

# Polymorphisms of UDP-Glucuronosyltransferase Gene and Irinotecan Toxicity: A Pharmacogenetic Analysis<sup>1</sup>

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## ABSTRACT

Irinotecan unexpectedly causes severe toxicity of leukopenia or diarrhea. Irinotecan is metabolized to form active SN-38, which is further conjugated and detoxified by UDP-glucuronosyltransferase (UGT) 1A1 enzyme. Genetic polymorphisms of the *UGT1A1* would affect an interindividual variation of the toxicity by irinotecan via the alternation of bioavailability of SN-38. In this case-control study, retrospective review of clinical records and determination of *UGT1A1* polymorphisms were performed to investigate whether a patient with the variant *UGT1A1* genotypes would be at higher risk for severe toxicity by irinotecan. All patients previously received irinotecan against cancer in university hospitals, cancer centers, or large urban hospitals in Japan. We identified 26 patients who experienced severe toxicity and 92 patients who did not. The relationship was studied between the multiple variant genotypes (*UGT1A1*\*28 in the promoter and *UGT1A1*\*6, *UGT1A1*\*27, *UGT1A1*\*29, and *UGT1A1*\*7 in the coding region) and the severe toxicity of grade 4 leukopenia ( $\leq 0.9 \times 10^9/\text{liter}$ ) and/or grade 3 (watery for 5 days or more) or grade 4 (hemorrhagic or dehydration) diarrhea. Of the 26 patients with the severe toxicity, the genotypes of *UGT1A1*\*28 were homozygous in 4 (15%) and heterozygous in 8 (31%), whereas 3 (3%) homozygous and 10 (11%) heterozygous were found among the 92 patients without the severe toxicity. Multivariate analysis suggested that the genotype either heterozygous or homozygous for *UGT1A1*\*28 would be a significant risk factor for severe toxicity by irinotecan ( $P < 0.001$ ; odds ratio, 7.23; 95% confidence interval, 2.52–22.3). All 3 patients heterozygous for *UGT1A1*\*27 encountered severe toxicity. No statistical association of *UGT1A1*\*6 with the occurrence of severe toxicity was observed. None had *UGT1A1*\*29 or *UGT1A1*\*7. We suggest that determination of the *UGT1A1* genotypes might be clinically useful for predicting severe toxicity by irinotecan in cancer patients. This research warrants a prospective trial to corroborate the usefulness of gene diagnosis of *UGT1A1* polymorphisms prior to irinotecan chemotherapy.

## INTRODUCTION

Irinotecan<sup>3</sup> (CPT-11) is a camptothecin analogue with strong anti-tumor activity through an inhibition of topoisomerase I. Although the drug is now used widely, especially for colorectal and lung cancers (1–4), patients and oncologists have grave concerns about the dose-limiting toxicity of irinotecan, resulting in leukopenia and/or diarrhea (4–6). Severe, occasionally fatal, toxicity happens sporadically, even in a better risk patient who participates in well-controlled clinical

trials (1–4). Indeed, during a period of its clinical trials, the deaths of 55 patients of 1245 were attributed to side effects (5, 6). The Ministry of Health and Welfare in Japan has allowed irinotecan to be used at a medical institution that is sufficiently equipped to provide emergency treatment for these adverse reactions and under the supervision of specialists thoroughly experienced in chemotherapy (6). In addition, all patients treated with irinotecan have to be studied and reported during its Post Marketing Surveillance until January 2000, and each patient must be judged appropriate for the administration of the drug using the checklist on registering (6). Now, an innovative way of predicting the toxicity is strongly required.

Irinotecan is metabolized by carboxylesterase to form an active SN-38, which is further conjugated and detoxified by UGT (EC 2.4.1.17) to yield its  $\beta$ -glucuronide (7). The glucuronide is excreted in the small intestine via bile, where bacterial glucuronidase resolves the glucuronide into the former SN-38 and glucuronic acid (8). Interindividual differences in pharmacokinetics of SN-38 are suggested to cause the variation in drug effect (9, 10). On the other hand, there are two UGT enzymes in humans, UGT1 and UGT2, and the UGT1 family consists of one gene along with multiple promoters and the first exons which are spliced to the mutual exon 2 (11). Thus, the substrate specificity of the enzyme depends on the first exon. The *UGT1A1* gene is composed of a promoter and the first exon closest to exons 2–5 (11, 12). UGT1A1 enzyme, which is primarily responsible for conjugating bilirubin, can glucuronidate drugs (e.g., ethinylestradiol), xenobiotic compounds (e.g., phenols, anthraquinones, and flavones), and endogenous steroids (13). At present, >30 genetic variations in a promoter region and exons have been known to decrease the enzyme activity, leading to constitutional unconjugated jaundice, Crigler-Najjar or Gilbert's syndrome (12). Recent *in vitro* analyses have revealed that the UGT1A1 isoform would be responsible for the glucuronidation of SN-38 and that the genetic variation would associate with the decreased activity of SN-38 glucuronidation as well as bilirubin (14, 15). Additionally, we have suggested an interindividual difference in the pharmacokinetics of SN-38 and SN-38 glucuronide, depending on the *UGT1A1* genotype (16). Thus, we speculated that the variant genotypes would increase the toxicity by irinotecan via excessive accumulation of its active metabolite SN-38.

