

# UGT1A1\*6, UGT1A7\*3 and UGT1A9\*1b polymorphisms are predictive markers for severe toxicity in patients with metastatic gastrointestinal cancer treated with irinotecan-based regimens

CHENGXU CUI<sup>1\*</sup>, CHANG SHU<sup>2\*</sup>, DANDAN CAO<sup>2\*</sup>, YI YANG<sup>2</sup>, JUNBAO LIU<sup>1</sup>, SHUPING SHI<sup>1</sup>, ZHUJUN SHAO<sup>1</sup>, NAN WANG<sup>1</sup>, TING YANG<sup>1</sup>, HAO LIANG<sup>2</sup>, SHANSHAN ZOU<sup>2</sup> and SONGNIAN HU<sup>2</sup>

<sup>1</sup>Department of Medical Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences, Beijing 100021;

<sup>2</sup>CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, Beijing 100101, P.R. China

Received March 30, 2015; Accepted August 3, 2016

DOI: 10.3892/ol.2016.5130

**Abstract.** Irinotecan-induced severe neutropenia and diarrhea, which remain unpredictable, has restrained the dose and clinical efficiency of irinotecan administration. In the present study, a total of 70 irinotecan-treated patients with histologically confirmed metastatic gastrointestinal cancer were enrolled. Despite genotyping well-reported alleles, direct sequencing was specifically adopted to avoid ethnic heterogeneity and to identify novel variations. The promoter (-1000 bp) and exon 1 regions of UDP glucuronosyltransferase family 1 member A complex locus (UGT1A1) gene family members UGT1A1, UGT1A7 and UGT1A9 were sequenced, and comprehensive analysis of their genetic polymorphisms was performed to determine the association between inherited genetic variations and irinotecan-induced toxicity. A total of 23 different genetic variants were detected in the present study, including 2 novel polymorphisms. The results of the present study revealed that UGT1A1\*6 and UGT1A7\*3 are risk factors for irinotecan-induced severe neutropenia, and UGT1A9\*1b

is associated with severe diarrhea. These results may provide biomarkers for the selection of the optimal chemotherapy for Chinese patients with metastatic gastrointestinal cancer.

## Introduction

Irinotecan, also termed Camptosar and CPT-11, is a type of semi-artificial camptothecin compound that is one of the effective cytotoxic antineoplastic agents used to treat colorectal cancer (1). Irinotecan can be activated to SN-38 subsequent to hydrolysis of carboxylesterase *in vivo*, and derived into an inhibitor of topoisomerase I, which can synchronously be inactivated into SN-38G by UDP glucuronosyltransferase family 1 member A complex locus (UGT1A) (2). Thus, irinotecan interrupts the replication and transcription of DNA by preventing the relegation of single-stranded DNA and causes the death of tumor cells following a series of biotransformations. Irinotecan received authorization from the US Food and Drug Administration (FDA) in 1998, and has been approved as the primary therapy of metastatic adult colorectal cancer, combined with 5-fluorouracil (5-FU) and leucovorin (LV) (3). However, severe neutropenia and diarrhea, the primary toxicities of irinotecan, have restrained the dose and clinical efficiency of irinotecan (4). It has been reported that the curative effect and negative toxicity are each dependent on ethnicity (5-7).

The UGT1A gene family has 13 members in *Homo sapiens*, and is located on Chr2 (2q37). Each member is composed of a unique exon 1, followed by four common exons (exons 2-5). Therefore, each functional protein of the UGT1A family has a specific N-terminus, which is determined by the first exon sequence, to recognize the corresponding substrate. In addition, the last four mutual exons provide the identical C termini, which consists of 245 amino acids and guarantee the combination with uridine diphosphate glucuronic acid (8). UGT1A1, UGT1A7 and UGT1A9 have been shown to be the major effective factors during the inactivation of SN-38 (9,10), and genetic polymorphism in the UGT1A family has also been

*Correspondence to:* Dr Chengxu Cui, Department of Medical Oncology, Cancer Institute and Hospital, Chinese Academy of Medical Sciences, 17 South Panjiayuan Lane, Beijing 100021, P.R. China

E-mail: cuichengxu@cscsco.org.cn

Dr Songnian Hu, CAS Key Laboratory of Genome Sciences and Information, Beijing Institute of Genomics, Chinese Academy of Sciences, Building 104, 1 Beichen West Road, Beijing 100101, P.R. China

E-mail: husn@big.ac.cn

\*Contributed equally

**Key words:** irinotecan, UGT1A, polymorphisms, neutropenia, diarrhea

shown to affect the variability of irinotecan toxicity during clinical application (11,12).

