**6	2.964	1.664–5.280	0.001
Combined genotypes	2.812	1.487–5.317	0.001
Chemotherapy regimen	1.205	0.323–4.495	0.781
Chemotherapy line	2.78	1.024–7.546	0.045
Han			
Sex	1.648	0.305–8.908	0.562
Age	1.289	0.410–4.054	0.664
ECOG	5.984	0.524–68.298	0.15
Location of primary tumor	1.477	0.451–4.834	0.519
Degree of differentiation	0.456	0.125–1.666	0.235
CEA level	0.563	0.131–2.419	0.44
*UGT1A1**28	1.275	0.324–5.026	0.728
*UGT1A1**6	1.296	0.334–5.114	0.787
Combined genotypes	3.802	1.594–9.071	0.003
Chemotherapy regimen	0.127	0.015–1.076	0.058
Chemotherapy line	2.145	0.722–6.379	0.17

Exp exponential, *CI* confidence interval, *ECOG* Cooperative Oncology Group, *CEA* carcinoembryonic antigen

who have a significant impact on OS. It has been proposed that *UGT1A* variants together with *UGT1A1**28 might help predicting the outcome of CRC patients treated with FOLFIRI [11]. It has been found that homozygous *UGT1A1**28 allele carriers have increased survival rates and higher tumor response rates [44]. The response rate was higher in *28 allele/*28 allele patients compared with *1 allele/*1 allele in metastatic CRC patients treated with FOLFIRI, but a nonsignificant survival advantage was observed for *28 allele/*28 allele when compared with *1 allele/*1 allele patients [45]. However, many studies reported contrary points or even slight evidence regarding the association of response rates and OS and genotypes of *UGT1A1**28 and *UGT1A1**6 in Chinese patients with advanced CRC in irinotecan-based regimens [36, 42, 46]. *UGT1A1**28 polymorphism cannot be considered as a reliable predictor of TR and PFS in CRC patients treated with IRI-based chemotherapy in a meta-analysis in Caucasians [47]. Therefore, the association between clinical response nor prognosis and *UGT1A1* gene polymorphisms needs to be further confirmed by more well-designed studies with the ultimate goal of achieving personalized irinotecan-based chemotherapy.

In summary, *UGT1A1* gene polymorphism can predict irinotecan-induced adverse reactions in advanced CRC patients in individuals from Xinjiang Uygur ethnicity and Han ethnicity. *UGT1A1* gene polymorphism is associated with clinical response and prognosis of irinotecan-based chemotherapy in persons from Uygur ethnicity, and only associated with prognosis in persons from Han nationality. However, the recruited subjects received FOLFIRI regimen including irinotecan and 5-FU. Both irinotecan and 5-FU may induce similar side effects (severe neutropenia and diarrhea). Therefore, part of the toxic side effects in our study may be possibly caused by 5-FU, and the specific effect of *UGT1A1* polymorphism in each treatment regimen should be further investigated. In addition, more fully and strongly clinical research evidence is needed to reflect the detection of the relevant genetic polymorphisms which can be used to correctly guide the clinical safety and effectiveness of the use of irinotecan and to achieve the purpose of predicting serious adverse reactions.

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Compliance with ethical standards

Conflict of interest The authors have declared that no conflict of interest.

References

1. Jemal A, Bray F, Center MM et al (2011) Global cancer statistics. CA Cancer J Clin 61:69–90
2. Liu S, Zheng R, Zhang M et al (2015) Incidence and mortality of colorectal cancer in China, 2011. Chin J Cancer Res 27:22–28
3. Morand Bounaix, du Puch C, Nouaille M, Giraud S et al (2016) Chemotherapy outcome predictive effectiveness by the Oncogramme: pilot trial on stage-IV colorectal cancer. J Transl Med 14:10
4. Gustavsson B, Carlsson G, Machover D et al (2015) A review of the evolution of systemic chemotherapy in the management of colorectal cancer. Clin Colorectal Cancer 14:1–10
5. Hammond WA, Swaika A, Mody K (2016) Pharmacologic resistance in colorectal cancer: a review. Ther Adv Med Oncol 8:57–84
6. (2010) Chemotherapy of metastatic colorectal cancer. Prescrire Int 19(109):219–224
7. Kim MJ, Han SW, Lee DW et al (2016) Splenomegaly and its associations with genetic polymorphisms and treatment outcome in colorectal cancer patients treated with adjuvant FOLFOX. Cancer Res Treat. doi:10.4143/crt.2015.296
8. Yu M, Tong X, Qi B et al (2014) Berberine enhances chemosensitivity to irinotecan in colon cancer via inhibition of NFkappaB. Mol Med Rep 9:249–254