Genotypes involved in Gilbert's syndrome rather than Crigler-Najjar syndrome II would be clinically important for explanation of patient-patient variations in the reaction to a drug that is mainly conjugated by UGT1A1. Hyperbilirubinemia in a patient with Gilbert's syndrome is usually milder than that in Crigler-Najjar syndrome II, and 3–10% of the general population are estimated to have Gilbert's syndrome (17). Moreover, genotypes found in Gilbert's syndrome are also noted in seemingly healthy individuals and do not always cause hyperbilirubinemia (18–22), probably because of non-genetic factors including diet and therapeutic drug use. Thus, cancer patients carrying the genotypes associated with Gilbert's syndrome may be possible candidates for irinotecan chemotherapy.

This study retrospectively investigated the impact of the genetic

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<sup>3</sup> The abbreviations used are: irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; UGT, UDP-glucuronosyltransferase; CI, confidence interval; RFLP, restriction fragment length polymorphism.

polymorphism of *UGT1A1* on the likelihood of severe toxicity in patients receiving irinotecan in cancer chemotherapy. The genotype analyses were centered on those associated with Gilbert's syndrome (Table 1). Two types of variant genotypes have been reported in this syndrome. One is a 2-bp insertion (TA) in the TATA box in the promoter [normal (TA)<sub>6</sub>TAA], resulting in the sequence (TA)<sub>7</sub>TAA, *UGT1A1*\*28 (12, 18, 19), and the other is a heterozygous (sometimes homozygous) single nucleotide change in the coding region (23), all of which have been reported to reduce *UGT1A1* activity (19, 24, 25). Our hypothesis is that a patient with the variant genotypes would be at higher risk for severe hematological toxicity and/or diarrhea because of a relatively increased bioavailability of active unconjugated SN-38, and that some of the unexpected severe toxicity might be explained by the genetic factor. The goal of the present study is to explore a clinical advantage of determining *UGT1A1* polymorphisms prior to irinotecan chemotherapy for predicting the toxicity.

## MATERIALS AND METHODS

**Patients and Clinical Information.** The subjects were Japanese cancer patients who had received irinotecan-containing chemotherapy in the participating institutes from July 1994 to June 1999 (median, October 1998). They all gave informed consent in writing between July 1998 and June 1999 (median, January 1999) for their peripheral blood to be used for the research. According to the strict requirement of the Ministry (6), each patient was primarily ensured to have an adequate bone marrow function, as a leukocyte count of  $3 \times 10^9$ /liter or more and a platelet count of  $100 \times 10^9$ /liter or more before the use of irinotecan. In addition, patients who had evidence of active infection, watery diarrhea, paralytic ileus, pulmonary interstitial pneumonia or fibrosis, massive ascites or pleural effusion, apparent jaundice, or anamnesis of hypersensitivity to irinotecan were excluded from the irinotecan use. After the administration of irinotecan, the complete blood count, platelet count, and serum chemistry were assessed at least once a week in accordance with the warnings issued by the Ministry. Other appropriate investigations depended on each assigned physician. Almost all subjects were treated as in-patients, and bilirubin levels were always measured after overnight fasting.

We retrospectively reviewed the clinical records including patient characteristics (age, gender, primary disease and previous treatments, evidence of distant metastasis, Eastern Cooperative Oncology Group performance status, and major complications), dosage, and schedule of irinotecan administration, concurrent use of other drugs or radiotherapy, and observed toxicity after irinotecan infusion. We counted the number of days when patients received granulocyte-colony stimulating factors or loperamide hydrochloride, which is commonly prescribed for irinotecan-induced diarrhea in Japan. Prophylactic uses of granulocyte-colony stimulating factor could not be clearly distinguished from those for neutropenia. Because the dose-limiting toxicity of irinotecan results in leukopenia and diarrhea (4), we defined "severe toxicity" in this research as leukopenia of grade 4 ( $\leq 0.9 \times 10^9$ /liter) and/or diarrhea of grade 3 or worse (grade 3, watery for 5 days or more; grade 4, hemorrhagic or dehydration), classified in accordance with the Japan Society for Cancer Therapy criteria (26). The other toxicity was not included in the analysis because anemia would be influenced by miscellaneous patients' backgrounds including gastrointestinal lesions or nutritious status, and because simultaneous uses of cisplatin or carboplatin probably result in extremely exacerbated nausea/vomiting or thrombocytopenia, respectively. Serum total bilirubin levels were obtained just prior to irinotecan administration along with the highest of those after initiation of the therapy. The study was approved by the Ethical

Committees of Nagoya University School of Medicine and the participating institutes.

