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ORIGINAL RESEARCH

Relationship between *UGT1A1*6/*28* gene polymorphisms and the efficacy and toxicity of irinotecan-based chemotherapy

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Purpose: A retrospective study was performed to analyze the relationship between uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) **6/*28* gene polymorphisms and adverse reactions associated with irinotecan (CPT-11)-based chemotherapy. The correlation between *UGT1A1* polymorphisms and the clinical efficacy of CPT-11 was also analyzed, along with the influence of age and tumor type.

Patients and methods: Patients administered a CPT-11-based regimen in the Beijing Cancer Hospital from April 2015 to September 2016 were included in our study (n=81). Blood samples for detecting *UGT1A1* were collected from each patient after various administration regimens.

Results: Colorectal cancer patients with the *UGT1A1*6* mutant genotype had a significantly higher risk of severe delayed diarrhea than that of wild-type individuals when administered a CPT-11 dose ≥130 mg/m^2 (P=0.042); the same phenomenon was observed when the *UGT1A1*6* and *UGT1A1*28* mutant genotypes were considered together (P=0.028). However, in lung cancer patients administered a low dose of CPT-11, *UGT1A1*6/*28* variants were not significantly associated with severe neutropenia or delayed diarrhea. Furthermore, adult patients with the *UGT1A1*6* mutation were more likely to develop severe delayed diarrhea than did wild-type adults (P=0.013); however, the difference was not significant in elderly patients. No significant differences in tumor response were found among the different genotypes (P>0.05).

Conclusion: Thus, age and tumor type influence our ability to predict adverse reactions based on *UGT1A1* gene polymorphisms in cancer patients. Further, *UGT1A1* gene polymorphisms are not correlated with the efficacy of CPT-11-based regimens.

Keywords: CPT-11, uridine diphosphate glucuronosyltransferase 1A1, SN-38, digital fluorescence molecular hybridization

Plain language summary

Pharmacogenetic testing of uridine diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) **6/*28* is recommended in clinical practice prior to the administration of irinotecan (CPT-11)-based regimens; however, the research results are not uniform. We need to conduct more research and perform more rigorous experiments to verify the results. In this study, we analyzed the relationship between *UGT1A1*6/*28* gene polymorphisms and adverse reactions, as well as the clinical efficacy of CPT-11 and the influence of age and tumor type. We found that colorectal cancer patients with the *UGT1A1*6* mutant genotype had a significantly higher risk of severe delayed diarrhea than that of wild-type individuals when administered a CPT-11 dose ≥130 mg/m^2 (P=0.042). Further, the same phenomenon was observed when the *UGT1A1*6* and *UGT1A1*28* mutant genotypes were considered together (P=0.028); however, no significant difference in lung cancer patients was observed (all P>0.05). Furthermore, adult patients with the *UGT1A1*6*

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mutation were more likely to develop severe delayed diarrhea than did wild-type adults (P=0.013). No significant differences in tumor response were found among the different genotypes (P>0.05). Moreover, *UGT1A1* gene polymorphisms were not correlated with the efficacy of CPT-11-based regimens.

Introduction

Irinotecan (CPT-11), a semisynthetic camptothecin (CPT) derivative, was initially isolated from *Camptotheca acuminata* in the early 1960s.[1] It exerts high antineoplastic activity via inhibition of topoisomerase I (topo I) and is widely used to treat solid tumors in lung, colorectal, gastric, esophageal, cervical, ovarian, and other types of cancers.[2–4] However, CPT-11 can cause many adverse reactions, including delayed diarrhea and neutropenia, which have limited its clinical application.

CPT-11 is hydrolyzed to 7-ethyl-10-hydroxy-camptothecin (SN-38) by carboxylesterases. SN-38 is an active form of CPT-11 and a thousand times more toxic than CPT-11.[5–7] SN-38 is converted into an inactive SN-38 glucuronide (SN-38G) by uridine diphosphate-glucuronosyl transferases (UGTs) in the liver. Therefore, UGTs play a major role in SN-38 glucuronidation and may be related to CPT-11-induced adverse reactions.

Recently, single nucleotide polymorphisms (SNPs) have been found to be essential genomic resources that can significantly influence responses to pharmacotherapy, and they can be used to predict whether a drug will produce adverse reactions. As UGTs play an important role in the metabolism of CPT-11, mutations in the UGT genes may decrease UGT activity, which subsequently affects the pharmacokinetics and toxicity of CPT-11.[8,9]

UGTs are divided into 2 families (*UGT1* and *UGT2*), which in turn are further divided into 3 subfamilies (*UGT1A*, *UGT2A*, and *UGT2B*).[1,10] The UGT1A enzyme is present as 3 isozymes, namely, UGT1A1, UGT1A7, and UGT1A9.[11] *UGT1A1*6* and *UGT1A1*28* are considered to be the most important alleles for preventing severe SN-38-induced adverse reactions such as neutropenia and delayed diarrhea. Asians with mutant genotypes are more likely to develop neutropenia and delayed diarrhea; however, Caucasians with mutant genotypes are more likely to develop neutropenia.[12] Further, the prevalence of the UGT1A1*6 polymorphism is higher in Asian populations than that in Caucasian populations. Therefore, we considered the UGT1A1*6 polymorphism in Asian populations. Previously, studies have shown that UGT1A1*6/*28 gene polymorphisms can be used to assess the risk of neutropenia; Chinese people are more likely to suffer delayed diarrhea than Japanese and Thai people.[12–16]

In this study, we used digital fluorescence molecular hybridization (DFMH) to determine the *UGT1A1* genotypes of cancer patients treated with CPT-11 in our hospital. We determined the relationship between *UGT1A1*6* and *UGT1A1*28* gene polymorphisms and the clinical efficacy and toxicity of CPT-11. In addition, we categorized the patients according to age and tumor type to examine the influence of these factors on the efficacy and toxicity of CPT-11.

