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ORIGINAL ARTICLE

Genetic linkage of *UGT1A7* and *UGT1A9* polymorphisms to *UGT1A1*6* is associated with reduced activity for SN-38 in Japanese patients with cancer

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Abstract
Purpose The phenotypic effects of *UGT1A7* and *UGT1A9* genetic polymorphisms on the in vivo pharmacokinetics of irinotecan were examined.
Methods Eighty-four Japanese patients with cancer who received irinotecan-based chemotherapy were enrolled. Polymorphisms present in *UGT1A7* (*T* to *G* transversion at −57 and *UGT1A7*2* to **9*), *UGT1A9* (9 or 10 repeat of *T* at −118 [−118(*T*)9 or 10] and *UGT1A9*2* to **5*), and *UGT1A1* (*UGT1A1*6*, *UGT1A1*27*, and *UGT1A1*28*) were analyzed for all patients. Pharmacokinetics of irinotecan were examined in 52 patients.
Results The most frequent haplotype (haplotype I, 56.7%, 95% CI 53.1–60.4) consisted of polymorphisms related to normal catalytic or transcriptional activity [*T* at −57 and **1* of *UGT1A7*, −118(*T*)10 of *UGT1A9*, and *UGT1A1*1*]. The second most frequent haplotype (haplotype II, 15.0%, 95% CI 12.4–18.3) consisted of polymorphisms related to reduced catalytic or transcriptional activity [−57*T* > *G* and **3* of *UGT1A7* and −118(*T*)9 of *UGT1A9* linked to *UGT1A1*6*]. The $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios in three patients homozygous for haplotype II were significantly higher than those in 20 patients with I/I diplotype ($P = 0.011$). Neither of these patients had *UGT1A1*28*.
Conclusion Genetic linkage of *UGT1A7* and *UGT1A9* polymorphisms to *UGT1A1*6*, related to reduced catalytic and transcriptional activities of UGTs, is associated with the decreased glucuronosyltransferase activity for SN-38 in Japanese patients with cancer.

Keywords Irinotecan · SN-38 · Polymorphism · *UGT1A7* · *UGT1A9* · *UGT1A1*6*

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Introduction

Irinotecan is a camptothecin analogue with high-antitumor efficacy that acts by inhibiting topoisomerase I. Irinotecan is a prodrug metabolized to its active metabolite SN-38, which is further conjugated by hepatic UDP-glucuronosyltransferase (UGT) 1A1, to yield the more polar, inactive SN-38 glucuronide (SN-38G) [1]. A (TA)7 within the promoter of the human *UGT1A1* gene (*UGT1A1*28*) has been associated with reduced glucuronidation capacity as well as with irinotecan-related dose-limiting toxicity, most commonly diarrhea and neutropenia [2–4].

Although UGT1A7 and UGT1A9 also participate in the glucuronidation of SN-38 in vitro [5–7], the in vivo roles of these UGTs remain poorly understood as compared with that of UGT1A1. UGT1A7 and UGT1A9, as well as UGT1A1 are encoded by a single *UGT1A* gene located on chromosome 2q37. UGT1A7 is expressed exclusively in the oropharynx, esophagus, stomach, and pancreas [8–12], but is absent in the liver [13]. In contrast, UGT1A9 is expressed in the liver, kidney, small intestine, colon, and reproductive organs such as the testis and ovary [8–10].

Functionally significant genetic polymorphisms have been described for *UGT1A7* and *UGT1A9* [6, 14, 15]. *UGT1A7*3*, **4*, **5*, **8*, and **9* and *UGT1A9*3* and **5*

reduce catalytic activity for SN-38 [6, 14, 15]. Polymorphisms affecting transcriptional activity have also been identified [16, 17]. Lankisch et al. [16] have shown that a *T* to *G* transversion at −57 (−57*T* > *G*), located in the putative TATA box of the *UGT1A7* gene, is related to a reduction in promoter activity to 30%. The 9 repeat of *T* allele at −118 [−118(*T*)9], resulting in lower transcriptional activity than −118(*T*)10, has been found in the 5′-flanking region of the *UGT1A9* gene [17]. Clinically, Carlini et al. [18] have shown that *UGT1A7*3/*3* is significantly associated with a good antitumor response to irinotecan and lack of severe gastrointestinal toxicity in patients with metastatic colorectal cancer. Furthermore, they have proposed that homozygosity for the presence of −118(*T*)9 sites in the *UGT1A9* gene is significantly related to enhanced response and reduced toxicity. These results have suggested that *UGT* genotypes causing low catalytic or transcriptional activity are associated with better responses to irinotecan. This hypothesis is consistent with the notion that low-catalytic activities or expression levels of UGTs might increase plasma concentrations of SN-38, enhancing the clinical response to irinotecan. However, the increased plasma concentrations of SN-38 seen in patients with *UGT* gene polymorphisms seem unlikely to relate to the reduced toxicity.