**Genotyping.** Blood sampling and genetic analyses were performed after irinotecan administration in each patient. Genomic DNA was prepared from whole blood (100–200  $\mu$ l) using the QIAamp Blood kit (Qiagen, Hilden, Germany). We researched the following variant sequences (Table 1; Ref. 12): a two-extra-nucleotide insertion (TA) within the TATA box resulting in the sequence (TA)<sub>7</sub>TAA (–39 to –53, *UGT1A1*\*28; Refs. 18 and 19); a transition (+211 from the initial site of the transcription, G to A) at codon 71 in exon 1 that changes glycine to arginine (G71R, *UGT1A1*\*6; Refs. 23 and 27); a transversion (+686, C to A) at codon 229 in exon 1 that alters proline to glutamine (P229Q, *UGT1A1*\*27; Ref. 23); a transversion (+1099, C to G) at codon 367 in exon 4 that converts arginine to glycine (R367G, *UGT1A1*\*29; Ref. 23); and a transversion (+1456, T to G) at codon 486 in exon 5 that transforms tyrosine into aspartic acid (Y486D, *UGT1A1*\*7; Ref. 27).

*UGT1A1*\*28 was distinguished from the most common allele (*UGT1A1*\*1) by direct sequencing (–147 to +106) of 253–255 bp produced by PCR using the method described previously (18, 20). Cycle sequencing was performed with a dye terminator sequence reaction (ABI Prism DNA Sequencing kit; Perkin-Elmer, Foster City, CA) using an ABI PRISM 310 Genetic Analyzer. The remaining variant sequences were distinguished from *UGT1A1*\*1 by PCR-RFLP assay. For the analysis of exon 1, the first-step PCR amplification of a 923-bp fragment containing the exon 1 was performed in accordance with the reported method (21). Subsequently, for the analysis of *UGT1A1*\*6, the second set of PCR amplifications was carried out using nested primers designed to amplify a 235-bp segment. The mismatched forward and the reverse primer was 5'-CTAGCACCTGACGCCTCGTGTGACATCAGAGCC-3' (+178 to +210; underlining indicates mismatched site) and 5'-CCATGAGCTCCTTGTGTGC-3' (+393 to +412), respectively. The forward primer was designed to introduce a *MspI* (Takara Shuzo Co., Ltd., Otsu, Japan) restriction site in *UGT1A1*\*1 (+209 to +212), not in *UGT1A1*\*6. The 1000-fold diluted product of the first PCR was subjected to nested PCR in a volume of 50  $\mu$ l containing 0.2 mM of each deoxynucleoside triphosphate, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl<sub>2</sub>, 0.5  $\mu$ M of each primer, and 1.3 unit of Taq polymerase (Takara Shuzo Co., Ltd.). PCR conditions were: 95°C for 5 min followed by 25 cycles of 94°C for 30 s, 60°C for 40 s, and 72°C for 40 s (PCR Thermal Cycler MP; Takara Shuzo Co., Ltd.). A 1- $\mu$ l PCR product was digested with 4 units of *MspI* for 1 h at 37°C. DNA from *UGT1A1*\*1 was digested into 203- and 32-bp fragments, DNA from *UGT1A1*\*6 gave an undigested 235-bp fragment, and DNA from the heterozygous genotype gave all three fragments. For the sequence of *UGT1A1*\*27, another set of the second PCR amplifications was performed using hemi-nested primers 5'-AGTACCTGTCTCTGCCAC-3' (+485 to +503) and 5'-GTCCCACTCCAATACAC-3' (+865 to +867 and intron 1), designed to amplify a 399-bp segment. Two *BsrI* (New England Biolabs, Inc., Beverly, MA) restriction sites exist in *UGT1A1*\*27 (+552 to +556 and +684 to +688), but only one site (+552 to +556) exists in *UGT1A1*\*1. The set of PCR amplifications was identical with that for *MspI* RFLP described above. Digestion of PCR products with 2.5 units of *BsrI* for 1 h at 65°C gave 199-, 132- and 68-bp fragments from *UGT1A1*\*27 or 331- and 68-bp from *UGT1A1*\*1. The heterozygous genotype gave all four fragments.