Patients and methods

Patients

All 81 patients administered a CPT-11-based regimen in the Beijing Cancer Hospital from April 2015 to September 2016 were recruited. For inclusion in our study, patients were required to have at least 2 cycles of CPT-11-based chemotherapy. The study was reviewed and approved by the research and medical ethics committee of Beijing Cancer Hospital. Written informed consent was obtained from each patient after a brief description of the purpose and protocols of the study.

Patient treatments

Lung cancer

The following treatments were used for lung cancer patients: 1) irinotecan mono-therapy, which included intravenous infusion of 60 mg/m^2 CPT-11 on days 1, 8, and 15 (repeated every 4 weeks); 2) irinotecan plus cisplatin (IP) regimen, which included intravenous infusion of 60 mg/m^2 CPT-11 on days 1, 8, and 15 and 60 mg/m^2 cisplatin on days 1 and 2 (repeated every 4 weeks); and 3) IP regimen plus bevacizumab, which included the same dosage regimen as IP and 5 mg/kg bevacizumab on day 1 (repeated every 3 weeks) (Table 1).

Colorectal cancer

The following treatments were used for colorectal cancer patients: 1) FOLFIRI regimen, which included intravenous infusion of 180 mg/m^2 CPT-11, 300 mg folinic acid, and 400 mg/m^2 5-fluorouracil (FU) on day 1, then continuous infusion of 2,500 mg/m^2 5-FU by infusion pump for 46 h (repeated every 2 weeks); 2) FOLFIRI regimen plus bevacizumab/cetuximab, which included the same dosage regimen as FOLFIRI, plus intravenous infusion of 250 mg/m^2 cetuximab on day 1, repeated each week, or 5 mg/kg bevacizumab on day 1, repeated every 2 weeks; 3) IP regimen, which included intravenous infusion of 130 mg/m^2 CPT-11 on day 1 and 70 mg/m^2 cisplatin on days 1 and 2 (repeated every 3 weeks); 4) CPT-11 plus bevacizumab, which

Table 1 Treatment regimens

Tumor	Regimen	No. of patients (n=81)	Dose of CPT-11 (mg/m^2)	Days on which CPT-11 was administered	Cycle (weeks)
Lung cancer	IP	36	60	1, 8, 15	4
	CPT-11 monotherapy	8	60	1, 8, 15	4
	IP plus bevacizumab	1	60	1, 8, 15	3
Colorectal cancer	IP	1	130	1	3
	FOLFIRI	22	180	1	2
	FOLFIRI plus bevacizumab/cetuximab	2	180	1	2
	CPT-11 plus bevacizumab	1	180	1	3
	CPT-11, capecitabine plus bevacizumab	1	180	1	2
Esophageal cancer	IP	2	130	1	3
	FOLFIRI	1	180	1	2
	CPT-11 plus apatinib mesylate	6	150	1	2

Abbreviations: CPT-11, irinotecan; IP, irinotecan plus cisplatin; FOLFIRI, folinic acid, 5-fluorouracil plus irinotecan.

included intravenous infusion of 180 mg/m^2 CPT-11 on day 1 and 5 mg/kg bevacizumab on day 1 (repeated every 3 weeks); and 5) CPT-11, capecitabine plus bevacizumab, which included intravenous infusion of 180 mg/m^2 CPT-11 on day 1, 1,000 mg/m^2 capecitabine on days 1–7, and 5 mg/kg bevacizumab on day 1 (repeated every 2 weeks) (Table 1).

Esophageal cancer

The following treatments were used for esophageal cancer patients: 1) IP regimen, which included intravenous infusion of 130 mg/m^2 CPT-11 on day 1 and 60 mg/m^2 cisplatin on days 2 and 3 (repeated every 3 weeks); 2) CPT-11 plus apatinib mesylate, which included intravenous infusion of 150 mg/m^2 CPT-11 on day 1 and 250 mg apatinib mesylate once daily (repeated every 2 weeks); and 3) FOLFIRI regimen, which included the same dosage regimen as previously mentioned (Table 1).

Evaluation criteria for drug toxicity

Toxicity was evaluated based on the National Cancer Institute Common Toxicity Criteria for Adverse Events (NCI-CTCAE) Version 3.0. Adverse reactions attributable to CPT-11 include neutropenia, leukopenia, delayed diarrhea, nausea, and vomiting. These reactions were divided into 4 stages in accordance with the NCI-CTCAE, with stages III and IV representing severe reactions.

Evaluation criteria for tumor response

The Response Evaluation Criteria in Solid Tumors (RECIST) is a standardized set of criteria for measuring tumor responses. Tumor response was assessed by computed tomography (CT) after every 2 cycles of treatment. The cases were defined as a complete response (CR), partial response (PR), or progressive disease (PD).

UGT1A1 gene detection

Blood samples were collected from each patient and placed in blood collection tubes containing ethylenediaminetetraacetic acid (EDTA) to prevent coagulation. The blood samples were then transferred to an Eppendorf (EP) tube and lysed using ammonium chloride (Sino-Era Jiyin Tech Co., Ltd., Beijing, China) for 5 min. The samples were then centrifuged at 700× *g* for 5 min to obtain plasma samples. Leukocyte genomic DNA was extracted directly from the blood samples using a nucleic acid purification kit (Sino-Era Jiyin Tech Co., Ltd.) and vortexed for 1 min. After 15 min of stasis, universal sequencing kits (Sino-Era Jiyin Tech Co., Ltd.) for individual gene loci were used to determine the *UGT1A1* genotype. *UGT1A1*6* and *UGAT1A1*28* gene polymorphisms were then identified by DFMH using fluorescent probes (Tianlong Science and Technology Co., Ltd., Xi'an, China).

DFMH is a new technique that is based on the principle of fluorescence in situ hybridization (FISH). Specific DNA strains containing fluorophore-labeled nucleotides can be used as probes to identify complementary sequences.

