

Pharmacogenetic impact of polymorphisms in the coding region of the *UGT1A1* gene on SN-38 glucuronidation in Japanese patients with cancer

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Pharmacogenetic testing for UDP-glucuronosyltransferase (*UGT*) *1A1*28*, a promoter variant of the *UGT1A1* gene, is now carried out clinically to estimate the risk of irinotecan-associated toxicity. We studied the clinical significance of *UGT1A1*6* and *UGT1A1*27*, two variants in exon 1 of the *UGT1A1* gene that are found mainly in Asians. The study group comprised 46 Japanese patients who received various regimens of chemotherapy including irinotecan at doses from 50 to 180 mg/m². Pharmacogenetic relationships were explored between the *UGT1A1* genotype and the ratio of the area under the plasma concentration–time curve (AUC) of the active metabolite of irinotecan (SN-38) to that of SN-38 glucuronide (SN-38G), used as a surrogate for *UGT1A1* activity (AUC_{SN-38}/AUC_{SN-38G}). No patient was homozygous for *UGT1A1*28*, and none had *UGT1A1*27*. Two were heterozygous for *UGT1A1*28*. Two were homozygous and 15 heterozygous for *UGT1A1*6*, all of whom were wild type with respect to *UGT1A1*28*. Two patients were simultaneously heterozygous for *UGT1A1*28* and *UGT1A1*6*, present on different chromosomes. The other 25 patients had none of the variants studied. The two patients simultaneously heterozygous for *UGT1A1*28* and *UGT1A1*6* and the two patients homozygous for *UGT1A1*6* had significantly higher AUC_{SN-38}/AUC_{SN-38G} ratios than the others ($P = 0.0039$). Concurrence of *UGT1A1*28* and *UGT1A1*6*, even when heterozygous, altered the disposition of irinotecan remarkably, potentially increasing susceptibility to toxicity. Patients homozygous for *UGT1A1*6* should also be carefully monitored. *UGT1A1* polymorphisms in the coding region of the *UGT1A1* gene should be genotyped in addition to testing for *UGT1A1*28* to more accurately predict irinotecan-related toxicity, at least in Asian patients. (*Cancer Sci* 2006; 97: 1255–1259)

Irinotecan is a camptothecin analog with potent antitumor activity resulting from the inhibition of topoisomerase. This anticancer drug is now used widely to treat colorectal, lung and other types of cancer.^(1,2) Dose-limiting toxicity of irinotecan includes severe leukopenia, neutropenia and diarrhea.^(3,4) Post-marketing studies of approximately 14 000 patients with cancer who received irinotecan in Japan have estimated that the incidence of grade 3 or higher leukopenia is 23.8% for irinotecan monotherapy and 38.3% for irinotecan combined with other drugs, whereas that of grade 3 or higher diarrhea is approximately 10%, regardless of regimen.⁽³⁾

Genetic polymorphisms of UDP-glucuronosyltransferase (*UGT*) *1A1*, a key metabolizing enzyme of irinotecan, are important determinants of individual variations in susceptibility to toxicity.⁽⁵⁾ Irinotecan is a prodrug that is metabolized by carboxylesterase to its principal active metabolite, SN-38. SN-38 is subsequently conjugated mainly by *UGT1A1* to a more polar, inactive glucuronide (SN-38G). Severe toxicity is attributed, at least in part, to increased exposure to SN-38 caused by decreased *UGT1A1* activity due to genetic polymorphisms.

Pharmacogenetic studies of irinotecan toxicity have therefore focused on genetic polymorphisms of the *UGT1A1* gene, especially *UGT1A1*28*, a variant sequence in the promoter region.^(6,7) Multiple studies have significantly linked this variant to irinotecan-related toxicity.⁽⁸⁾ The United States Food and Drug Administration has required that the package insert of irinotecan states that *UGT1A1*28* is associated with an increased risk of toxicity; to decrease this risk, genetic testing is encouraged. The package insert also recommends that the starting dose of irinotecan is reduced by at least one level for patients who are homozygous for *UGT1A1*28*; however, whether this reduction is sufficient remains unclear. Firm recommendations for patients who are heterozygous for *UGT1A1*28* have yet to be established. Such patients are likely to have an intermediate *UGT1A1* activity leading to an increased risk of neutropenia, but clinical evidence supporting the use of lower doses of irinotecan is lacking.