9. Fujita K, Sparreboom A (2010) Pharmacogenetics of irinotecan disposition and toxicity: a review. Curr Clin Pharmacol 5:209–217
10. O'Dwyer PJ, Catalano RB (2006) Uridine diphosphate glucuronosyltransferase (UGT) 1A1 and irinotecan: practical pharmacogenomics arrives in cancer therapy. J Clin Oncol 24:4534–4538
11. Cecchin E, Innocenti F, D'Andrea M et al (2009) Predictive role of the UGT1A1, UGT1A7, and UGT1A9 genetic variants and their haplotypes on the outcome of metastatic colorectal cancer patients treated with fluorouracil, leucovorin, and irinotecan. J Clin Oncol 27:2457–2465
12. Fujita K, Kubota Y, Ishida H, Sasaki Y (2015) Irinotecan, a key chemotherapeutic drug for metastatic colorectal cancer. World J Gastroenterol 21:12234–12248
13. Inoue K, Sonobe M, Kawamura Y et al (2013) Polymorphisms of the UDP-glucuronosyl transferase 1A genes are associated with adverse events in cancer patients receiving irinotecan-based chemotherapy. Tohoku J Exp Med 229:107–114
14. Tsunedomi R, Hazama S, Fujita Y et al (2014) A novel system for predicting the toxicity of irinotecan based on statistical pattern recognition with UGT1A genotypes. Int J Oncol 45:1381–1390
15. Fukui T, Mitsufuji H, Kubota M et al (2011) Prevalence of topoisomerase I genetic mutations and UGT1A1 polymorphisms associated with irinotecan in individuals of Asian descent. Oncol Lett 2:923–928
16. Innocenti F, Grimsley C, Das S et al (2002) Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. Pharmacogenetics 12:725–733
17. Kaniwa N, Kurose K, Jinno H et al (2005) Racial variability in haplotype frequencies of UGT1A1 and glucuronidation activity of a novel single nucleotide polymorphism 686C > T (P229L) found in an African-American. Drug Metab Dispos 33:458–465
18. Marques SC, Ikediobi ON (2010) The clinical application of UGT1A1 pharmacogenetic testing: gene-environment interactions. Hum Genomics 4:238–249
19. Liu X, Cheng D, Kuang Q, Liu G, Xu W (2014) Association of UGT1A1*28 polymorphisms with irinotecan-induced toxicities in colorectal cancer: a meta-analysis in Caucasians. Pharmacogenomics J 14:120–129
20. Chen YJ, Hu F, Li CY et al (2014) The association of UGT1A1*6 and UGT1A1*28 with irinotecan-induced neutropenia in Asians: a meta-analysis. Biomarkers 19:56–62
21. Premawardhena A, Fisher CA, Liu YT et al (2003) The global distribution of length polymorphisms of the promoters of the glucuronosyltransferase 1 gene (UGT1A1): hematologic and evolutionary implications. Blood Cells Mol Dis 31:98–101
22. Zhang A, Xing Q, Qin S et al (2007) Intra-ethnic differences in genetic variants of the UGT-glucuronosyltransferase 1A1 gene in Chinese populations. Pharmacogenomics J 7:333–338
23. Fujita K, Ando Y, Nagashima F et al (2007) Genetic linkage of UGT1A7 and UGT1A9 polymorphisms to UGT1A1*6 is associated with reduced activity for SN-38 in Japanese patients with cancer. Cancer Chemother Pharmacol 60:515–522
24. Hazama S, Mishima H, Tsunedomi R et al (2013) UGT1A1*6, 1A7*3, and 1A9*22 genotypes predict severe neutropenia in FOLFIRI-treated metastatic colorectal cancer in two prospective studies in Japan. Cancer Sci 104:1662–1669
25. Matsuoka A, Ando Y (2015) UGT1A1 genotyping for proper use of irinotecan. Rinsho Byori 63:876–882
26. Cleeland CS, Gonin R, Baez L, Loehrer P, Pandya KJ (1997) Pain and treatment of pain in minority patients with cancer. The Eastern Cooperative Oncology Group Minority Outpatient Pain Study. Ann Intern Med 127:813–816
27. Kaba H, Fukuda H, Yamamoto S, Ohashi Y (2004) Reliability at the National Cancer Institute-Common Toxicity Criteria version 2.0. Gan To Kagaku Ryoho 31:1187–1192
28. Nishino M, Jagannathan JP, Ramaiya NH, Van den Abbeele AD (2010) Revised RECIST guideline version 1.1: what oncologists want to know and what radiologists need to know. AJR Am J Roentgenol 195:281–289