Considering the complex mechanism involved in the association between the UGT1A family and toxicity of irinotecan, the FDA has instructed the manufacturer of irinotecan to revise the label and add the caution of relative toxicity and dose titration in patients with the UGT1A1\*28 polymorphism, as well as the advice for patients to undergo allelic detection (13). For patients from the Asian population, UGT1A1\*6 is considered to be another potential risk factor that is responsible for the toxicity among the Asian population in addition to the UGT1A1\*28 allele (11,12,14). The Japanese Pharmaceuticals and Medical Devices Agency package insert for irinotecan states that individuals who are homozygous for the UGT1A1\*6 or UGT1A1\*28 alleles, or heterozygous with the UGT1A1\*6/\*28 genotype, are at an increased risk of severe adverse events, particularly neutropenia (15). The impact of UGT1A7 polymorphism on irinotecan-induced toxicity has also been well-studied (16-18). In addition, UGT1A9\*22 has been reported to be associated with the toxic potency of irinotecan (18-20). Considering the ethnicity-specific distribution of genetic polymorphisms, the predictive markers for severe toxicity in Chinese patients treated with irinotecan-based regimens have not been affirmatively reported by studies yet.

In the present study, direct sequencing was adopted to avoid ethnic heterogeneity and to identify novel variations. The first exon regions of UGT1A1, UGT1A7 and UGT1A9 were sequenced, and comprehensive analysis of genetic polymorphisms was performed to determine the association between inherited genetic variations and irinotecan-induced toxicity. The major aim of the current study was to identify toxicity predictable markers of Chinese metastatic gastrointestinal cancer patients treated with irinotecan-based regimens.

## Patients and methods

**Patients.** In total, 70 patients with histologically-confirmed metastatic gastrointestinal cancer were enrolled from the Cancer Institute and Hospital, Chinese Academy of Medical Sciences (Beijing, China) between January 2012 and December 2014. All patients were treated with the irinotecan-based folinic acid, fluorouracil (5-FU) and irinotecan (FOLFIRI) or capecitabine and irinotecan (XELIRI) regimens. The study protocol was approved by the Ethics Committee of Beijing Chao-Yang Sanhuan Cancer Hospital (Beijing, China), which is affiliated with the Cancer Institute and Hospital of the Chinese Academy of Medical Sciences. Written informed consent was obtained from all patients prior to entering the study.

**Eligibility criteria.** All patients were diagnosed with histologically-confirmed gastrointestinal cancer and were undergoing their first irinotecan-based chemotherapy. The patients were aged >18 years and had an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0-2 and a life expectancy of  $\geq 3$  months.

**Chemotherapy.** The patients were treated with one of the following FOLFIRI-based regimens until progressive disease (PD), unacceptable toxicity, patient refusal or a medical

decision to discontinue treatment. Patients treated with the FOLFIRI regimen received 180 mg/m<sup>2</sup> irinotecan intravenously (IV), 400 mg/m<sup>2</sup> LV IV and 400 mg/m<sup>2</sup> 5-FU bolus, followed by 2,400 mg/m<sup>2</sup> 5-FU IV, all administered on day 1, every 2 weeks. Patients treated with the XELIRI regimen received 120 mg/m<sup>2</sup> irinotecan IV on days 1 and 8, and 800 mg/m<sup>2</sup> oral capecitabine twice per day on days 1-14 every 3 weeks.

**Genotyping and genetic analysis.** Peripheral venous blood samples were obtained from 70 patients with metastatic gastrointestinal cancer, who were treated with irinotecan-based regimens (37 with FOLFIRI and 33 with XELIRI) for isolation of genomic DNA. Genomic DNA was isolated using the QIAamp DNA blood mini kit (Qiagen, Inc., Valencia, CA, USA). The promoter (-1000 bp) and exon 1 regions of UGT1A1, UGT1A7 and UGT1A9 were resequenced to screen for single nucleotide polymorphisms (SNPs), using the DYEnamic ET terminator cycle sequencing kit (GE Healthcare, Chalfont St. Giles, UK) on the ABI Prism 3730xl DNA analyzer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). Polymerase chain reaction (PCR) was performed in a final volume of 15  $\mu$ l, containing 30 ng genomic DNA, 1 $\times$  PCR Takara buffer (Mg<sup>2+</sup> plus; Takara Biotechnology Co., Ltd., Dalian, China), 2.5 pmol of each primer, 25 pmol deoxynucleotide triphosphates and 1 U of Taq DNA polymerase (Takara Biotechnology Co., Ltd.). Following pre-denaturation at 93°C for 3 min, amplification was performed under the following conditions for 32 cycles: Denaturation at 95°C for 30 sec; annealing at 58°C for 40 sec; and extension at 72°C for 2 min. The primer sequences used are shown in Table I (21).