The clinical consequences of *UGT1A1*6* and *UGT1A1*27*, single nucleotide polymorphisms in exon 1 of the *UGT1A1* gene, also remain poorly understood. These variants are found mainly in Asians.⁽⁹⁾ Unlike *UGT1A1*28*, case-control studies of Japanese patients have failed to demonstrate a significant relationship between *UGT1A1*6* alone and severe irinotecan-related toxicity.⁽¹⁰⁾ However, the study suggested that the presence of *UGT1A1*6* and *UGT1A1*27* in addition to *UGT1A1*28* might increase susceptibility to irinotecan-related toxicity considerably. To better understand the clinical significance of these variants, especially the more frequent variant of *UGT1A1*6*, we examined how the coexistence of *UGT1A1*6* or *UGT1A1*27* and *UGT1A1*28* alters the pharmacokinetics of irinotecan in Japanese patients.

Materials and Methods

Patients. Forty-six Japanese patients with cancer who received irinotecan monotherapy or various regimens of irinotecan-based chemotherapy from June 2003 through April 2006 were studied. Some patients with metastatic colorectal cancer were also enrolled in a phase I study of a regimen containing irinotecan, fluorouracil (FU), and leucovorin (LV) (FOLFIRI), carried out at Saitama Medical School (Saitama, Japan). Toxicity was assessed prospectively according to the common terminology criteria for adverse events, version 3.0 (http://ctep.cancer.gov/reporting/ctc_v30.html). Tumor response was not included as a study variable because of various primary diseases and prior therapies. All subjects gave informed consent in writing for their peripheral blood samples and medical information to be used in this study. This study was approved by the Institutional Review Board of Saitama Medical School.

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Treatments. Irinotecan as a monotherapy was given weekly at doses of 50, 75 or 100 mg/m² for the first 3 weeks of a 4-week cycle or every 2 weeks at a dose of 150 mg/m² until the onset of disease progression or intolerable toxicity. For combination chemotherapy, a 100-mg/m² dose of irinotecan was administered with bolus FU 500 mg/m² and LV 10 mg/m² (*L* isomer form) weekly for the first 4 weeks of a 6-week cycle (IFL). The FOLFIRI regimen, administered at 2-week intervals, comprised irinotecan starting at 150 or 180 mg/m² and LV 200 mg/m² administered over the course of 2 h, followed by FU 400 mg/m² as a bolus injection and FU 2400 mg/m² as a 46-h continuous infusion. Regimens containing irinotecan plus cisplatin (IP) were repeated every 4 weeks. Irinotecan was given at a dose of 50–70 mg/m², followed 2 h later by a 120-min infusion of cisplatin 80 mg/m² with adequate hydration on day 1. The same dose of irinotecan was given again on day 15. For all regimens, 50–180 mg/m² of irinotecan was dissolved in 250 mL of 5% dextrose solution and infused over the course of 90 min.

Pharmacokinetic analysis. Blood samples for pharmacokinetic analysis were usually obtained during the first course of treatment with irinotecan. The samples were taken from the arm opposite the infusion site, at the beginning of irinotecan infusion and 0, 0.25, 0.5, 1, 2, 4, 8 and 24 h after the end of the infusion. The blood samples were centrifuged immediately, and the plasma was stored at –80°C until analysis. Total (lactone and carboxylate) plasma concentrations of irinotecan, SN-38 and SN-38G were analyzed by a modified reverse-phase high-performance liquid chromatographic (HPLC) method.⁽¹¹⁾ A 150- μ L plasma sample was mixed with 300 μ L of methanol, 5% perchloric acid (50:50, v/v) and camptothecin, serving as an internal standard, in a vortex mixer. The mixture was centrifuged at 20 600 *g* for 10 min, and 200 μ L of the supernatant was injected into an HPLC system (Hitachi model 7000 series; Hitachi, Tokyo, Japan), equipped with a TSK-gel ODS-120T analytical column (4.6 \times 250 mm, 4 μ m; TOSOH, Tokyo, Japan). HPLC was carried out at 40°C at a flow rate of 1.0 mL/min. Irinotecan, SN-38 and SN-38G were determined fluorometrically (excitation 355 nm; emission 515 nm). The total (lactone and carboxylate) concentrations of irinotecan, SN-38 and SN-38G in plasma were quantified. The mobile phase consisted of 75 mM ammonium acetate (pH 4.75) for solvent A, and acetonitrile for solvent B; a 20-min run was carried out with a linear gradient of 85–65% for solvent A.