The sequence of *UGT1A1*\*29 was also identified using a nested PCR-RFLP assay. The first-step PCR amplification encompassing exons 2, 3, and 4 was performed according to the reported method with minor modifications (21). The mismatched forward and the reverse primers for the second PCR amplification designed to amplify a 285-bp segment was 5'-TCCTCCCTATTTTGCATCTCAGGTCACCCGATG<sup>u</sup>CC-3' (intron 3 and +1085 to +1098; underlining indicates mismatched site) and 5'-TGAATGCCATGACCAAA-3' (intron 4), respectively. The forward primer was designed to introduce a *CfrI*31 (Takara Shuzo Co., Ltd.) restriction site in *UGT1A1*\*1 (+1095 to +1099) but not in *UGT1A1*\*29. The PCR reaction mixture was the same as that used in the second PCR examination for *UGT1A1*\*6. A PCR product was digested with *CfrI*31 enzyme. DNA from *UGT1A1*\*1 was digested into 252- and 33-bp fragments, and DNA from *UGT1A1*\*29 gave an undigested 285-bp fragment. For detection of *UGT1A1*\*7, the PCR amplification for a 579-bp fragment of exon 5 was carried out using the primer described previously (21). The reaction mixture was the same as that used in the second PCR assay for *UGT1A1*\*6. There is a *BsrI* restriction site in the sequence of *UGT1A1*\*1 (+1452 to +1456) but not in *UGT1A1*\*7. After incubation with *BsrI* enzyme, DNA from

Table 1 Variant *UGT1A1* alleles analyzed in this study<sup>a</sup>

Allele	Nucleotide change	Effect on protein	Exon
<i>UGT1A1</i> *28	(TA) <sub>7</sub> TAA	Reduced expression	Promoter
<i>UGT1A1</i> *6	211G→A	G71R	1
<i>UGT1A1</i> *27	686C→A	P229Q	1
<i>UGT1A1</i> *29	1099C→G	R367G	4
<i>UGT1A1</i> *7	1456T→G	Y486D	5

<sup>a</sup>The most common (wild-type) *UGT1A1* allele is regarded as *UGT1A1*\*1.

Table 2 Baseline characteristics of patients

	Leukopenia (grade 4) and/or diarrhea (grade 3 or worse) <sup>a</sup>		P
	Experienced (n = 26)	Not experienced (n = 92)	
Gender (men/women)	14/12	66/26	0.085 <sup>b</sup>
Median age (range, yr)	60 (38–76)	61 (41–75)	>0.2 <sup>c</sup>
Performance status			>0.2 <sup>b</sup>
0	8 (31%)	31 (34%)	
1	15 (58%)	51 (55%)	
≥2	3 (12%)	10 (11%)	
Primary disease			>0.2 <sup>b</sup>
Small cell lung	4 (15%)	17 (18%)	
Non-small cell lung	16 (62%)	49 (53%)	
Colorectal	3 (12%)	18 (20%)	
Other	3 (12%)	8 (9%)	
Distant metastases	21 (81%)	68 (74%)	>0.2 <sup>b</sup>
Previous treatment			>0.2 <sup>b</sup>
None	12 (46%)	36 (39%)	
Systemic chemotherapy	12 (46%)	47 (51%)	
Surgery	8 (31%)	34 (37%)	
Radiotherapy	3 (12%)	17 (18%)	
Complications			>0.2 <sup>b</sup>
Diabetes	2 (8%)	8 (9%)	
Liver diseases	3 (12%)	6 (7%)	

<sup>a</sup> Japan Society for Cancer Therapy criteria.

<sup>b</sup>  $\chi^2$  test.

<sup>c</sup> Mann-Whitney U test.

*UGT1A1\*1* was digested into 365- and 214-bp fragments, and DNA from *UGT1A1\*7* gave an undigested 579-bp fragment.

Restriction fragments were analyzed by 4% agarose gel electrophoresis and ethidium bromide staining. The representative genotyping results of every variant genotype were confirmed by direct sequencing analyses.

**Statistical Analysis.** Possible factors analyzed to assess associations with the severe toxicity or the polymorphisms of *UGT1A1* were as follows; gender, age, performance status, primary disease, presence of distant metastasis, previous treatments, complications of diabetes or liver diseases, chemotherapy regimens, concurrent radiotherapy, and the intended schedule and dosage for each infusion of irinotecan. The chemotherapy regimens were categorized into three groups: irinotecan alone, irinotecan plus platinum (cisplatin or carboplatin), and irinotecan plus other agents (paclitaxel, docetaxel, etoposide, mitomycin C, or 5-fluorouracil). The correlation or association between potential variables was assessed using  $\chi^2$  test or Fisher's exact test for categorical variables, or with Mann-Whitney U test for continuous ones. Possible variables that seemed to be associated with severe toxicity ( $P < 0.1$ ) were considered for inclusion in an unconditional multiple logistic regression analysis. We did not include the following factors in the multivariate analysis because they were highly dependent on the outcome of chemotherapy: total actual dosage and uses of granulocyte colony-stimulating factor and loperamide hydrochloride. The variables in the final models were chosen using forward and backward stepwise procedures at the significance level of 0.25 and 0.1, respectively. The importance of the genetic polymorphism for occurrence of severe toxicity was verified when controlling for the other variables. We performed these analyses using JMP version 3.0.2 software (SAS Institute, Inc., Cary, NC). A difference was considered statistically significant when the two-tailed  $P$  was  $< 0.05$ .