Base calling, quality assessment and polymorphism determination from DNA sequencing were analyzed using Phred, Phrap, Consed (<http://www.phrap.org/phredphrapconsed.html>) and PolyPhred (<http://droog.gs.washington.edu/polyphred/>). Haploview 4.2 (Broad Institute of MIT and Harvard, Cambridge, MA, USA (22)) was used to estimate allele frequencies, test the Hardy-Weinberg equilibrium, measure pairwise linkage disequilibrium (LD), establish haplotypes, estimate haplotype frequency, and analyze the association between the haplotypes and toxicity. LD blocks were defined by the 'solid spine of LD' algorithm, and LD structure was exhibited using GOLD heatmap color scheme (23,24). The allele designations were defined according to the UGT Allele Nomenclature Committee (25).

**Statistical analysis.** Patients underwent baseline evaluations, including physical examination, complete medical history, ECOG PS, complete blood count, and hepatic and renal function tests. Toxicity evaluations were performed according to National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 criteria. Associations between the clinical features and toxicity were analyzed by two-tailed Fisher's exact test or Student's t-test. In addition, associations between the alleles or genotypes and toxicity were analyzed by two-tailed Fisher's exact test.  $P < 0.05$  was considered to indicate a statistically significant difference, as calculated by PLINK v1.07 (<http://pngu.mgh.harvard.edu/~purcell/plink>) (26). Due to the exploratory nature of the present study, no adjustments were made for multiple comparisons.

Table I. Primer sequences.

Genes	Primer ID	Primer sequences
UGT1A1	U1_E1AF	TCGTCCTTCTTCCTCTCTGG
	U1_E1AR	GCAGTGCATGCAAGAAGAAT
	U1_E1BF	TGTCTGGCTGTTCCCACTTA
	U1_E1BR	CCAGAAGATGATGCCAAAGA
	U1_PF	GGTCATTCTTACCCCAGCA
	U1_PR	AAAGCTGTCAGTCCACAAAGG
UGT1A7	U7_E1AF	AAACTCATATTGCAGCACAGG
	U7_E1AR	AAGTCAAAAATACCATTGGATGAA
	U7_E1BF	GGAAGATCACTGAATTGCACAG
	U7_E1BR	TTCCTCTGGGGGTAGTGTAGAA
	U7_PF	TCTTTCCGTGCAACATGAGA
	U7_PR	CACATTCAGTCCCAATGATTTA
UGT1A9	U9_E1AF	CCAAGGCAAAGACCATAAGCTA
	U9_E1AR	CAAACCTCCTGCAATTTGAAAAA
	U9_E1BF	CATATACCCTGGAGGATCTGGA
	U9_E1BR	CTGACGAGTACACGCATTGG
	U9_PF	CCTCTGACCTCAAGGAGTGC
	U9_PR	CAATGATTTACCCAAAAGAACAAG

F, forward; R, reverse; UGT1A, UDP glucuronosyltransferase family 1 member A complex locus.

## Results

In total, 70 patients with histologically-confirmed metastatic gastrointestinal cancer, consisting of 37 patients with metastatic colorectal cancer and 33 patients with metastatic gastric cancer, were enrolled in the present study. The rates of grade 3-4 toxicity were as follows: Neutropenia, 18.57% (n=13); and diarrhea, 10% (n=7). These results are in accordance with a previous study of Asian patients (6). The clinical features of patients and the incidence of severe toxicity of the irinotecan-based regimens FOLFIRI and XELIRI are summarized in Table II.

A total of 23 different genetic variants were detected in the present study, all of the variants were consistent with the Hardy-Weinberg equilibrium ( $P>0.05$ ). Genetic variations in the UGT1A1, UGT1A7 and UGT1A9 genes were compared with the genomic reference sequence AF039138.1. The amino acids were located in the reference sequences NP\_000454.1 (UGT1A1), NP\_061950.2 (UGT1A7) and NP\_066307.1 (UGT1A9). Of these variants, 2 novel polymorphisms were identified: M06 and M09, which are located in the promoter regions of UGT1A9 and UGT1A7, respectively. UGT1A1\*6, which is found only in Asian populations, had the highest frequency [minor allele frequency (MAF), 22.5%] of the SNPs in UGT1A1 in the present study. The frequency of the UGT1A1\*28 SNP (MAF, 9.3%) was comparable to the frequency previously found in the Asian population (MAF, 7.0%), and was much lower compared with the frequency in Caucasian patients (MAF, 31.6%), as previously reported (11,20). Other known UGT1A alleles, UGT1A1\*80, UGT1A1\*81, UGT1A7\*2, UGT1A7\*3 and UGT1A9\*1b (UGT1A9\*22), were found concurrently. Table III lists the summary of identified polymorphisms in UGT1A1, UGT1A7 and UGT1A9.

Table II. Clinical features of 70 patients with histologically-confirmed metastatic gastrointestinal cancer.