The lower limit of quantification was 5 ng/mL (7.4 nM) for irinotecan and 0.5 ng/mL (1.2 nM and 0.88 nM, respectively) for SN-38 and SN-38G. The intra-assay and interassay coefficients of variation were less than 10% for irinotecan and the metabolites.

The area under the plasma time–concentration curve (AUC; μ mol·h/L) from the beginning of the infusion of irinotecan to the time of obtaining the last blood sample was calculated by a linear trapezoidal rule. The ratio of the AUC of SN-38 to that of SN-38G (AUC_{SN-38}/AUC_{SN-38G}) was used as a surrogate for UGT1A1 enzyme activity *in vivo*.

Genotyping. Genomic DNA was extracted from peripheral blood, which had been stored at –80°C until analysis, with the use of a QIAamp Blood Kit (QIAGEN, Hilden, Germany).

To analyze exon 1, first-step PCR amplification of a 923-bp fragment containing exon 1 was carried out as reported previously.⁽¹⁰⁾

For analysis of UGT1A1*6, the second set of polymerase chain reaction (PCR) amplifications was carried out with nested primers designed to amplify a 235-bp segment. The mismatched forward primer and the reverse primer were 5'-CTAGCACCTGACGCCTCGTTGTACATCAGAGCC-3' (+178 to +210; underlining indicates mismatched site) and 5'-CCATGAGCTCCTTGTTGTGC-3' (+393 to +412), respectively. The forward primer was designed to introduce an *Msp*I (Takara Shuzo Co., Otsu, Japan) restriction site into the wild-type sequence from

+209 to +212. A 1000-fold diluted product of the first PCR was subjected to nested PCR in a volume of 50 μ L containing 0.2 mM of each deoxynucleoside triphosphate, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, 0.5 μ M of each primer and 1.3 units of *Taq* polymerase (AmpliTaQ Gold; Perkin-Elmer, Foster City, CA, USA). The PCR conditions were 95°C for 15 min followed by 25 cycles of 94°C for 30 s, 55°C for 40 s and 72°C for 40 s. A 1- μ L PCR product was digested with 4 units of *Msp*I for 1 h at 37°C. DNA from the wild-type sequence was digested into 203- and 32-bp fragments, whereas DNA from UGT1A1*6 gave an undigested 235-bp fragment.

For the sequence of UGT1A1*27, another set of the second PCR amplifications was carried out using hemi-nested primers 5'-AGTACCTGTCTCTGCCAC-3' (+485 to +503) and 5'-GTCCCACTCCAATACACAC-3' (+865 to +867 and intron 1), designed to amplify a 399-bp segment. Two *Bsr*I (New England Biolabs, Ipswich, MA, USA) restriction sites exist in UGT1A1*27 (+552 to +556 and +684 to +688), but only one site (+552 to +556) exists in wild type. The method of PCR amplification was identical to that for the *Msp*I restriction fragment length polymorphism, as described above. Digestion of the PCR products with 2.5 units of *Bsr*I for 1 h at 65°C gave 199-, 132- and 68-bp fragments from UGT1A1*27, or 331- and 68-bp fragments from wild type.

UGT1A1*28 was distinguished from wild type by direct sequencing (–147 to +106) of the 253–255 bp produced by PCR.

A set of PCR amplifications of a 1030-bp fragment containing UGT1A1*28 and UGT1A1*6 was used to determine the location of these variant sequences on the respective alleles. The reaction mixture was similar to that described previously,⁽⁷⁾ except that the concentration of MgCl₂ was 2.0 mM, and the amount of *Taq* polymerase was 1.3 units. The PCR primers were 5'-AAGTGAAGTCCCTGCTACCTT-3' (–147 to –127) and 5'-GTCCCACTCCAATACACAC-3' (+865 to +867 and intron 1). The PCR conditions were 95°C for 15 min, followed by 30 cycles of 94°C for 30 s, 58°C for 40 s and 72°C for 40 s. The PCR fragments obtained were subcloned into pT7 Blue T-Vector (Novagen, Darmstadt, Germany), and sequence analysis was used to examine the gene arrangement of the variants on homologous chromosomes.

Statistical analysis. The statistical significance of differences in the AUC_{SN-38}/AUC_{SN-38G} was assessed using the Mann–Whitney *U* test. This and other statistical analyses were carried out with SPSS for Windows, version 12.0 J (SPSS Japan, Tokyo Japan). Differences were considered statistically significant when the two-tailed *P*-value was less than 0.05.