To gain better insight into the in vivo roles of UGT1A7 and UGT1A9 in SN-38 glucuronidation, we studied the relation between genotypes of *UGT1A7* and *UGT1A9* and the pharmacokinetics of irinotecan. First, we examined genetic polymorphisms present in *UGT1A7*, *UGT1A9*, and *UGT1A1* in 84 Japanese patients with cancer who received irinotecan-based chemotherapy. We then studied the relation between the genotype and the pharmacokinetics of irinotecan in 52 of these patients.

Patients and methods

Materials

Irinotecan, SN-38, and SN-38G were kindly supplied by Yakult Honsha (Tokyo, Japan). All chemicals and solvents were of the highest grade commercially available.

Patients

The study group comprised 84 Japanese patients (males/females, 52/32) with cancer (50 colons, 18 stomachs, seven ovaries, seven lungs, and two others) who received irinotecan monotherapy or various regimens of irinotecan-based combined chemotherapy from November 2004 through June 2006. A subset of the patients in the present study was included in the previous study [19]. The median age of the patients was 62 years (35–85). All patients gave informed consent in writing for their peripheral blood samples and medical information to be used for research. The study protocol was approved by the Institutional Review Board of Saitama Medical School.

Treatments

For monotherapy, irinotecan was given weekly at a dose of 100 mg/m^2 for the first 3 weeks of a 4-week cycle, or every 2 weeks at a dose of 150 mg/m^2. In combination with fluorouracil and leucovorin (IFL regimen), 100 mg/m^2 of irinotecan was administered weekly for the first 4 weeks of a 6-week cycle. In the FOLFIRI regimen, irinotecan was administered at 2-week intervals at doses of 150 or 180 mg/m^2. In combination with cisplatin (IP regimen), irinotecan was given at a dose of 50–70 mg/m^2 on day 1 of a 4-week cycle and at the same dose on day 15. For each of these regimens, irinotecan (50–180 mg/m^2) was infused over the course of 90 min.

Genotyping

Genomic DNA was extracted from 200 µl of peripheral blood, which had been stored at −80°C until analysis, with the use of a QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany).

UGT1A1

Two polymorphisms (G71R [**6*] and P229Q [**27*]) were analyzed by the polymerase chain reaction-restriction fragment length polymorphism method (PCR-RFLP) described by Ando et al. [2]. The TATA box polymorphism (TA)7 (**28*) was determined by the direct sequencing method described by Ando et al. [2].

UGT1A7

The *UGT1A7* promoter sequence was amplified by PCR, and sequence analysis was performed to determine −57*T* > *G* as described by Lankisch et al. [16], with minor modifications. Briefly, the reaction mixture consisted of 2.5 mM MgCl2 and 1.25 u of AmpliTaq Gold polymerase in a final volume of 50 µl.

Genotype of exon 1 was determined by direct sequencing of a PCR product that spans all of the polymorphic sites (N129K and R131K [**2*], N129K, R131K and W208R [**3*], W208R [**4*], G115S [**5*], E139D [**6*], N129K, R131K and E139D [**7*], N129K, R131K, E139D, and W108R [**8*] and G115S, N129K, and R131K [**9*]) as described by Carlini et al. [18], with minor modifications. Briefly, the reaction mixture consisted of 2.5 mM MgCl2

and 1.25 u of AmpliTaq Gold polymerase in a final volume of 50 µl.

UGT1A9

Five polymorphisms (−118(*T*)9 or 10 [based on assigning the A in the translation start codon as +1] [18], C3Y [**2*], M33T [**3*], Y242X [**4*], and D256N [**5*]) were evaluated by direct DNA sequencing of a PCR amplicon spanning all of the polymorphic sites as described by Carlini et al. [18], with minor modifications. Briefly, the reaction mixture consisted of 2.5 mM MgCl2 and 1.25 u of AmpliTaq Gold polymerase in a final volume of 50 µl.