Features	Value, n (%)
Age, years	
Median	54
Range	25-77
Gender	
Male	48 (68.57)
Female	22 (31.43)
Tumor location	
Colon	37 (52.86)
Stomach	33 (47.14)
ECOG PS	
0	19 (27.14)
1	46 (65.71)
2	5 (7.14)
TNM stage IV	70 (100.00)
Regimen	
FOLFIRI	37 (52.86)
XELIRI	33 (47.14)
Neutropenia grade 3-4	13 (18.57)
Diarrhea grade 3-4	7 (10.00)

ECOG PS, Eastern Cooperative Oncology Group performance status; TNM, tumor-node-metastasis classification of malignant tumours; FOLFIRI, folinic acid, fluorouracil and irinotecan; XELIRI, capecitabine and irinotecan.

Table III. Summary of identified polymorphisms in UGT1A1, UGT1A7 and UGT1A9.

ID	Location	db SNP ID	Change	Allele	Function	MAF	H-Wpval
M01	UGT1A9 promoter	rs3806598	A>C	-	-	0.243	0.747
M02	UGT1A9 promoter	rs45440791	T>C	-	-	0.007	1.000
M03	UGT1A9 promoter	rs2741045	C>T	-	-	0.007	1.000
M04	UGT1A9 promoter	rs2741046	T>A	-	-	0.007	1.000
M05	UGT1A9 promoter	rs3832043	T9>T10	*1b	-	0.616	0.818
M06	UGT1A9 promoter	-	C>T	-	-	0.007	1.000
M07	UGT1A9 exon 1	rs151216459	G>T	-	Synonymous	0.014	1.000
M08	UGT1A7 promoter	rs4530361	A>G	-	-	0.250	0.633
M09	UGT1A7 promoter	-	T>C	-	-	0.007	1.000
M10	UGT1A7 promoter	rs28946877	C>T	-	-	0.143	1.000
M11	UGT1A7 promoter	rs7586110	T>G	-	-	0.246	0.296
M12	UGT1A7 exon 1	rs7577677	C>A	-	Synonymous	0.243	0.314
M13	UGT1A7 exon 1	rs17868323	T>G	*2&*3	N129K	0.407	1.000
M14	UGT1A7 exon 1	rs17863778	C>A	*2&*3	R131K	0.407	1.000
M15	UGT1A7 exon 1	rs17868324	G>A	*2&*3	R131Q	0.407	1.000
M16	UGT1A7 exon 1	rs139969318	A>G	-	Synonymous	0.007	1.000
M17	UGT1A7 exon 1	rs11692021	T>C	*3	W208R	0.25	0.633
M18	UGT1A7 exon 1	rs45462096	C>T	-	Synonymous	0.014	1.000
M19	UGT1A7 exon 1	rs17864686	G>A	-	Synonymous	0.157	1.000
M20	UGT1A1 promoter	rs887829	C>T	*80	-	0.107	1.000
M21	UGT1A1 promoter	rs873478	G>C	*81	-	0.036	1.000
M22	UGT1A1 promoter	rs3064744	TA6>TA7	*28	-	0.093	1.000
M23	UGT1A1 exon 1	rs4148323	G>A	*6	G71R	0.225	0.550

MAF, minor allele frequency; UGT1A, UDP glucuronosyltransferase family 1 member A complex locus; H-Wpval, Hardy-Weinberg P-value.

In total, 6 SNPs exhibited an association with grade 3-4 neutropenia, including M01 ( $P=0.009$ ), M08 ( $P=0.011$ ), M10 ( $P=0.025$ ), M17 ( $P=0.011$ ), M19 ( $P=0.014$ ) and M23 ( $P=0.016$ ) (Table IV). In addition, a significant association was observed between the UGT1A9\*1b polymorphism (M05) and severe diarrhea ( $P=0.045$ ; Table IV). Of these 7 SNPs, two are functional mutations, consisting of UGT1A7\*3 ( $P=0.011$ ; OR, 3.391) and UGT1A1\*6 ( $P=0.016$ ; OR, 3.373). The hotspot allele UGT1A1\*28, which has been previously reported (20), was not significantly associated with grade 3-4 neutropenia or severe diarrhea ( $P=1.000$  and 0.620, respectively; Table IV). The present study established haplotypes of those 7 SNPs and calculated the association with irinotecan-induced toxicity (Table V). Of the 7 haplotypes that were assessed, haplotypes H03, H04, H05 and H06 were observed to be associated with a higher risk of grade 3-4 neutropenia ( $P=0.037$ ,  $P=0.043$ ,  $P=0.097$  and  $P=0.004$ , respectively). In addition, H04 was found to be associated with grade 3-4 diarrhea ( $P=0.001$ ).