Results

A total of 46 patients were studied (Table 1). Performance status was generally good; most patients had gastrointestinal tract cancers. Fourteen patients received IP, 20 FOLFIRI, three IFL and nine irinotecan monotherapy. Two of the 14 patients treated with IP, which is one of the experimental arms of an ongoing randomized phase III trial against unresectable gastric cancer in Japan (JCOG9912), were enrolled into JCOG9912, and the other 12 patients treated with IP also fulfilled the recruitment criteria of JCOG9912 except for having no prior chemotherapy.

Genomic DNA from all patients was genotyped to examine UGT1A1 polymorphisms. No patient was homozygous for UGT1A1*28 or had UGT1A1*27 (Table 2). Two patients were heterozygous for UGT1A1*28. Two patients were homozygous and 15 were heterozygous for UGT1A1*6, all of whom were wild type with respect to UGT1A1*28. Two patients were simultaneously heterozygous for UGT1A1*6 and UGT1A1*28. Sequencing of the subcloned products obtained from the two

Table 1. Patient characteristics (n = 46)

Characteristic	n
Median age (years)	62 (range: 42–85)
Sex	
Female	20
Male	26
Performance status	
0	28
1	16
2	2
Primary organ	
Gastric	14
Colorectal	25
Other	7
Previous treatments	
Surgery	31
Radiotherapy	3
Systemic chemotherapy	38
1 regimen	30
2 regimens	8
None	6

Table 2. UGT1A1 genetic profiles and plasma concentration–time curve (AUC) ratios

UGT1A1*6	UGT1A1*28	n [†]	AUC _{SN-38} /AUC _{SN-38G}
-/-	-/-	24	0.43 (0.08–1.39) [‡]
+/-	-/-	15	0.61 (0.26–1.42) [‡]
+/+	-/-	2	1.40, 1.10
-/-	+/-	2	0.48, 0.44
+/-	-/+	2	1.04, 2.16

[†]Total n = 45. One patient was excluded because of an incomplete set of blood samples; [‡]median (range).

patients who were heterozygous for both *UGT1A1**6 and *UGT1A1**28 revealed that the variants existed on different chromosomes (*UGT1A1**6/*UGT1A1**28 diplotype), which was consistent with the results of Saeki *et al.*⁽¹¹⁾ The other 25 patients had none of the variants studied.

Complete sets of blood samples were obtained in 45 of the 46 patients. In the remaining one patient who received FOLFIRI, a blood sample could not be obtained for technical reasons at the end of the infusion. Pharmacokinetic profiling showed that SN-38G levels were higher than SN-38 levels; AUC_{SN-38}/AUC_{SN-38G} was therefore usually under 1 (Table 2; Fig. 1A). There was no apparent relationship between AUC_{SN-38}/AUC_{SN-38G} and the dose of irinotecan (data not shown). In the patient who was excluded from AUC analysis because of the missing blood sample, SN-38G levels were higher than SN-38 levels at all other time points. This patient had none of the variants studied.

In the two patients who had the heterozygous *UGT1A1**6/*UGT1A1**28 diplotype, SN-38G levels were lower than or approximated SN-38 levels. Consequently, their AUC_{SN-38}/AUC_{SN-38G} ratios were higher than those of the patients who had no variants or who were heterozygous for either *UGT1A1**6 or *UGT1A1**28 alone (Table 2; Figs 1B,C, 2). One of these patients who received the FOLFIRI regimen at an irinotecan dose of 150 mg/m² was studied again, after obtaining additional informed consent, to confirm reproducibility of the pharmacokinetic profile. The dose of irinotecan was reduced from 150 mg/m² in the first course to 100 mg/m² in the second course because of grade 4 neutropenia during the first course. The patient had