The conditions for sequencing by fluorescence molecular hybridization were as follows: 1) for the *UGT1A1*6* system, initial pre-degeneration at 95°C was performed for 10 min, followed by 50 cycles of degeneration at 95°C for 30 s and revival at 62°C for 75 s and 2) for the *UGT1A1*28* system, initial pre-degeneration at 95°C was performed for 10 min, followed by 50 cycles of degeneration at 95°C for 30 s and revival at 64°C for 75 s.

UGT1A1 genotype analysis

*UGT1A1*6* (G/G, G/A, and A/A) and *UGT1A1*28* (TA6/6, TA6/7, and TA7/7) genotypes were used as quality control samples (Sino-Era Jiyin Tech Co., Ltd.) to set the range of parameters, which were determined by measuring the time

to reach the specific fluorescent signal intensity (ST). ST1 and ST2 values reflect the interaction between the 2 probes and template and the different times to reach the specific fluorescent intensity, respectively; therefore, we set the parameter range by calculating the difference using the following formula:

$$\Delta ST = ST1 - ST2$$

Depending on the ΔST value, a threshold for the fluorescent signal can be set to facilitate genotype determination. The ΔST value ranges for the *UGT1A1*6/*28* genotypes were calculated using quality control samples. The specific genotype corresponds to a specific range of ΔST. Therefore, we determined the genotype of each patient using the ΔST value ranges of the *UGT1A1*6* and *UGT1A1*28* genotypes. Results of *UGT1A1*6/*28* quality control gene polymorphism tests are shown in Figure 1A–F.

Statistics

Data were analyzed using SPSS Version 22.0 software (IBM Corporation, Armonk, NY, USA). Genotyping data were analyzed for deviation from the Hardy–Weinberg equilibrium using the chi-square test. Differences in the incidence of adverse reactions and the clinical efficacy of CPT-11 were also analyzed using the chi-square test and Fisher's exact test. All statistical analyses were 2-sided tests, and a *P*-value ≤0.05 was defined as statistically significant.

Results

Distribution of *UGT1A1* gene polymorphisms

Complete genotyping was performed for all 81 patients, of whom 67 were men (82.72%) and 14 were women (17.28%). Thirty three patients (40.74%) were ≥60 years old, and 48 patients (59.26%) were <60 years old; the median patient age was 58 years (range: 28–79 years). The clinical characteristics of the patients are shown in Table 2, and results of the *UGT1A1*6* and *UGT1A1*28* genotyping are shown in Figure 2A–F. The *UGT1A1*28* genotype was divided into 3 groups: wild-type (TA6/6), heterozygous mutant (TA6/7), and homozygous mutant (TA7/7), with 61 (75.31%), 16 (19.75%), and 4 (4.49%) of the cases, respectively. The *UGT1A1*6* genotype was similarly divided into wild-type (G/G), heterozygous mutant (G/A), and homozygous mutant (A/A) groups, with 62 (76.54%), 17 (20.99%), and 2 (2.47%) of the cases, respectively. In total, there were 9 possible genotype combinations when both *UGT1A1*6* and *UGT1A1*28* were taken into consideration. These genotypes were divided into 3 categories depending on the number of mutations: double wild-type (G/G and TA6/6, n=44, 54.32%), single-site mutation (G/G and TA6/7 or G/A and TA6/6, n=29, 35.80%), and 2-site mutations (G/G and TA7/7, G/A and TA6/7, or A/A and TA6/6, n=8, 9.88%). However, we were unable to find the remaining 3 combinations (G/A and TA7/7, A/A and TA6/7, and A/A and TA7/7) in this study. The distribution of *UGT1A1* gene polymorphisms is shown in Table 3. The genotype distributions of *UGT1A1*6/*28* were in accordance with the Hardy–Weinberg equilibrium ($P>0.05$).

Correlation between *UGT1A1* genotype and adverse reactions

The different adverse reactions attributable to CPT-11 in patients, such as leukopenia, neutropenia, delayed diarrhea, and nausea and vomiting, were recorded in this study. In total, 32 patients (39.51%) suffered from leukopenia, 28 (34.57%) from neutropenia, 39 (48.15%) from nausea and vomiting, and 26 (32.10%) from delayed diarrhea. The results show that mutant *UGT1A1*6* and *UGT1A1*28* genotypes, as well as mutant *UGT1A1*6/*28* genotypes considered together, increase the risk of severe diarrhea over that of wild-type individuals; however, the differences were not significant ($P=0.305$, $P=0.707$, and $P=0.354$, respectively). In addition, there were no significant differences in the incidence of severe leukopenia, neutropenia, or nausea and vomiting in patients with *UGT1A1* gene polymorphisms (Table 4).

Correlation between *UGT1A1* genotype and adverse reactions with different tumors

The patients were divided into 2 groups according to tumor type. One group included 45 lung cancer patients treated with a low dose of CPT-11 (<130 mg/m^2), and the other group comprised 27 colorectal cancer patients treated with a high dose of CPT-11 (≥130 mg/m^2). The relationship between *UGT1A1* genotype and adverse reactions with different cancers is shown in Table 5. In lung cancer patients, the risk of grade I/II nausea and vomiting was higher in those with the *UGT1A1*6* mutant genotypes (40.00%; 4/10) than those with the wild-type *UGT1A1* (17.14%; 6/35). *UGT1A1*28* mutant genotypes (18.18%; 2/11) resulted in a higher risk of grade I/II neutropenia than the wild-type genotype (5.88; 2/34); however, the difference was not statistically significant ($P=0.247$). Thus, neither the wild-type nor mutant genotypes were significantly associated with severe adverse reactions in lung cancer patients. However, the outcome was