Pairwise LD analysis was performed with the detected variations having a MAF >10%. LD blocks were defined by the 'solid spine of LD' algorithm, and LD structure was exhibited by the GOLD heatmap color scheme. As shown in Fig. 1, a linkage block was observed across the sequenced region in the present metastatic gastrointestinal cancer patients. The variants detected in the UGT1A7 and UGT1A9 regions were closely associated (block 1). Out of the 6 SNPs that exhibited an association with grade 3-4 neutropenia, 5 SNPs were located in the UGT1A7-UGT1A9

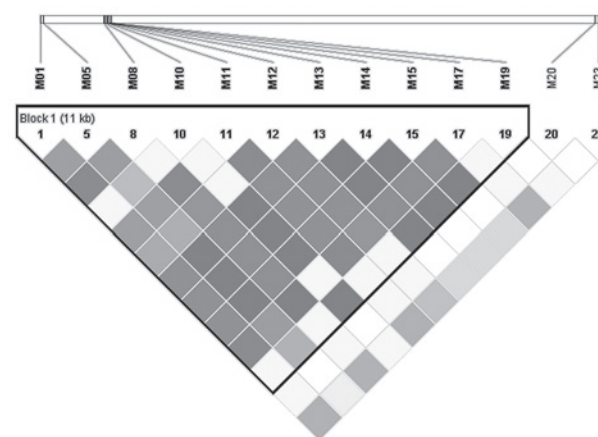


Figure 1. Pairwise linkage disequilibrium associations between UGT1A polymorphisms in metastatic gastrointestinal cancer patients. A denser color indicates greater linkage. An area corresponding to a haplotype block is exhibited in frame. UGT1A, UDP glucuronosyltransferase family 1 member A complex locus.

block (block 1). In addition, M17 (UGT1A7\*3) was the only functional variant among the 5 SNPs. It was hypothesized that the significant association with grade 3-4 neutropenia of the other 4 SNPs was attributed to UGT1A7\*3.

In total, 2 functional mutations exhibited a significant association with grade 3-4 neutropenia, including UGT1A7\*3



Table IV. Association between UGT1A polymorphisms and severe toxicity.

SNP ID	db SNP ID	Neutropenia grade 3-4			Diarrhea grade 3-4		
		P-value	OR	95% CI	P-value	OR	95% CI
M01	rs3806598	0.009	3.584	1.457-8.821	0.745	1.280	0.374-4.379
M02	rs45440791	1.000	0.000	NA	1.000	0.000	NA
M03	rs2741045	1.000	0.000	NA	0.100	NA	NA
M04	rs2741046	1.000	0.000	NA	0.100	NA	NA
M05	rs3832043	1.000	1.003	0.417-2.411	0.045	3.273	1.033-10.37
M06	-	0.186	NA	NA	1.000	0.000	NA
M07	rs151216459	1.000	0.000	NA	1.000	0.000	NA
M08	rs4530361	0.011	3.391	1.384-8.312	0.339	1.778	0.553-5.714
M09	-	1.000	0.000	NA	0.100	NA	NA
M10	rs28946877	0.025	0.000	NA	1.000	1.000	0.206-4.845
M11	rs7586110	0.081	2.292	0.923-5.693	1.000	0.818	0.214-3.124
M12	rs7577677	0.077	2.344	0.944-5.819	1.000	0.836	0.219-3.19
M13	rs17868323	0.659	1.314	0.558-3.099	0.084	2.925	0.925-9.245
M14	rs66534818	0.659	1.314	0.558-3.099	0.084	2.925	0.925-9.245
M15	rs17868324	0.659	1.314	0.558-3.099	0.084	2.925	0.925-9.245
M16	rs139969318	0.186	NA	NA	1.000	0.000	NA
M17	rs11692021	0.011	3.391	1.384-8.312	0.339	1.778	0.553-5.714
M18	rs45462096	1.000	0.000	NA	1.000	0.000	NA
M19	rs17864686	0.014	0.000	NA	0.236	2.400	0.679-8.481
M20	rs887829	0.737	0.647	0.137-3.062	0.647	1.449	0.292-7.199
M21	rs873478	0.584	0.000	NA	0.414	2.346	0.244-22.59
M22	rs3064744	1.000	0.780	0.162-3.754	0.620	1.742	0.345-8.802
M23	rs4148323	0.016	3.373	1.350-8.431	0.191	0.241	0.03-1.92

SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; UGT1A, UDP glucuronosyltransferase family 1 member A complex locus.

Table V. Correlation between UGT1A haplotypes and severe toxicity.