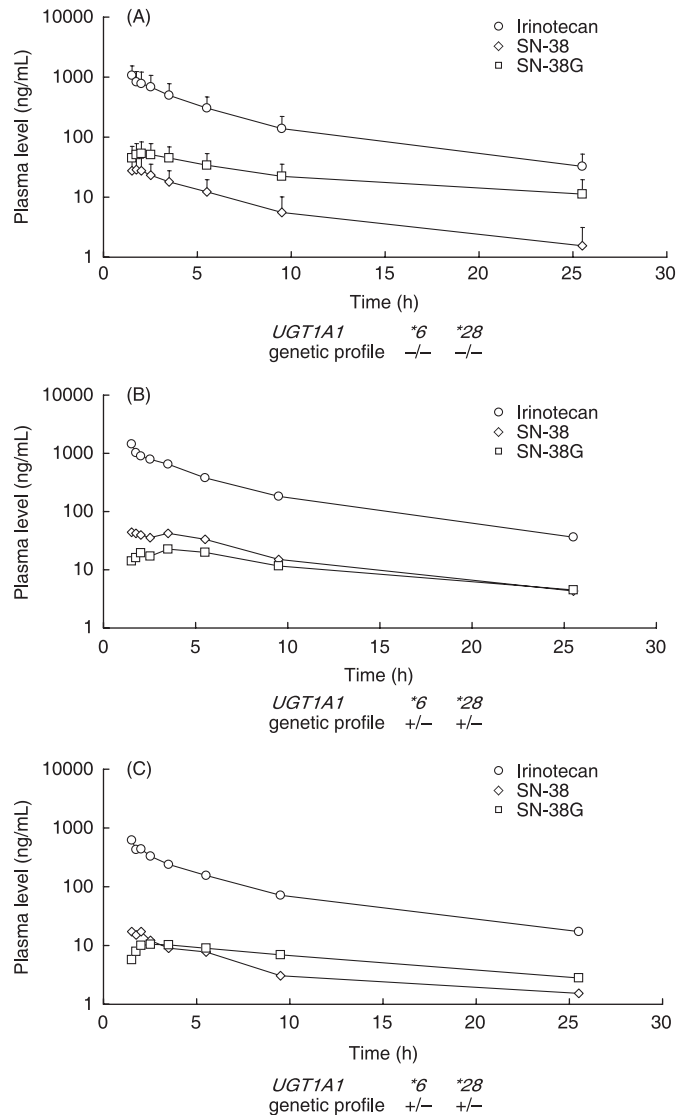


Fig. 1. Pharmacokinetic profiles of patients who received irinotecan. (A) The profile of 24 patients without *UGT1A1**28, *UGT1A1**6 and *UGT1A1**27 showed that SN-38 glucuronide (SN-38G) levels were higher than the SN-38 levels reported in previous studies. Each data point indicates mean \pm SD. (B) The patient with colon cancer received irinotecan at a dose of 150 mg/m² in FOLFIRI. (C) The patient with gastric cancer received irinotecan at a dose of 70 mg/m² in IP. SN-38G levels were lower than SN-38 levels in these two patients who were heterozygous for *UGT1A1**6 and *UGT1A1**28.

grade 2 neutropenia in the second course. The pharmacokinetic profiles were similar for the first and second courses of treatment; the AUC_{SN-38}/AUC_{SN-38G} was 2.16 in the first course and 1.56 in the second. The other patient who had the heterozygous *UGT1A1**6/*UGT1A1**28 diplotype received IP safely, with no apparent toxicity.

Two patients were homozygous for *UGT1A1**6. Their AUC_{SN-38}/AUC_{SN-38G} ratios were relatively higher than those of the other patients (Table 2; Fig. 2). One received irinotecan at a dose of 180 mg/m² in the FOLFIRI regimen and had grade 2 neutropenia in the first course of treatment. Pharmacokinetic studies were repeated in this patient with the same dose of irinotecan after obtaining additional informed consent. The patient had grade 3 neutropenia in the second course. Similar pharmacokinetic profiles were obtained; the AUC_{SN-38}/AUC_{SN-38G} ratio was 1.40