29. Xiao XG, Xia S, Zou M et al (2015) The relationship between UGT1A1 gene polymorphism and irinotecan effect on extensive-stage small-cell lung cancer. Onco Targets Ther 8:3575–3583
30. Zhou CF, Ma T, Su Y et al (2013) UGT1A1 gene polymorphisms and the toxicities of FOLFIRI in Chinese Han patients with gastrointestinal cancer. Anticancer Agents Med Chem 13:235–241
31. Chantry AS, Quaranta S, Ciccolini J, Lacarelle B (2014) Clinical application, limits and perspectives of pharmacogenetic and pharmacokinetic analysis of anticancer drugs. Ann Biol Clin (Paris) 72:527–542
32. Chibaudel B, Maindrault-Goebel F, Bachet JB et al (2016) PEPCOL: a GERCOR randomized phase II study of nanoliposomal irinotecan PEP02 (MM-398) or irinotecan with leucovorin/5-fluorouracil as second-line therapy in metastatic colorectal cancer. Cancer Med 5:676–683
33. Ando Y, Hasegawa Y (2005) Clinical pharmacogenetics of irinotecan (CPT-11). Drug Metab Rev 37:565–574
34. Soeda H, Shimodaira H, Gamoh M et al (2014) Phase II trial of cetuximab plus irinotecan for oxaliplatin- and irinotecan-based chemotherapy-refractory patients with advanced and/or metastatic colorectal cancer: evaluation of efficacy and safety based on KRAS mutation status (T-CORE0801). Oncology 87:7–20
35. Cheng L, Li M, Hu J et al (2014) UGT1A1*6 polymorphisms are correlated with irinotecan-induced toxicity: a system review and meta-analysis in Asians. Cancer Chemother Pharmacol 73:551–560
36. Wang Y, Shen L, Xu N et al (2012) UGT1A1 predicts outcome in colorectal cancer treated with irinotecan and fluorouracil. World J Gastroenterol 18:6635–6644
37. Atasilp C, Chansriwong P, Sirachainan E et al (2016) Correlation of UGT1A1(*)28 and (*)6 polymorphisms with irinotecan-induced neutropenia in Thai colorectal cancer patients. Drug Metab Pharmacokinet 31:90–94
38. Sukasem C, Atasilp C, Chansriwong P et al (2016) Development of pyrosequencing method for detection of UGT1A1 polymorphisms in thai colorectal cancers. J Clin Lab Anal 30:84–89
39. Green PM, Giannelli F (1994) Direct sequencing of PCR-amplified DNA. Mol Biotechnol 1:117–124
40. Biason P, Masier S, Toffoli G (2008) UGT1A1*28 and other UGT1A polymorphisms as determinants of irinotecan toxicity. J Chemother 20:158–165
41. Cortejoso L, Garcia MI, Garcia-Alfonso P et al (2013) Differential toxicity biomarkers for irinotecan- and oxaliplatin-containing chemotherapy in colorectal cancer. Cancer Chemother Pharmacol 71:1463–1472
42. Li M, Wang Z, Guo J et al (2014) Clinical significance of UGT1A1 gene polymorphisms on irinotecan-based regimens as the treatment in metastatic colorectal cancer. Onco Targets Ther 7:1653–1661
43. Boyer JC, Etienne-Grimaldi MC, Thomas F et al (2014) Interest of UGT1A1 genotyping within digestive cancers treatment by irinotecan. Bull Cancer 101:533–553
44. Palomaki GE, Bradley LA, Douglas MP, Kolor K, Dotson WD (2009) Can UGT1A1 genotyping reduce morbidity and mortality in patients with metastatic colorectal cancer treated with irinotecan? An evidence-based review. Genet Med 11:21–34
45. Toffoli G, Cecchin E, Corona G et al (2006) The role of UGT1A1*28 polymorphism in the pharmacodynamics and

pharmacokinetics of irinotecan in patients with metastatic colorectal cancer. J Clin Oncol 24:3061–3068

46. Schulz C, Heinemann V, Schalhorn A et al (2009) UGT1A1 gene polymorphism: impact on toxicity and efficacy of irinotecan-based regimens in metastatic colorectal cancer. World J Gastroenterol 15:5058–5066

47. Liu X, Cheng D, Kuang Q, Liu G, Xu W (2013) Association between UGT1A1*28 polymorphisms and clinical outcomes of irinotecan-based chemotherapies in colorectal cancer: a meta-analysis in Caucasians. PLoS ONE 8:e58489