**RESULTS**

**Toxicity of Irinotecan.** We collected blood samples from the 118 patients with their clinical information (Tables 2 and 3). Nine (8%) and 38 (32%) patients experienced leukopenia of grade 4 ( $\leq 0.9 \times 10^9$ /liter) and grade 3 ( $1.9\text{--}1.0 \times 10^9$ /liter), respectively. Diarrhea was reported in 3 patients (3%) with grade 4 (hemorrhagic or dehydration) and 19 patients (16%) with grade 3 (watery for 5 days or more). Five of the 9 patients with grade 4 leukopenia also had grade 3/4 diarrhea, and 16 of the 22 patients with grade 3/4 diarrhea encountered grade 3/4 leukopenia. Then, we identified 26 patients who experienced severe toxicity and 92 patients who did not (Tables 2 and 3). During the first course, 19 of the 26 patients (73%) experienced severe toxicity.

Lower total amounts of actual irinotecan ( $< 300 \text{ mg/m}^2$ ) and more frequent use of granulocyte-colony stimulating factor or loperamide hydrochloride were observed in patients suffering from severe toxicity (Table 3). Complete or partial responses were observed more often in patients who experienced severe toxicity, although the difference was not significant (Table 3).

**Distribution of Genotypes.** The genotypes were determined in the all 118 patients including 9 patients whose *UGT1A1\*28* genotyping results have been reported elsewhere (16). The allele frequencies of *UGT1A1\*28* were 0.308 (95% CI, 0.182–0.433) and 0.087 (95% CI, 0.046–0.128), and those of *UGT1A1\*6* were 0.077 (95% CI, 0.004–0.149) and 0.136 (95% CI, 0.086–0.185) among the patients with and without severe toxicity, respectively (Table 4). The difference in allelic distribution between the patients with and without severe toxicity was significant for *UGT1A1\*28* ( $P < 0.001$ ) but not significant for *UGT1A1\*6* ( $P > 0.2$ ; GENESOP version 3.1d software, the Laboratoire de Génétique et Environnement, Montpellier, France). The co-occurrence of the polymorphisms was found in 5 patients: 2 patients were heterozygous for both *UGT1A1\*28* and *UGT1A1\*6*, and 3 patients were heterozygous for *UGT1A1\*27* and homozygous (2 patients) or heterozygous (1 patient) for *UGT1A1\*28*. We did not examine the *cis* or *trans* arrangement of the variant sequences in these 5 patients. None of the patients had *UGT1A1\*29* or *UGT1A1\*7*.

The total bilirubin levels prior to and the highest during the chemotherapy were obtained in 117 patients; the one patient missing the measurement had no apparent jaundice and was homozygous for *UGT1A1\*1*. The 2 patients heterozygous for both *UGT1A1\*28* and *UGT1A1\*6* had bilirubin levels within the normal range: 13.9 ( $\mu\text{mol/l}$ ) and 15.4 prior to therapy and 10.3 and 15.4 after initiation of

Table 3 Information on irinotecan chemotherapy

	Leukopenia (grade 4) and/or diarrhea (grade 3 or worse) <sup>a</sup>		P
	Experienced (n = 26)	Not experienced (n = 92)	
Regimens			0.015 <sup>b</sup>
Irinotecan alone	3 (12%)	32 (35%)	
Irinotecan and platinum	13 (50%)	45 (49%)	
Irinotecan and other anticancer drugs	10 (38%)	15 (16%)	
Concurrent radiotherapy	1 (4%)	8 (9%)	>0.2 <sup>c</sup>
Intended schedule			0.059 <sup>b</sup>
Weekly (days 1, 8, and 15)	15 (72%)	62 (67%)	
Every 3 or 4 weeks	8 (31%)	11 (12%)	
Twice every 4 weeks	3 (12%)	19 (21%)	
Intended irinotecan dosage for each infusion ( $\text{mg/m}^2$ )			>0.2 <sup>b</sup>
<60	9 (35%)	18 (20%)	
60	8 (31%)	34 (37%)	
>60	9 (35%)	40 (43%)	
Total actual dosage ( $\text{mg/m}^2$ )			0.010 <sup>b</sup>
<300	15 (58%)	24 (26%)	
301–600	7 (27%)	46 (50%)	
>600	4 (15%)	22 (24%)	
Use of granulocyte colony-stimulating factor (days)			<0.001 <sup>b</sup>
0	5 (19%)	61 (66%)	
1–14	11 (42%)	13 (14%)	
≥15	10 (38%)	18 (26%)	
Use of loperamide hydrochloride (days)			0.002 <sup>b</sup>
0	4 (15%)	51 (55%)	
1–7	15 (58%)	28 (30%)	
≥8	7 (27%)	13 (14%)	
Objective response			>0.2 <sup>b</sup>
Complete or partial	11 (48%)	31 (39%)	
Stable disease	8 (35%)	36 (45%)	
Progressive disease	4 (17%)	13 (16%)	
Not measurable	3	12	

<sup>a</sup> Japan Society for Cancer Therapy criteria.