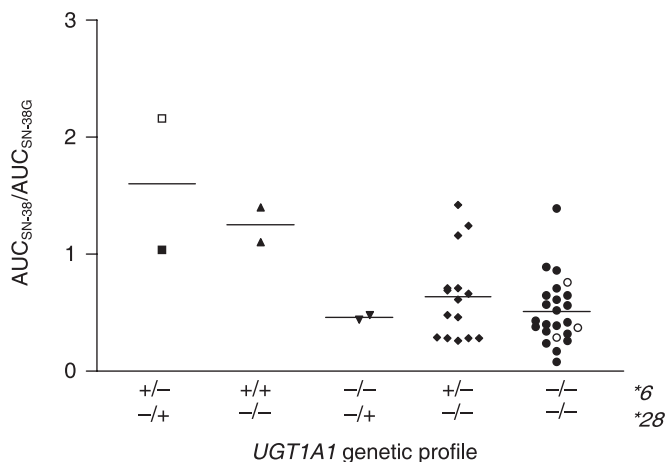


Fig. 2. Ratio of the area under the plasma concentration–time curve (AUC) for SN-38 to the AUC for SN-38 glucuronide (SN-38G), used as a surrogate for UGT1A1 activity (AUC_{SN-38}/AUC_{SN-38G}). Results are shown according to *UGT1A1* genetic profile for 45 Japanese patients who received various regimens of irinotecan chemotherapy. Open symbols in the figure represent the patients suffering from grade 4 neutropenia. Lines indicate median values. One patient was excluded because of an incomplete set of blood samples.

in the first course and 2.73 in the second. The other patient received irinotecan monotherapy at a dose of 75 mg/m² and had grade 3 nausea and grade 1 neutropenia. AUC_{SN-38}/AUC_{SN-38G} in this patient was 1.10. The AUC_{SN-38}/AUC_{SN-38G} ratios in the four patients who were heterozygous for *UGT1A1**28/*UGT1A1**6 or homozygous for *UGT1A1**6 were significantly higher than those in the other 41 patients ($P = 0.0039$).

Among the 45 patients, four patients suffered from grade 4 neutropenia. Two of them received FOLFIRI regimen. One of the patients with *UGT1A1**28/*UGT1A1**6 received a FOLFIRI regimen including irinotecan 150 mg/m², and the absolute AUC for SN-38 was the highest (1.06 μmol·h/L) among all patients who received FOLFIRI (median 0.56 μmol·h/L; range 0.49–1.06 μmol·h/L; irinotecan dose 150 or 180 mg/m²; $n = 19$). One who had wild-type *UGT1A1* alleles received FOLFIRI with irinotecan 180 mg/m², and the absolute AUC for SN-38 was not high (0.66 μmol·h/L). Another two who harbored wild-type *UGT1A1* alleles received IP regimens with irinotecan 60 or 70 mg/m². The AUCs for SN-38 were, respectively, 0.39 μmol·h/L and 0.26 μmol·h/L, similar to the values in the other patients given IP (median 0.30 μmol·h/L; range 0.20–0.68 μmol·h/L; $n = 12$). No patient had grade 3 or higher diarrhea in this study.

Discussion

The present study demonstrated that patients who were heterozygous for both *UGT1A1**6 and *UGT1A1**28 (*UGT1A1**6/*UGT1A1**28 diplotype) had markedly lower SN-38 glucuronidation activity than those who were heterozygous for either of these variants, let alone patients who had neither variant. Previous pharmacokinetic studies of irinotecan have reported that *UGT1A1**6 decreases SN-38 glucuronidation, despite the effects being somewhat lower than *UGT1A1**28 does.^(12,13) Heterozygous *UGT1A1**28 status is considered to result in UGT activity intermediate between that of wild type and that of homozygous *UGT1A1**28. Thus, the presence of both *UGT1A1**6 and *UGT1A1**28, even when heterozygous, apparently lowers SN-38 glucuronidation activity additively, resulting in a phenotypic effect similar to that associated with homozygous *UGT1A1**28. The mechanisms responsible for this add-on effect are unclear. *UGT1A1**6 and *UGT1A1**28 decrease

UGT1A1 activity via different mechanisms; the former variant lessens protein function directly, whereas the latter reduces transcriptional activity of the promoter. Genetic effects on UGT activity compensate for each other when these variants exist separately. In contrast, compensation might be hindered by the coexistence of *UGT1A1**6 and *UGT1A1**28, resulting in remarkably lower UGT activity.

Our results suggest that patients who have the *UGT1A1**6/*UGT1A1**28 diplotype are at elevated risk for severe irinotecan-related toxicity due to increased exposure to SN-38. This notion is supported by the fact that one of the four patients with severe toxicity had the *UGT1A1**6/*UGT1A1**28 diplotype. The AUC for SN-38 in this patient was higher than that of any other patients who received the same FOLFIRI regimen. We therefore believe that patients who have the *UGT1A1**6/*UGT1A1**28 diplotype should be treated carefully, similar to those who are homozygous for *UGT1A1**28. Furthermore, 2.5–4.6% of the Japanese population is estimated to have both *UGT1A1**6 and *UGT1A1**28,^(13–15) which is similar to, or slightly higher than, the percentage homozygous for *UGT1A1**28. These findings suggest that pharmacogenetic testing for irinotecan toxicity should include not only *UGT1A1**28, but also the coding region variant *UGT1A1**6, at least in Japanese patients.