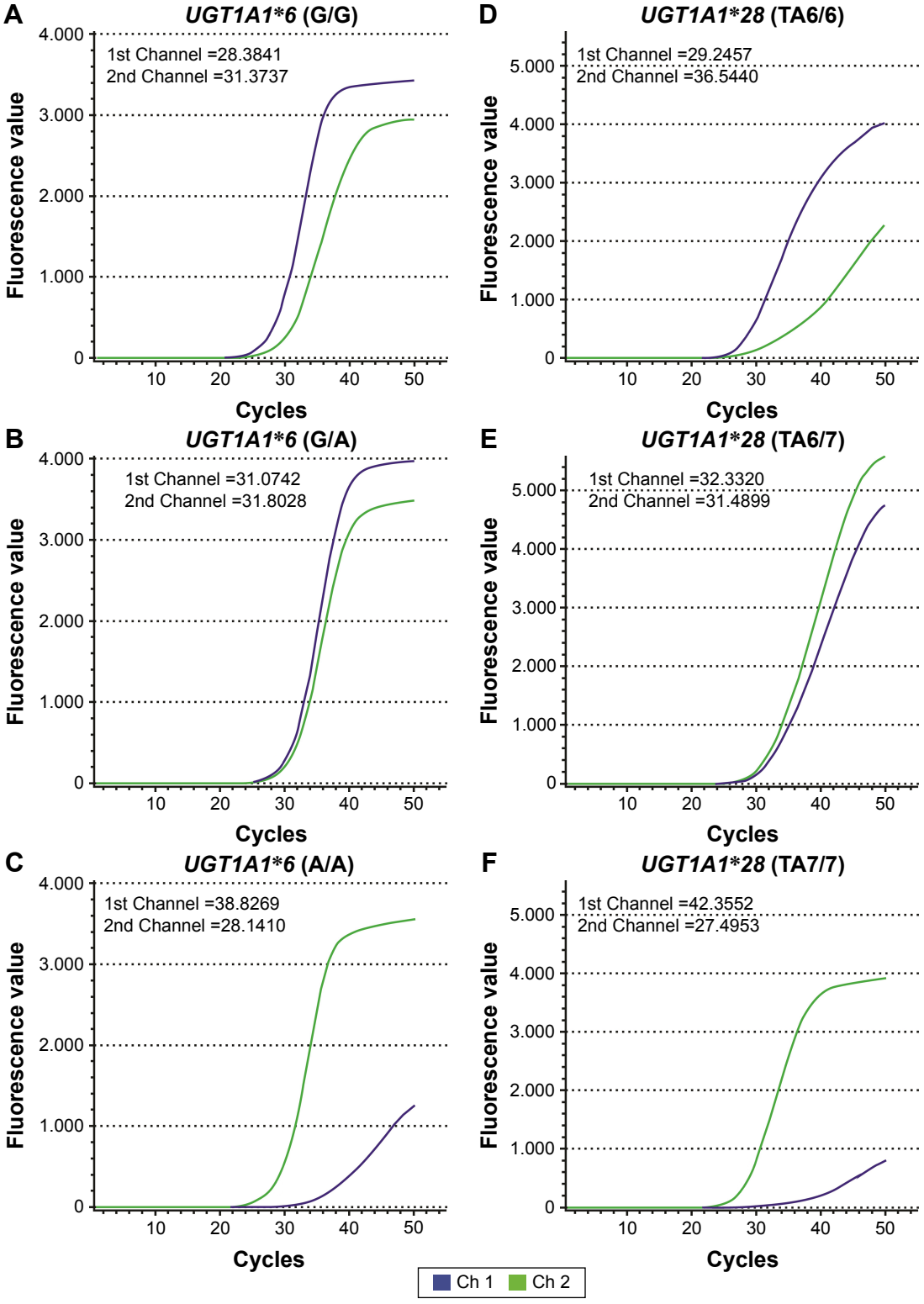

Figure 1 Detection of gene polymorphisms in *UGT1A1*6* and *UGT1A1*28* quality control genes by DFMH.

Notes: *UGT1A1* gene polymorphisms were detected using different fluorescent probes specific for *UGT1A1*6* or *UGT1A1*28* wild-type or mutant genotypes. The time to reach the specific fluorescence ST was determined for each channel using the fluorescence detector software, Microseq (Tianlong Science and Technology Co., Ltd., Xi'an, China). ΔST was calculated to set the range of ΔST values indicative of each *UGT1A1* polymorphism, which could then be used to determine the genotype of each patient. The results were as follows: (**A**) *UGT1A1*6* (G/G), ΔST =−2.9896; (**B**) *UGT1A1*6* (G/A), ΔST =−0.7286; (**C**) *UGT1A1*6* (A/A), ΔST =10.6859; (**D**) *UGT1A1*28* (TA6/6), ΔST =−7.2983; (**E**) *UGT1A1*28* (TA6/7), ΔST =0.8421; and (**F**) *UGT1A1*28* (TA7/7), ΔST =14.8599. The maximum value of ΔST is 5. Therefore, the ΔST values of *UGT1A1*6* (G/A), *UGT1A1*6* (G/G), and *UGT1A1*6* (A/A) were between −2.0657 and 5.000, <−2.0657, and >5.000, respectively, and the ΔST values of *UGT1A1*28* (TA6/7), *UGT1A1*28* (TA6/6), and *UGT1A1*6* (TA7/7) were between −3.5868 and 5.000, <−3.5868, and >5.000, respectively.

Abbreviations: DFMH, digital fluorescence molecular hybridization; ST, signal intensity.