<sup>b</sup>  $\chi^2$  test.

<sup>c</sup> Fisher's exact test.

Table 4 Associations of *UGT1A1* genotypes and bilirubin levels with severe toxicity

	Leukopenia (grade 4) and/or diarrhea (grade 3 or worse) <sup>a</sup>		P
	Experienced (n = 26)	Not experienced (n = 92)	
<i>UGT1A1</i> *28 <sup>b</sup>			<0.001 <sup>c</sup>
-/-	14 (54%)	79 (86%)	
+/-	8 (31%)	10 (11%)	
+/+	4 (15%)	3 (3%)	
<i>UGT1A1</i> *6 <sup>b</sup>			>0.2 <sup>c</sup>
-/-	22 (85%)	69 (75%)	
+/-	4 (15%)	21 (23%)	
+/+	0 (0%)	2 (2%)	
Total bilirubin levels (μmol/l)			
Prior to therapy	8.6 (6.8–13.7) <sup>d</sup>	8.6 (6.8–12.0) <sup>e</sup>	>0.2 <sup>f</sup>
Highest after infusion	16.2 (11.8–26.5)	13.7 (10.3–18.8) <sup>e</sup>	0.071 <sup>f</sup>

<sup>a</sup> Japan Society for Cancer Therapy criteria.

<sup>b</sup> Symbols of (-/-), (+/-), and (+/+) denote homozygous absence of the variant allele, heterozygous, and homozygous for the variant allele, respectively.

<sup>c</sup> GENEPOP version 3.1d software, the Laboratoire de Génétique et Environnement, Montpellier, France.

<sup>d</sup> Median (interquartile range).

<sup>e</sup> One patient missed the bilirubin measurement during the treatment.

<sup>f</sup> Mann-Whitney U test.

therapy, respectively. Except for these 2 patients, the differences in the bilirubin levels among the genotypes were statistically significant prior to the therapy (P = 0.031, Kruskal-Wallis test) and after the initiation of therapy (P < 0.001; Table 5). There was no significant association between the genotypes and objective responses (data not shown).

**Genotypes and Toxicity.** Simple logistic regression analysis showed that the genotype either heterozygous or homozygous for *UGT1A1*\*28 proved to be a significant predictor of severe toxicity (odds ratio, 5.21; 95% CI, 1.98–13.96; P < 0.001; Table 4). Conversely, no statistical association of *UGT1A1*\*6 with the occurrence of severe toxicity was observed (odds ratio, 0.55; 95% CI, 0.15–1.61; P > 0.2).

Besides the variant genotypes, the factors that seemed to affect severe toxicity adversely (P < 0.1) were gender, chemotherapy regimen, and intended schedule of irinotecan infusion (Tables 2 and 3). These factors were assessed for correlation or association. Significant association was found between chemotherapy regimen and intended schedule (P < 0.001, χ<sup>2</sup> test); in other words, 12 of 19 patients (63%) treated with irinotecan of 3- or 4-week cycle had received additional anticancer drugs. Because the chemotherapy regimen was the variable with stronger relationship with severe toxicity, we considered the factor of chemotherapy regimen for inclusion in the model. The other correlation or association among chemotherapy regimen, gender, and *UGT1A1*\*28 genotype was not significant. The stepwise procedures identified female gender and use of other anticancer drugs (apart from platinum) as important variables for the occurrence of severe toxicity besides the *UGT1A1*\*28 genotype (Table 6). After adjustment with

these two variables, the importance of the *UGT1A1*\*28 genotype was verified (Table 6).

Among the 5 patients who had both grade 4 leukopenia and grade 3 or worse diarrhea concurrently, 2 had both *UGT1A1*\*28 and *UGT1A1*\*27, 2 were heterozygous for *UGT1A1*\*6, and one had none of the variant genotypes analyzed (homozygous for *UGT1A1*\*1). On the other hand, it is noteworthy that 4 of 5 patients (80%) who had the variant sequences both in the promoter (*UGT1A1*\*28) and in exon 1 (*UGT1A1*\*6 or *UGT1A1*\*27) suffered from life-threatening toxicity. There were 3 patients who did not encounter severe toxicity among the 7 patients homozygous for *UGT1A1*\*28. One of the 3 patients received chronically ursodesoxycholic acid and trepibutone for the treatment of cholelithiasis and, additionally, rifampin and isoniazid for 2 weeks, 1 month before the irinotecan infusion. Another ceased chemotherapy because of vomiting of blood caused by gastric ulcer after one-time infusion of irinotecan. The 2 patients homozygous for *UGT1A1*\*6 could be treated without severe toxicity, and all 3 patients heterozygous for *UGT1A1*\*27 experienced severe toxicity (Table 4).