Although the patients who were homozygous for *UGT1A1**6 had lower SN-38 glucuronidation activity than the others in this study, *UGT1A1**6 alone appears to have only a limited effect on irinotecan-related toxicity. Indeed, *UGT1A1**6 was not associated with severe toxicity, and even patients who were homozygous for *UGT1A1**6 could be treated safely in a previous study.⁽¹⁰⁾ Thus, patients who are heterozygous or even homozygous for *UGT1A1**6 alone can most likely tolerate conventional starting doses of irinotecan, unless they have other conditions associated with an increased risk of toxicity. In *in vitro* expression studies, however, homozygosity and heterozygosity for *UGT1A1**6 were associated with approximately 30 and 60% reductions in UGT activity, respectively.⁽¹⁶⁾ Otherwise, *UGT1A1**6 could be partially linked to other genetic polymorphisms that decrease overall UGT activity. We have recently identified genetic linkages of *UGT1A7* and *UGT1A9* polymorphisms to *UGT1A1**6, including those related to lower catalytic or transcriptional activities of UGT enzymes (K. Fujita *et al.*, unpublished data, 2006). In addition, a previous pharmacokinetic study showed that the patients who had *UGT1A1**6 alone showed slightly but not significantly decreased SN-38 glucuronidation activity,⁽¹³⁾ consistent with the results of our study. As for hyperbilirubinemia, *UGT1A1**6 is a significant contributory factor to unconjugated hyperbilirubinemia, including Gilbert's syndrome, especially among Japanese neonates.^(14,15) These findings suggest that patients who have *UGT1A1**6, especially when homozygous, are at increased risk for irinotecan-related toxicity. Although definitive evidence of this risk is lacking, we believe that toxicity should be monitored rigorously during irinotecan chemotherapy in patients who have *UGT1A1**6 alone.

The genetic basis for irinotecan-related toxicity apparently differs among distinct ethnic populations. That is to say, the same genetic variants affect toxicity differently. *UGT1A1* genetic polymorphisms differ considerably among genetically distinct populations; the allele frequency of *UGT1A1**28 is several times higher in whites (0.3–0.4) than in Asians (around 0.15).^(17,18) *UGT1A1**6 and *UGT1A1**27 have been identified only in Asians (0.11–0.23 for *UGT1A1**6 and 0.01–0.03 for *UGT1A1**27).^(6,9,12,14) As for hyperbilirubinemia, bilirubin levels are generally higher in Asians than in whites, at least among infants, suggesting that the genetic basis for hyperbilirubinemia differs between these two ethnic groups.^(19–21) The neonatal hyperbilirubinemia found in Japanese is significantly related to *UGT1A1**6, not *UGT1A1**28,^(14,15) whereas that occurring in whites during the first 2 days of life is associated with homozygosity

for *UGT1A1**28.⁽²²⁾ In the present study, the total bilirubin levels observed in the patients who were homozygous for *UGT1A1**6 and heterozygous for both of *UGT1A1**6 and *UGT1A1**28 were not significantly higher than those observed in the other patients. The complex genetic basis for *UGT1A1* activity makes it difficult to compare irinotecan-related toxicity among different ethnic groups. In addition, other factors potentially related to irinotecan-associated toxicity include patients' age, organ functions, prior treatments, dosing schedule and concurrently administered drugs. However, because genotype-based individualized chemotherapy with irinotecan is now practiced widely, studies comparing irinotecan toxicity among the different ethnic populations appear to be warranted.

In conclusion, our study showed that the coexistence of two *UGT1A1* variants, the promoter variant *UGT1A1**28 and the coding region variant *UGT1A1**6, remarkably altered the disposition of irinotecan, potentially increasing susceptibility to

toxicity, even when these variants were heterozygous. Patients who had the *UGT1A1**6/*UGT1A1**28 diplotype should therefore be treated similarly to those who are homozygous for *UGT1A1**28. Genotyping for *UGT1A1**6 in addition to *UGT1A1**28 is necessary to predict the risk of irinotecan-related toxicity, at least in Asian patients. Genomic information is hereafter expected to play an increasingly important role in optimizing the use of irinotecan therapy.

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