Table 2 Clinical characteristics of patients with irinotecan-based treatment in this study

Characteristics	No. of patients (n=81)	Frequency (%)
Mean age, years (range)	58 (28–79)	
Gender		
Male	67	82.72
Female	14	17.28
ECOG performance status		
0	47	58.02
1	31	38.27
2	2	2.47
3	1	1.23
Type of tumor		
Lung cancer	45	55.56
Colorectal cancer	27	33.33
Esophageal cancer	9	11.11
Habit of smoking and drinking		
Smoking	20	24.69
Drinking	5	6.17
Smoking and drinking	31	38.27
None	25	34.57
Line of treatment		
First line	30	37.04
Second line	47	58.02
Third line	4	4.94
Cycle of treatment		
Second cycle	34	41.98
Third cycle	24	29.63
≥ fourth cycle	23	28.40

Abbreviation: ECOG, Eastern Cooperative Oncology Group.

different in the colorectal cancer group. The risk of severe delayed diarrhea was significantly higher in patients with the *UGT1A1*6* mutant genotypes (42.86%; 3/7) than those with the wild-type genotype (5.00%; 1/20) (P=0.042). Similarly, severe delayed diarrhea was higher in patients with the mutant *UGT1A1*28* genotypes (28.57%; 2/7) than those with the wild-type genotype (10.00%; 2/20); however, the difference was not significant (P=0.269). When the *UGT1A1*6* and *UGT1A1*28* mutant genotypes were considered together, the incidence of severe delayed diarrhea was significantly higher than that of the wild-type genotype (P=0.028).

Correlation of *UGT1A1* genotype and adverse reactions in aging patients

The patients were divided into 2 groups depending on their age. One group included 33 elderly patients (≥60 years old), and the other comprised 48 adult patients (<60 years old). The relationship between age and adverse reactions associated with different *UGT1A1* genotypes is shown in Table 6. In adult patients, the incidence of severe delayed diarrhea was higher in patients with the *UGT1A1*6* mutant genotypes than those with the wild-type genotype (P=0.013), while the *UGT1A1*28* genotype had no significant effect on delayed diarrhea. When the *UGT1A1*6* and *UGT1A1*28* mutant genotypes were considered together (12.50%; 3/24), the risk of delayed diarrhea was higher than that of the wild-type genotype (0%; 0/24); however, the difference was not statistically significant (P=0.233). In elderly patients, the incidence of grade I/II nausea and vomiting was higher in patients with the *UGT1A1*6* mutant genotypes than those with the wild-type genotype (P=0.039). Patients with *UGT1A1*28* polymorphisms were more likely to suffer from grade I/II neutropenia; however, the difference was not significant (P=0.093). The incidence of severe delayed diarrhea in elderly patients carrying a *UGT1A1*28* mutation (16.67%; 1/6) was higher than in those with wild-type *UGT1A1* (7.41%; 2/27; P=0.464). No significant differences in adverse reactions associated with CPT-11 in elderly patients were observed when *UGT1A1*6* and *UGT1A1*28* were considered together.

Correlation of *UGT1A1* genotype and tumor response

Tumor response was assessed in 30 (66.67%; 30/45) patients with lung cancer and 19 (70.37%; 19/27) patients with colorectal cancer, respectively (Table 7). PR, stable disease (SD), and PD were observed in 6, 17, and 7 lung cancer cases and 4, 12, and 3 colorectal cancer cases, respectively. Colorectal patients with *UGT1A1*28* mutant genotypes had a good tumor response in the PR group; however, the difference was not significant (P=0.178).

Discussion

CPT-11 causes obvious adverse reactions, including myelosuppression and delayed diarrhea, which limit its clinical application. The rapid growth of pharmacogenetics has shown that differences in drug metabolism between individuals are associated with genetic polymorphisms. Pharmacogenetic research has shown that genetic disparities play a major role in pharmacokinetics and can explain the clinical profile of many drugs, especially antineoplastic agents.[17–19] For example, *UGT1A1* plays an important role in SN-38 metabolism. The different *UGT1A1*6/*28* genotypes result in different metabolic rates, and elevated SN-38 blood concentrations induce adverse reactions. Therefore, it may be possible to predict the likelihood of SN-38-induced adverse reactions in patients using *UGT1A1*6/*28* genotyping.