Haplotype ID	UGT1A haplotype							Frequency	P-value	
	M01	M05	M08	M10	M17	M19	M23		Neutropenia grade 3-4	Diarrhea grade 3-4
H01	A	10T	A	C	T	G	G	0.555	0.269	0.114
H02	C	9T	G	C	C	G	A	0.176	0.183	0.274
H03	A	9T	A	T	T	A	G	0.120	0.037	0.783
H04	C	9T	G	C	C	G	G	0.044	0.043	0.001
H05	A	10T	A	C	T	G	A	0.030	0.097	0.502
H06	C	10T	G	C	C	G	A	0.015	0.004	0.629
H07	A	9T	G	C	C	G	G	0.014	0.494	0.059

UGT1A, UDP glucuronosyltransferase family 1 member A complex locus.

(P=0.011; OR, 3.391; 95% CI, 1.384-8.312) and UGT1A1\*6 (P=0.016; OR, 3.373; 95% CI, 1.350-8.431). The risk of severe neutropenia associated with UGT1A7\*3 and UGT1A1\*6 genotypes (Table VI), and the combined effects of those two alleles, was analyzed (Table VII). In the present study, patients with one UGT1A7\*3 allele had an OR of 4.444 (95% CI, 1.060-18.627) for the risk of severe neutropenia.

Compared with patients heterozygous for UGT1A7\*3, patients homozygous for UGT1A7\*3 demonstrated a 5-fold increase in risk, with an OR of 23.333 (95% CI, 1.609-338.419). The OR for patients homozygous for UGT1A1\*6 (OR, 12.333; 95% CI, 0.607-250.508) was two times higher than the OR of patients heterozygous for UGT1A1\*6 (OR, 6.167; 95% CI, 1.486-25.586). Combined effects analysis of those 2 alleles

Table VI. Risk of severe toxicity associated with UGT1A7\*3, UGT1A1\*6 and UGT1A9\*1b.

SNP ID	Allele	Genotypes <sup>a</sup>	Severe toxicity, n	Overall, n	P-value	OR	95% CI
M17	UGT1A7*3	0	3 <sup>b</sup>	38			
		1	8 <sup>b</sup>	29	0.046	4.444	1.060-18.627
		2	2 <sup>b</sup>	3	0.035	23.333	1.609-338.419
M23	UGT1A1*6	0	3 <sup>b</sup>	40			
		1	9 <sup>b</sup>	27	0.010	6.167	1.486-25.586
		2	1 <sup>b</sup>	2	0.184	12.333	0.607-250.508
M05	UGT1A9*1b	0	1 <sup>c</sup>	27			
		1	3 <sup>c</sup>	31	0.617	2.786	0.272-28.496
		2	3 <sup>c</sup>	11	0.065	9.750	0.886-107.249

<sup>a</sup>Number of alleles carried by the patients. <sup>b</sup>Number of patients developing neutropenia among all the patients in the same genotype group.

<sup>c</sup>Number of patients developing diarrhea among all the patients in the same genotype group. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; UGT1A, UDP glucuronosyltransferase family 1 member A complex locus.

Table VII. Risk of severe neutropenia associated with combined effects of UGT1A7\*3 and UGT1A1\*6.

SNP ID	Allele	Genotypes	Severe neutropenia, n	Overall, n	P-value	OR	95% CI
M17	UGT1A7*3	0	3 <sup>a</sup>	38 <sup>b</sup>	0.014	5.556	1.371-22.507
		1+2	10 <sup>a</sup>	31 <sup>b</sup>			
M23	UGT1A1*6	0	3 <sup>a</sup>	40 <sup>b</sup>	0.010	6.491	1.595-26.423
		1+2	10 <sup>a</sup>	29 <sup>b</sup>			
M17&M23		0	2 <sup>a</sup>	34 <sup>b</sup>	0.012	7.333	1.485-36.209
		Others	11 <sup>a</sup>	35 <sup>b</sup>			

<sup>a</sup>Number of patients developing toxicity among all the patients in the same genotype group. <sup>b</sup>Number of alleles carried by the patient. SNP, single nucleotide polymorphism; OR, odds ratio; CI, confidence interval; UGT1A, UDP glucuronosyltransferase family 1 member A complex locus.

revealed that patients carrying the UGT1A7\*3 and UGT1A1\*6 alleles had a higher OR (OR, 7.333; 95% CI, 1.485-36.209) than the OR of patients carrying either UGT1A7\*3 (OR, 5.556; 95% CI, 1.371-22.507) or UGT1A1\*6 (OR, 6.491; 95% CI, 1.595-26.423). However, UGT1A9\*1b was not significantly associated with the risk of severe toxicity in the heterozygous (P=0.617) or homozygous (P=0.065) state.

## Discussion

Polymorphisms in the UGT1A family have been demonstrated to repeatedly affect the variability of irinotecan-induced toxicity, and the results are dependent on ethnicity. The toxicity of irinotecan remains unpredictable and requires additional investigation (11,12,20).