There was a significant increase in the bilirubin levels after irinotecan infusion in both the patients who did (P < 0.001, Wilcoxon signed-rank test) and did not (P < 0.001; Table 4) encounter the severe toxicity. The increase in bilirubin levels after the initiation of therapy tended to be worse in the patients who experienced severe toxicity than in those who did not (P = 0.071, Mann-Whitney U test; Table 4).

DISCUSSION

The variant genotype in the promoter region, *UGT1A1*\*28, was significantly related to the severe toxicity induced by irinotecan, whereas with *UGT1A1*\*6 in exon 1, it was not. The multivariate analysis suggested that the patients who have *UGT1A1*\*28 would be seven times as likely to encounter severe toxicity from irinotecan than those who do not have it (Table 6). Although the use of other anticancer drugs also significantly affected severe toxicity, it was not beyond the *UGT1A1*\*28 genotype (Tables 2 and 6). The effects of female gender on severe toxicity did not reach significant levels in the current analysis (Tables 2 and 6). These findings clarify the clinical importance of *UGT1A1*\*28 for *UGT1A1* conjugation activity, especially in acute exposure to irinotecan.

We should mention that several biases might modify the distri-

Table 6 Multiple logistic regression analysis

Term	β <sup>a</sup>	SE	χ <sup>2</sup>	P	Odds ratio (95% CI)
Intercept	0.763	0.591			
<i>UGT1A1</i> *28	1.979	0.550	12.95	0.0003	7.23 (2.52–22.3)
Regimen <sup>b</sup>	1.510	0.557	7.36	0.0067	4.52 (1.53–13.9)
Female	0.849	0.508	3.10	0.0782	2.45 (0.90–6.75)

<sup>a</sup> β, coefficient.

<sup>b</sup> Regimen of irinotecan plus other anticancer drugs apart from platinum.

Table 5 Association of *UGT1A1* genotypes with total bilirubin levels in 115 patients

Genotypes <sup>a</sup>			n	Total bilirubin levels (μmol/l) <sup>b</sup>	
TATA box <i>UGT1A1</i> *28	Codon 71 <i>UGT1A1</i> *6	Codon 229 <i>UGT1A1</i> *27		Prior to therapy	Highest after infusion
-/-	-/-	-/-	67	8.6 (6.8–12.0)	13.7 (10.3–17.1)
-/-	+/-, +/+ <sup>c</sup>	-/-	25	10.3 (8.6–13.7)	15.4 (11.1–25.7)
+/-	-/-	-/-, +/- <sup>d</sup>	16	8.6 (6.8–10.3)	18.0 (12.0–23.1)
+/+	-/-	-/-, +/- <sup>e</sup>	7	12.0 (6.8–20.5)	34.2 (22.2–42.8)

<sup>a</sup> Symbols of (-/-), (+/-), and (+/+) denote homozygous absence of the variant allele, heterozygous, and homozygous for the variant allele, respectively.

<sup>b</sup> Median (interquartile range).

<sup>c</sup> Two patients homozygous for *UGT1A1*\*6 had bilirubin levels of 23.9 and 11.8 prior to therapy and 18.8 and 23.9 following initiation of therapy, respectively.

<sup>d</sup> One patient who had both *UGT1A1*\*28 and *UGT1A1*\*27 had bilirubin levels of 13.7 prior to therapy and 17.1 after initiation of therapy, respectively.

<sup>e</sup> Two patients homozygous for *UGT1A1*\*28 and heterozygous for *UGT1A1*\*27 had bilirubin levels of 10.3 and 44.5 prior to therapy and 47.9 and 42.8 after initiation of therapy, respectively.

butions of the *UGT1A1* polymorphisms in this study: (a) patients with high bilirubin levels would usually be precluded from irinotecan treatment because of a suspicion of liver dysfunction that may cause severe toxicity; (b) if our hypothesis is correct, potential patients who have the variant genotypes might die of fatal toxicity by irinotecan and inevitably be excluded from the analysis. This speculation is compatible with the fact that no patients had either *UGT1A1*\*29 or *UGT1A1*\*7, which occurs in common exons of *UGT1A* isoforms, resulting in a substantial reduction of their functional activities. Conversely, the patients who experienced severe toxicity from irinotecan would be more inclined to participate in this research than those who did not. Nevertheless, we consider that the patients analyzed here could approximate a population of Japanese cancer patients, because the incidence of severe toxicity in the patients of this research was comparable with those in the previous Phase II or III trials of irinotecan chemotherapy in Japan (2, 3). Thus, this retrospective research warrants a prospective trial to corroborate the usefulness of gene diagnosis of *UGT1A1* polymorphisms prior to irinotecan chemotherapy.