Statistical analysis

Allele and genotype frequencies for each polymorphic allele in the *UGT1A7*, *UGT1A9*, and *UGT1A1* genes were determined by using SNPAlyze 5.0 (Dynacom, Yokohama, Japan). The significance of deviations from Hardy–Weinberg equilibrium was tested with the program SNPAlyze 5.0. Linkage disequilibrium analysis to make pairwise two-dimensional map of correlation coefficient r^2 and Lewontin's coefficient *D*' among single nucleotide polymorphisms, haplotype and diplotype configurations (combinations of haplotypes) analyses were also performed by an expectation-maximization-based algorithm using SNPAlyze 5.0. Polymorphisms not in Hardy–Weinberg equilibrium were excluded from the haplotype analysis.

The statistical significance of differences in the ratio of the AUC for SN-38 to that for SN-38G ($AUC_{SN\text{-}38}$/$AUC_{SN\text{-}38G}$) was assessed with the Mann–Whitney *U*-test. This and other statistical analyses were performed with SPSS for Windows, version 12.0J (SPSS Japan Inc., Tokyo Japan). Differences were considered statistically significant when the two-tailed *P*-value was less than 0.05.

Pharmacokinetic analysis

Pharmacokinetic analysis was performed in 52 subjects of this study. Blood samples for pharmacokinetic analysis were obtained during the first cycle of treatment. If necessary, blood samples were obtained during subsequent cycles of treatment to analyze pharmacokinetics. The blood 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 samples were immediately centrifuged, 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 reverse-phase high-performance liquid chromatography (HPLC) as described by Araki et al. [19]. The lower limit of quantification for irinotecan was 5 ng/ml (7.4 nM), and those for SN-38 and SN-38G were 0.5 ng/ml (1.2 and 0.88 nM). The intraassay and interassay coefficients of variation for irinotecan and the metabolites were less than 10%.

The area under the time-versus-concentration curve (AUC, µM h) from the beginning of the infusion to the time of obtaining the last blood sample was calculated by the linear trapezoidal rule, using a computer program (WinNonlin version 4.01 software, Pharsight Corporation, Mountain View, Calif).

Results

Polymorphisms in *UGT1A7*, *UGT1A9*, and *UGT1A1* in Japanese patients with cancer

The genotypes of *UGT1A7*, *UGT1A9*, and *UGT1A1* were determined in the 84 patients. The allele frequencies of the −57*T* > *G* allele of *UGT1A7* and the −118(*T*)9 allele of *UGT1A9*, associated with reduced transcriptional activity, were 25.0 and 38.1%, respectively. The allele frequency of these polymorphisms in Japanese was reported to be 22, and 34–40%, respectively [17, 20]. The frequency of *UGT1A7*3*, related to the reduced catalytic activity, and *UGT1A7*2* were 29.2 and 11.9%, respectively. Previous studies demonstrated that the frequencies of *UGT1A7*3* and *UGT1A7*2* in Japanese were about 25 and 15%, respectively [21, 22]. The allele frequencies of *UGT1A1*6* and *UGT1A1*28* were 22.6 and 9.5%, respectively. The reported frequencies were 15.1 and 13.3%, respectively [23]. All allele frequencies, except for those of *UGT1A1*27* (0.6%) and *UGT1A9*5* (0.6%), were in Hardy–Weinberg equilibrium ($P > 0.05$). There was no patient harboring *UGT1A7*4–*9* and *UGT1A9*2–*4*.

Diplotypes or genotypes of *UGT1A7*, *UGT1A9*, and *UGT1A1* found in the patients are shown in Table 1. The frequencies of homozygosity for −57*T* > *G* of *UGT1A7* and of *UGT1A7*3/*3* were both 7.1%, and that of homozygosity for −118(*T*)9 of *UGT1A9* was 10.7%. The frequencies of *UGT1A1*6/*6* and *UGT1A1*6/*28* were both 4.8%.

Linkage disequilibrium analysis

The result of the linkage disequilibrium analysis is shown in Fig. 1. We found that −118(*T*)9 of *UGT1A9* was highly linked with *UGT1A7* variants to cause N129K and R131K ($r^2 = 0.88$, *D*' = 1). −57*T* > *G* of *UGT1A7* gene was linked with *UGT1A7*3* ($r^2 = 0.7$, *D*' = 0.93). *UGT1A1*6* was linked with *UGT1A7*3* ($r^2 = 0.55$, *D*' = 0.88) and −57*T* > *G* of *UGT1A7* ($r^2 = 0.48$, *D*' = 1). r^2 and *D*'-values seen between *UGT1A1*6* and −118(*T*)9 of *UGT1A9* were 0.29 and 0.79, respectively.