Direct sequencing was adopted to avoid ethnic heterogeneity and to identify novel variations. Subsequent to sequencing of the promoter (-1000 bp) and exon 1 regions of UGT1A1, UGT1A7 and UGT1A9, comprehensive analysis of genetic polymorphisms in these alleles was performed in Chinese patients with metastatic gastrointestinal cancer treated using irinotecan-based regimens. A total of 23 different genetic variants were detected, including 2 novel polymorphisms. In total, 7 SNPs exhibited an association with grade 3-4 toxicity

(P<0.05). Finally, 3 polymorphisms were focused on, 2 of which were the functional mutations UGT1A7\*3 and UGT1A1\*6 that exhibited a significant association with grade 3-4 neutropenia (UGT1A7\*3: P=0.011, OR=3.391, 95% CI=1.384-8.312; and UGT1A1\*6: P=0.016, OR=3.373, 95% CI=1.350-8.431). Patients that carried the UGT1A7\*3 and UGT1A1\*6 alleles had an increased risk of grade 3-4 neutropenia (OR, 7.333; 95% CI, 1.485-36.209). Another key mutation is UGT1A9\*1b (-118 9T>10T), which is found predominantly in the Asian population, and leads to increased enzyme expression and glucuronidation rates (16,19). This was the only mutation significantly associated with grade 3-4 diarrhea (P=0.045; OR, 3.273; 95% CI, 1.033-10.37) in the present study.

UGT1A7\*3 is located on UGT1A7 exon 1, and a transition of T>C results in the 208th amino acid, tryptophan, changing to arginine. The UGT1A7\*3 allele has been reported to generate an enzyme with 50% reduced catalytic activity (27). It has also been reported that UGT1A7\*3 affects the pharmacokinetics of SN-38 in studies investigating both Caucasian and Asian patients (11,16,18,20). In the present study, patients homozygous for UGT1A7\*3 exhibited a 23.333-fold increased risk of grade 3-4 neutropenia (OR, 23.333; 95% CI, 1.609-338.419), while patients heterozygous for UGT1A7\*3 had an OR of 4.444 (95% CI, 1.060-18.627).

UGT1A1\*6, a unique allele in the Asian population, is located on UGT1A1 exon 1 (11,12). A transition of G>A results in a change of the 71st amino acid from glycine to arginine. Previous studies in the Asian population found that UGT1A1\*28/\*6 is a risk factor for irinotecan-induced toxicity (18,28). The OR for UGT1A1\*6-homozygous patients (OR, 12.333; 95% CI, 0.607-250.508) was increased two-fold compared with the OR for UGT1A1\*6-heterozygous patients (OR, 6.167; 95% CI, 1.486-25.586).

In conclusion, the UGT1A1\*6, UGT1A7\*3 and UGT1A9\*1b polymorphisms are predictive markers for severe toxicity in Chinese metastatic gastrointestinal cancer patients treated with irinotecan-based regimens.

## Acknowledgements

The present study was supported by the National Natural Science Foundation of China (grant no. 81402999).