We should be careful to understand the exact clinical importance of *UGT1A1*\*6 for toxicity by irinotecan. According to *in vitro* expression studies, *UGT1A1*\*6 in the homozygous and heterozygous genetic states decreases the enzyme activity to 32 and 60% of control, respectively (25). In addition, *UGT1A1*\*6 has been reported to be a significant risk factor for nonphysiological hyperbilirubinemia among Japanese neonates (21, 22). The genetic effect of *UGT1A1*\*6 might be somehow masked in the current retrospective research; otherwise, the reduced enzyme activity might be compensated in adults by acquired factors. Particularly, *UGT1A1*\*6 as well as *UGT1A1*\*27 might considerably affect the susceptibility to irinotecan when they coexist with a variant sequence in the promoter *UGT1A1*\*28.

The patients with variant *UGT1A1* genotypes were not always those who encountered the severe toxicity by irinotecan, and *vice versa*. Generally, pharmacogenetic variations definitely alter the relevant drug effects, as observed in a poor metabolizer of thiopurine *S*-methyltransferase or dihydropyrimidine dehydrogenase (28, 29). In this research, the genetic effect of the genotypes on toxicity by irinotecan would be relatively weak, because they are originally responsible for Gilbert's syndrome that shows mild hyperbilirubinemia compared with Crigler-Najjar syndrome. Moreover, the bioavailability of SN-38 depends on the capacity of not only *UGT1A1* but also carboxylesterase that metabolically transforms irinotecan to SN-38. However, determining the *UGT1A1* genotype would be clinically important for Japanese patients because >20% of them have the variant genotypes and possibly have an increased risk of severe toxicity. Besides *UGT1A1* polymorphisms, a recent report suggested that the *UGT1A7* isoform would glucuronidate SN-38 more than *UGT1A1* (30). However, because *UGT1A7* is absent in human liver (31), a primary organ for detoxifying *i.v.* irinotecan, the evidence of SN-38 glucuronidation by *UGT1A7* does not deny the role of *UGT1A1*. In fact, the significant increase in bilirubin levels after irinotecan infusion (Tables 4 and 5) clearly supported that the glucuronidation of SN-38 in the liver should competitively inhibit that of bilirubin, which is a major substrate of *UGT1A1*. Because *UGT1A7* is expressed in gastrointestinal tissue, it might be important especially for the efficacy against colon cancer and the impact on diarrhea by irinotecan. Although genetic polymorphism of *UGT1A7* and their relationships with the phenotypic activity have not yet been identified, more precise estimation of the clinical effects by irinotecan might be possible by investigating the genetic variations, if any.

The bilirubin level seems an inadequate parameter to predict severe toxicity by irinotecan. At the suggestion of other investigators (32), we also observed that the bilirubin levels were increased more in the

patients who experienced severe toxicity than in those who did not, although the difference was not significant (Table 4). Indeed, the differences in bilirubin levels among the genotypes were statistically significant but seemed clinically negligible as a tool to predict toxicity (Table 5). Furthermore, *UGT1A1*\*28 appeared to be important for Gilbert's syndrome but not sufficient for the complete manifestation of the syndrome (19). The clinical usefulness of the bilirubin level might be improved if patients abstain from drug and alcohol use and are strictly fasted, but it does not seem to be practical.

Inter-ethnic differences can be easily predicted in metabolic profiles and clinical effects of irinotecan, although racial differences in tolerability and clinical outcomes of irinotecan treatment have not been investigated directly. Great differences in the distributions of the *UGT1A1* polymorphisms between Caucasians and Japanese populations have been reported; the frequency of *UGT1A1*\*28 in Caucasians is higher than among Japanese (20, 24). This implies that Caucasians might be more susceptible to the drug than the Japanese. On the contrary, *UGT1A1*\*6 and *UGT1A1*\*27, the variant sequences in exon 1, have been identified only in the Japanese (19, 21, 22). Although the clinical significance for irinotecan chemotherapy of these genotypes in exon 1 remains uncertain, they might cause Japanese to be more sensitive to the drug than Caucasians. These findings suggest racial differences in the importance of *UGT1A1* genotypes in irinotecan toxicity.

Individualization of drug dosage is critical for cancer chemotherapy to reduce unnecessary toxicity and to improve its therapeutic efficacy because the therapeutic index is often narrow. Oncologists traditionally used to predict toxicity by drugs and to optimize the dosage based on the patient's physiological factors (*e.g.*, body surface area, age), pathological conditions (*e.g.*, performance status, organ functions), and clinical history (*e.g.*, previous treatments). Recently, pharmacokinetic and pharmacodynamic analyses in chemotherapy provide more objective information for predicting the clinical effects of drugs. Furthermore, we believe that pharmacogenetic analyses would be another clue for individualized chemotherapy. If there is the recognized difference in drug disposition and sensitivity caused by the polymorphic drug-metabolizing enzyme, the optimal dosage required for response with the least toxicity would be different in patients with the different genotypes. In the present study, the determination of the *UGT1A1* genotypes for irinotecan treatment was suggested to be clinically useful. We are planning a dose escalation study of irinotecan in cancer patients who have been determined to have the variant genotypes.

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