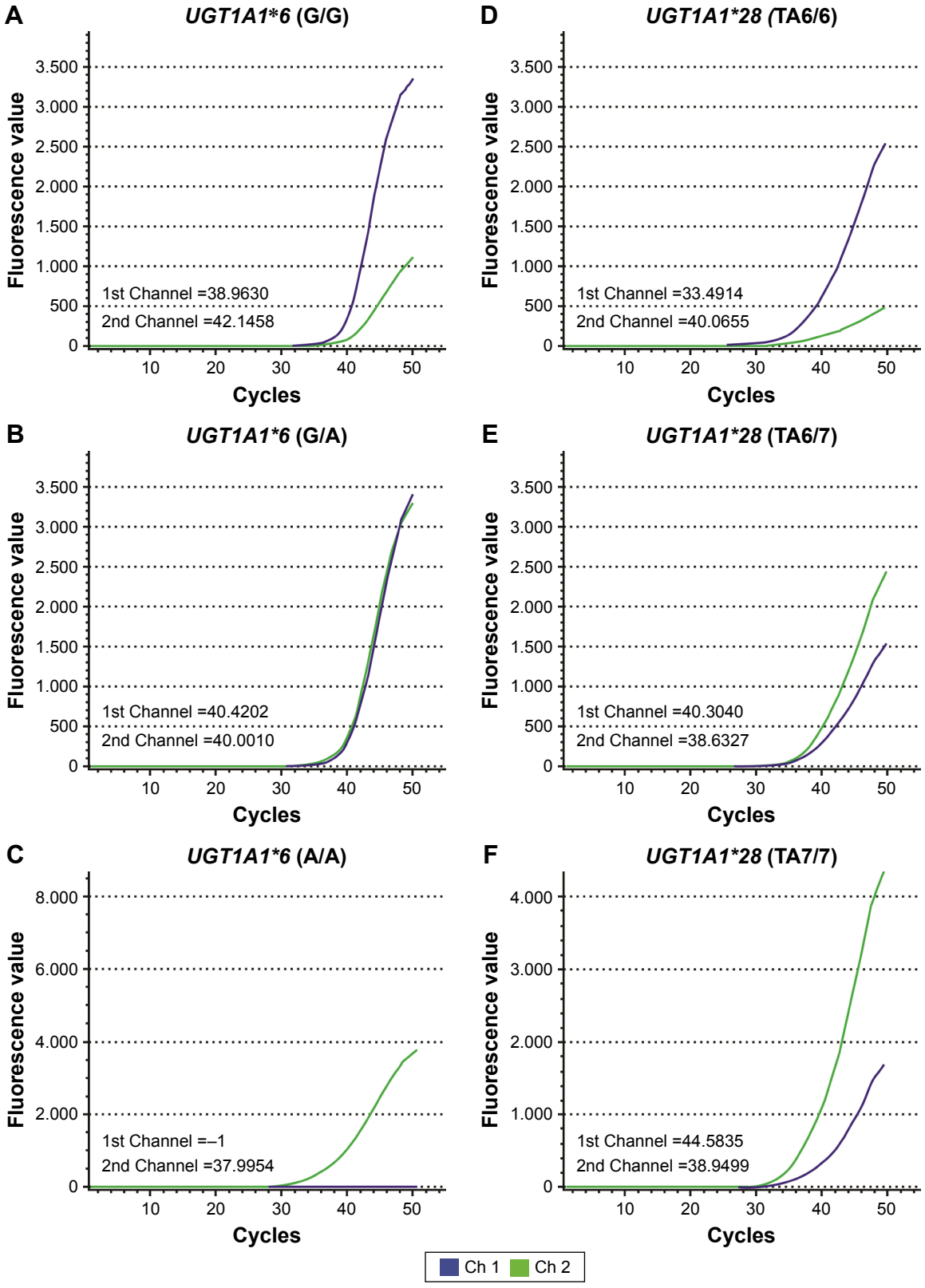

Figure 2 *UGT1A1* gene polymorphisms in patients.

Notes: Blood samples from the patients were analyzed by DFMH, and the results are as follows: (**A**) ΔST =–3.1828, representing *UGT1A1*6* (G/G); (**B**) ΔST =0.4192, representing *UGT1A1*6* (G/A); (**C**) ΔST =12.0046, representing *UGT1A1*6* (A/A); (**D**) ΔST =–6.5741, representing *UGT1A1*28* (TA6/6); (**E**) ΔST =1.6713, representing *UGT1A1*28* (TA6/7); and (**F**) ΔST =5.6336, representing *UGT1A1*28* (TA7/7).

Abbreviations: DFMH, digital fluorescence molecular hybridization; ST, signal intensity.

Table 3 Distribution of *UGT1A1* gene polymorphisms

Genotype	No. of patients (n=81)	Ratio (%)
*UGT1A1*6*		
G/G	62	76.54
G/A	17	20.99
A/A	2	2.47
*UGT1A1*28*		
TA6/6	61	75.31
TA6/7	16	19.75
TA7/7	4	4.94
UGT1A1 combinations		
G/G TA6/6	44	54.32
G/G TA6/7	14	17.28
G/A TA6/6	15	18.52
G/G TA7/7	4	4.94
G/A TA6/7	2	2.47
A/A TA6/6	2	2.47

Recently, the association between *UGT1A1* gene polymorphisms and adverse reactions, particularly delayed diarrhea and neutropenia, has been of interest worldwide. Many studies have shown that *UGT1A1*28* is significantly associated with CPT-11-induced toxicity, especially neutropenia.[20–24] Caucasians with mutant genotypes are more likely to have neutropenia.[12] However, *UGT1A1*6* is more prevalent in Asian countries than in Caucasian populations, suggesting that *UGT1A1*6* polymorphisms should be considered in addition to *UGT1A1*28* polymorphisms for more individualized CPT-11-based chemotherapy. In Japan, Hoskins et al[25] discovered that the risk of severe hematologic toxicity is higher in patients with *UGT1A1*28* homozygous mutations than in patients with wild-type or heterozygous genotypes at a dose >150 mg/m^2, but not <125 mg/m^2. However, Hirasawa et al[26] found that *UGT1A1*6/*28* mutations are associated with an increased risk of neutropenia and delayed diarrhea at a low dose of CPT-11. In China, Xu et al[3] discovered that mutant *UGT1A1*6/*28* genotypes significantly increase the incidence of delayed diarrhea, and the incidence of neutropenia is significantly increased when the *UGT1A1*6* and *UGT1A1*28* genotypes are considered together. Further, Chinese patients with *UGT1A1*6/*28* mutations are more likely to suffer delayed diarrhea than Japanese patients. Our results demonstrate that the *UGT1A1*6* mutant genotypes are associated with delayed diarrhea in colorectal cancer patients (P=0.042). In addition, when both *UGT1A1*6* and *UGT1A1*28* genotypes are taken into consideration, we discovered that the risk of severe delayed diarrhea is significantly higher in colorectal patients with mutant genotypes (P=0.028). However, in our study, there was no association between *UGT1A1* mutations and severe hematologic toxicity. Besides, we did not find *DPYD* mutations (rs55886062, rs67376798, or rs3918290) in our study; therefore, we could eliminate the effects of fluorouracil drug metabolism on adverse reactions.

The occurrence of cancer is increasing because of population growth and aging.[27] Hence, more attention should be paid to elderly patients, and individualized medication programs should be implemented to reduce the incidence of adverse reactions. In our study, *UGT1A1*6* mutations (G/A and A/A) increased the risk of grade I/II nausea and vomiting specifically in elderly patients (P=0.039). In contrast, adult patients with *UGT1A1*6* mutations had a significantly higher risk of severe delayed diarrhea (P=0.013); however, there were no significant differences in elderly patients. Differences in drug metabolism may not be obvious in elderly patients owing to reduced body function and metabolic enzyme activity.