## References

- Mathijssen RH, van Alphen RJ, Verweij J, Loos WJ, Nooter K, Stoter G and Sparreboom A: Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). *Clin Cancer Res* 7: 2182-2194, 2001.
- Guillemette C: Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *Pharmacogenomics J* 3: 136-158, 2003.
- Cunningham D, Pyrhönen S, James RD, Punt CJ, Hickish TF, Heikkilä R, Johannesen TB, Starkhammar H, Topham CA, Awad L, *et al*: Randomized trial of irinotecan plus supportive care versus supportive care alone after fluorouracil failure for patients with metastatic colorectal cancer. *Lancet* 352:1413-1418, 1998.
- Saltz LB, Cox JV, Blanke C, Rosen LS, Fehrenbacher L, Moore MJ, Maroun JA, Ackland SP, Locker PK, Pirota N, *et al*: Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. *N Engl J Med* 343: 905-914, 2000.
- Fuchs CS, Moore MR, Harker G, Villa L, Rinaldi D and Hecht JR: Phase III comparison of two irinotecan dosing regimens in second-line therapy of metastatic colorectal cancer. *J Clin Oncol* 21: 807-814, 2003.
- Zhang W, Zhao ZY, Wu Q, Li J, Fu Q, Cheng J, Xu N, Wu CP and Xu LG: Multicenter phase II study of modified FOLFIRI in patients in a Chinese population with advanced colorectal cancer (CRC) refractory to fluoropyrimidine and oxaliplatin. *J Clin Oncol* 23: 292s-292s, 2005.
- Maitland ML, Grimsley C, Kuttub-Boulos H, Witonsky D, Kasza KE, Yang L, Roe BA and Di Rienzo A: Comparative genomics analysis of human sequence variation in the UGT1A gene cluster. *Pharmacogenomics J* 6: 52-62, 2006.
- Gong QH, Cho JW, Huang T, Potter C, Gholami N, Basu NK, Kubota S, Carvalho S, Pennington MW, Owens IS and Popescu NC: Thirteen UDPglucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. *Pharmacogenetics* 11: 357-368, 2001.
- Iyer L, King CD, Whittington PF, Green MD, Roy SK, Tephly TR, Coffman BL and Ratain MJ: Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. *J Clin Invest* 101: 847-854, 1998.
- Gagné JF, Montminy V, Belanger P, Journault K, Gaucher G and Guillemette C: Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). *Mol Pharmacol* 62: 608-617, 2002.
- Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, Jang IJ, Lee DH and Lee JS: Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. *J Clin Oncol* 24: 2237-2244, 2006.
- Minami H, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, Kaniwa N, Sawada J, Hamaguchi T, Yamamoto N, *et al*: Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: Roles of UGT1A1\*6 and \*28. *Pharmacogenet Genomics* 17: 497-504, 2007.
- Pharmacia and Upjohn Company LLC: Camptosar - irinotecan hydrochloride injection, solution. <http://labeling.pfizer.com/ShowLabeling.aspx?id=533>.
- Sai K, Saeki M, Saito Y, Ozawa S, Katori N, Jinno H, Hasegawa R, Kaniwa N, Sawada J, Komamura K, *et al*: UGT1A1 haplotypes associated with reduced glucuronidation and increased serum bilirubin in irinotecan-administered Japanese patients with cancer. *Clin Pharmacol Ther* 75: 501-515, 2004.
- Bowcock AM, Anderson LA, Friedman LS, Black DM, Osborne-Lawrence S, Rowell SE, Hall JM, Solomon E and King MC: THRA1 and D17S183 flank an interval of <4 cM for the breast-ovarian cancer gene (BRCA1) on chromosome 17q21. *Am J Hum Genet* 52: 718-722, 1993.
- Fujita K, Ando Y, Nagashima F, Yamamoto W, Eodo H, Araki K, Kodama K, Miya T, Narabayashi M and Sasaki Y: 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, 2007.
- Lankisch TO, Schulz C, Zwingers T, Erichsen TJ, Manns MP, Heinemann V and Strassburg CP: Gilbert's syndrome and irinotecan toxicity: Combination with UDP-glucuronosyltransferase 1A7 variants increases. *Cancer Epidemiol Biomarkers Prev* 17: 695-701, 2008.
- Hazama S, Mishima H, Tsunedomi R, Okuyama Y, Kato T, Takahashi K, Nozawa H, Ando H, Kobayashi M, Takemoto H, *et al*: 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, 2013.
- Yamanaka H, Nakajima M, Katoh M, Hara Y, Tachibana O, Yamashita J, McLeod HL and Yokoi T: A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9\*22) and its effects on the transcriptional activity. *Pharmacogenetics* 14: 329-332, 2004.
- Cecchin E, Innocenti F, D'Andrea M, Corona G, De Mattia E, Bion P, Buonadonna A and Toffoli G: 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, 2009.
- Cui C, Shu C, Yang Y, Liu J, Shi S, Shao Z, Wang N, Yang T and Hu S: XELIRI compared with FOLFIRI as a second-line treatment in patients with metastatic colorectal cancer. *Oncol Lett* 8: 1864-1872, 2014.
- Barrett JC, Fry B, Maller J and Daly MJ: Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265, 2005.
- Barrett JC, Fry B, Maller J and Daly MJ: Haploview: Analysis and visualization of LD and haplotype maps. *Bioinformatics* 21: 263-265, 2005.
- Abecasis GR and Cookson WO: GOLD-graphical overview of linkage disequilibrium. *Bioinformatics* 16: 182-183, 2000.
- Uhlén M, Fagerberg L, Hallström BM, Lindskog C, Oksvold P, Mardinoglu A, Sivertsson Å, Kampf C, Sjöstedt E, Asplund A, *et al*: Proteomics. Tissue-based map of the human proteome. *Science* 347: 1260419, 2015.
- Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, Maller J, Sklar P, de Bakker PIW, Daly MJ and Sham PC: PLINK: A toolset for whole-genome association and population-based linkage analysis. *Am J Hum Genet* 81: 559-575.
- Guillemette C, Ritter JK, Auyeung DJ, Kessler FK and Housman DE: Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: Functional consequences of three novel missense mutations in the human UGT1A7 gene. *Pharmacogenetics* 10: 629-644, 2000.
- Wang H, Bian T, Jin T, Chen Y, Lin A and Chen C: Association analysis of UGT1A genotype and haplotype with SN-38 glucuronidation in human livers. *Pharmacogenomics* 15: 785-798, 2014.