Table 4 Relationship between *UGT1A1*6/*28* genotype and the incidence of adverse reactions associated with CPT-11

Genotyping	Leukopenia, n (%)		Neutropenia, n (%)		Nausea and vomiting, n (%)		Diarrhea, n (%)	
	Grades I–II	Grades III–IV	Grades I–II	Grades III–IV	Grades I–II	Grades III–IV	Grades I–II	Grades III–IV
G/G (n=62)	19 (30.65)	7 (11.29)	14 (22.58)	11 (17.74)	26 (41.94)	4 (6.45)	17 (27.41)	3 (4.84)
G/A (n=17)	5 (29.41)	1 (5.88)	0 (0)	3 (17.65)	8 (47.06)	0 (0)	3 (17.65)	3 (17.65)
A/A (n=2)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50.00)	0 (0)	0 (0)	0 (0)
P-value	1.000	1.000	0.067	1.000	0.908	0.614	0.739	0.305
TA6/6 (n=61)	18 (29.51)	7 (11.48)	10 (16.39)	11 (18.03)	27 (44.26)	3 (4.92)	15 (24.59)	4 (6.56)
TA6/7 (n=16)	5 (31.25)	1 (6.25)	3 (18.75)	2 (12.50)	7 (43.75)	1 (6.35)	4 (25.00)	2 (12.50)
TA7/7 (n=4)	1 (25.00)	0 (0)	1 (25.00)	1 (25.00)	1 (25.00)	0 (0)	1 (25.00)	0 (0)
P-value	1.000	1.000	0.757	0.646	0.844	1.000	1.000	0.707
Wild-type (n=44)	13 (29.55)	6 (13.64)	10 (22.73)	8 (18.18)	19 (43.18)	3 (6.38)	12 (27.27)	2 (4.55)
Single-site mutant (n=29)	10 (34.48)	2 (9.60)	3 (10.34)	5 (17.24)	13 (44.83)	1 (3.45)	7 (24.13)	3 (10.34)
Two-site mutant (n=8)	1 (12.50)	0 (0)	1 (12.50)	1 (12.50)	3 (37.50)	0 (0)	1 (12.50)	1 (12.50)
P-value	0.483	0.357	0.365	0.926	1.000	1.000	0.670	0.354

Table 5 Relationship between *UGT1A1*6/*28* genotype and the incidence of adverse reactions associated with different tumors

Tumor	Genotyping	Leukopenia, n (%)		Neutropenia, n (%)		Nausea and vomiting, n (%)		Diarrhea, n (%)	
		Grades I–II	Grades III–IV	Grades I–II	Grades III–IV	Grades I–II	Grades III–IV	Grades I–II	Grades III–IV
Lung cancer	G/G (n=35)	7 (20.00)	6 (17.14)	4 (11.43)	4 (11.43)	6 (17.14)	4 (11.43)	10 (28.57)	2 (5.71)
	G/A A/A (n=10)	3 (30.00)	0 (0)	0 (0)	0 (0)	4 (40.00)	0 (0)	2 (20.00)	0 (0)
P-value		0.811	0.379	0.561	0.561	0.270	0.561	0.893	1.000
Colorectal cancer	G/G (n=20)	7 (35.00)	1 (5.00)	5 (25.00)	6 (30.00)	15 (75.00)	0 (0)	5 (25.00)	1 (5.00)
	G/A A/A (n=7)	1 (14.29)	0 (0)	0 (0)	1 (14.29)	4 (57.14)	0 (0)	1 (14.29)	3 (42.86)
P-value		0.633	1.000	0.283	0.633	0.633	NG	1.000	0.042
Lung cancer	TA6/6 (n=34)	7 (20.59)	5 (14.71)	2 (5.88)	3 (8.82)	8 (23.53)	3 (8.82)	8 (23.53)	2 (5.88)
	TA6/7 TA7/7 (n=11)	3 (27.27)	1 (9.09)	2 (18.18)	1 (9.09)	2 (18.18)	1 (9.09)	4 (36.36)	0 (0)
P-value		0.963	1.000	0.247	1.000	1.000	1.000	0.657	1.000
Colorectal cancer	TA6/6 (n=20)	6 (30.00)	1 (5.00)	4 (20.00)	5 (25.00)	14 (70.00)	0 (0)	5 (25.00)	2 (10.00)
	TA6/7 TA7/7 (n=7)	2 (28.57)	0 (0)	1 (14.28)	2 (28.57)	5 (71.43)	0 (0)	1 (14.29)	2 (28.57)
P-value		1.000	1.000	1.000	1.000	1.000	NG	1.000	0.269
Lung cancer	Wild-type (n=24)	4 (16.67)	5 (20.83)	2 (8.33)	3 (12.50)	3 (12.50)	3 (12.50)	6 (25.00)	2 (8.33)
	Mutant type (n=21)	6 (28.57)	1 (4.76)	2 (9.52)	1 (4.76)	6 (28.57)	1 (4.76)	6 (28.57)	0 (0)
P-value		0.549	0.253	1.000	0.700	0.331	0.700	1.000	0.491
Colorectal cancer	Wild-type (n=15)	5 (33.33)	1 (6.67)	4 (26.67)	4 (26.67)	11 (73.33)	0 (0)	4 (26.67)	0 (0)
	Mutant type (n=12)	3 (25.00)	0 (0)	1 (8.33)	3 (25.00)	8 (66.67)	0 (0)	2 (16.67)	4 (33.33)
P-value		0.696	1.000	0.342	1.000	1.000	NG	0.662	0.028

Abbreviation: NG, not given.

Table 6 Relationship between *UGT1A1* genotype and the incidence of adverse reactions in patients of different ages

Age	Genotyping	Leukopenia, n (%)		Neutropenia, n (%)		Nausea and vomiting, n (%)		Diarrhea, n (%)	
		Grades I–II	Grades III–IV	Grades I–II	Grades III–IV	Grades I–II	Grades III–IV	Grades I–II	Grades III–IV
<60	G/G (n=36)	13 (36.11)	5 (13.89)	6 (16.67)	7 (19.44)	16 (44.44)	0 (0)	10 (27.78)	0 (0)
	G/A A/A (n=12)	3 (27.27)	1 (9.09)	0 (0)	2 (18.18)	3 (27.27)	0 (0)	0 (0)	3 (25.00)
P-value		0.724	1.000	0.313	1.000	0.394	NG	0.101	0.013
≥60	G/G (n=26)	6 (23.08)	2 (7.69)	8 (30.77)	4 (15.38)	10 (38.46)	4 (15.38)	7 (26.92)	3 (11.54)
	G/A A/A (n=7)	2 (33.33)	0 (0)	0 (0)	1 (16.67)	6 (85.71)	0 (0)	1 (14.28)	0 (0)
P-value		1.000	1.000	0.154	1.000	0.039	0.555	0.652	1.000
<60	TA6/6 (n=34)	11 (32.35)	5 (14.71)	6 (17.65)	6 (17.65)	11 (32.35)	0 (0)	7 (20.59)	2 (5.88)
	TA6/7 TA7/7 (n=14)	3 (27.27)	1 (9.09)	1 (9.09)	3 (21.43)	6 (42.86)	0 (0)	4 (28.57)	1 (7.14)
P-value		0.684	0.810	0.626	1.000	0.719	NG	0.826	1.000
≥60	TA6/6 (n=27)	7 (25.93)	2 (7.41)	4 (14.81)	5 (18.52)	16 (59.26)	3 (11.11)	8 (29.63)	2 (7.41)
	TA6/7 TA7/7 (n=6)	3 (50.00)	0 (0)	3 (50.00)	0 (0)	2 (40.00)	1 (20.00)	1 (16.67)	1 (16.67)
P-value		0.336	1.000	0.093	0.556	0.375	1.000	1.000	0.464
<60	Wild-type (n=24)	7 (29.17)	4 (16.67)	5 (20.83)	4 (16.67)	9 (37.50)	0 (0)	6 (25.00)	0 (0)
	Mutant type (n=24)	6 (33.33)	2 (11.11)	1 (5.56)	5 (20.83)	9 (37.50)	0 (0)	5 (20.83)	3 (12.50)
P-value		0.754	0.663	0.190	1.000	1.000	NG	1.000	0.233
≥60	Wild-type (n=20)	6 (30.00)	2 (10.00)	5 (25.00)	4 (20.00)	10 (50.00)	3 (15.00)	7 (35.00)	2 (10.00)
	Mutant type (n=13)	5 (38.46)	0 (0)	3 (23.08)	1 (9.09)	7 (53.85)	1 (9.09)	3 (23.07)	1 (7.69)
P-value		0.714	0.508	1.000	0.625	1.000	1.000	0.701	1.000

Abbreviation: NG, not given.

Table 7 Relationship between *UGT1A1* genotypes and tumor response

Tumor	Genotyping	PR, n (%)	*P*-value	SD, n (%)	*P*-value	PD, n (%)	*P*-value
Lung cancer	G/G (n=21)	3 (14.28)	0.320	13 (61.90)	0.443	5 (23.81)	1.000
	G/A A/A (n=9)	3 (33.33)		4 (44.44)		2 (22.22)	
	TA6/6 (n=23)	5 (21.74)	1.000	13 (56.52)	1.000	5 (21.74)	1.000
	TA6/7 TA7/7 (n=7)	1 (14.29)		4 (57.14)		2 (28.57)	
	Wild-type (n=14)	2 (14.29)	0.657	9 (64.29)	0.484	3 (21.43)	1.000
	Mutant type (n=16)	4 (25.00)		8 (50.00)		4 (25.00)	
Colorectal cancer	G/G (n=14)	4 (28.57)	0.530	8 (57.14)	0.603	2 (14.28)	1.000
	G/A A/A (n=5)	0 (0)		4 (80.00)		1 (20.00)	
	TA6/6 (n=15)	2 (13.33)	0.178	10 (66.67)	0.603	3 (20.00)	1.000
	TA6/7 TA7/7 (n=4)	2 (50.00)		2 (50.00)		0 (0)	
	Wild-type (n=11)	2 (18.18)	1.000	7 (63.64)	1.000	2 (18.18)	1.000
	Mutant type (n=8)	2 (25.00)		5 (62.50)		1 (12.50)	

Abbreviations: PR, partial response; SD, stable disease; PD, progressive disease.

There are currently no uniform conclusions regarding the correlation between *UGT1A1* gene polymorphisms and the clinical efficacy of CPT-11. Some studies have demonstrated that CPT-11 is more efficacious in patients with mutant genotypes.[28,29] However, most studies have found no correlation between different *UGT1A1* genotypes and clinical efficacy.[30,31] Further, a meta-analysis of 6,087 patients found that both homozygous and heterozygous *UGT1A1*28* mutant types had a higher response than wild-type patients, particularly Caucasians.[12] In our study, we observed that the tumor response in patients with *UGT1A1*28* mutant genotypes with colorectal cancer was better than in those with the wild-type genotype; however, the difference was not statistically significant (P=0.178).

A limitation of this study is its retrospective design; therefore, we did not conduct patient follow-ups once they discontinued treatment in our hospital. Consequently, we did not conduct a survival analysis in addition to the tumor response analysis to evaluate efficacy. Further, the small sample size of this study may impact the results. Owing to the low *UGT1A1*6* and *UGT1A1*28* mutation rates, we need to increase sample size and perform more rigorous experiments to verify the results of this study.

Conclusion

UGT1A1 is a potential pharmacogenetic marker for detecting the occurrence of severe delayed diarrhea, especially in colorectal cancer patients receiving a high dose of CPT-11. However, neither *UGT1A1*6* nor *UGT1A1*28* mutations were associated with severe neutropenia. *UGT1A1* gene polymorphisms cannot predict the clinical outcome of CPT-11-based regimens.

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Disclosure

The authors report no conflicts of interest in this work.

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