Table 1 Diplotypes or genotypes of the *UGT1A7*, *UGT1A9*, and *UGT1A1* genes found in Japanese patients with cancer

Gene	Genotype or diplotype	Number	Frequency (%)
UGT1A7	−57 *T/T*	48	57.1
	−57 *T/G*	30	35.8
	−57 *G/G*	6	7.1
	**1/*1*	26	31.0
	**1/*2*	14	16.7
	**2/*2*	1	1.2
	**1/*3*	33	39.2
	**2/*3*	4	4.8
	**3/*3*	6	7.1
UGT1A9	−118(*T*) 9/9	9	10.7
	−118(*T*) 9/10	46	54.8
	−118(*T*) 10/10	29	34.5
	**1/*1*	83	98.8
	**1/*5*	1	1.2
UGT1A1	**1/*1*[a]	38	45.1
	**1/*28*	12[b]	14.3
	**6/*28*	4	4.8
	**1/*6*	26	31.0
	**6/*6*	4	4.8

The number of patients was 84

[a] *UGT1A1*1* was defined as the allele not possessing **28*, **6*, and **27*

[b] The **28* and **27* were assumed to exist on the same allele [2, 22]

Haplotype structures of *UGT1A1*, *UGT1A7*, and *UGT1A9*

Since *UGT1A1*27* and *UGT1A9*5* were not in Hardy–Weinberg equilibrium ($P < 0.05$), these loci were excluded from haplotype analysis. Sai et al. [22] have found that *UGT1A1*6* and *UGT1A1*28* do not exist on the same allele. Therefore, we performed haplotype analysis with the *UGT1A1*1*, *UGT1A1*6*, and *UGT1A1*28* alleles.

Fifteen haplotypes estimated are shown in Table 2. The most frequent haplotype (haplotype I, 56.7%, 95% CI 53.1–60.4) consisted of polymorphisms related to normal catalytic or transcriptional activity. The second most frequent haplotype (haplotype II, 15.0%, 95% CI 12.4–18.3) consisted of polymorphisms related to reduced catalytic or transcriptional activity (−57*T* > *G* and **3* of *UGT1A7*, −118(*T*)9 of *UGT1A9*, and *UGT1A1*6*). In addition to the linkage disequilibrium analysis, these findings supported the genetic linkage among *UGT1A7* and *UGT1A9* polymorphisms and *UGT1A1*6*.

Relations between reduced glucuronidation capacity for SN-38 and haplotype structures of *UGT1A1*, *UGT1A7*, and *UGT1A9*

Pharmacokinetic analysis of irinotecan and its metabolites SN-38 and SN-38G was performed in 52 subjects. Their characteristics are summarized in Table 3. The distribution of $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios is shown in Fig. 2. The median of the $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios was 0.5. Diplotype configurations in the *UGT1A7*, *UGT1A9*, and *UGT1A1* genes were estimated by the haplotype analysis with the all of 84 Japanese patients with cancer. The diplotype configurations for 52 patients with pharmacokinetic data are shown in Table 4. The number of patient(s) showing $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratio(s) higher than 1.0 and the ratio(s) are also described in the Table 4. Three patients were homozygous for haplotype II. The $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios observed in these patients were 1.40, 1.10, and 1.11, respectively. The patient showing the 1.40 of $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$

		UGT1A9	*UGT1A7*				*UGT1A1*	
		-118(T)9	-57T>G	N129K	R131K	W208R	(TA)7	G71R
UGT1A9	-118(T)9		0.815	1	1	0.8475	0.8265	0.7877
UGT1A7	-57T>G	0.3598		1	1	0.9289	0.4696	0.7363
	N129K	0.8829	0.4783		1	1	0.809	1
	R131K	0.8829	0.4783	1		1	0.809	1
	W208R	0.4806	0.6985	0.5908	0.5908		0.5341	0.8773
UGT1A1	(TA)7	0.1169	0.0697	0.0988	0.0988	0.0729		1
	G71R	0.2947	0.4754	0.4194	0.4194	0.5464	0.0308	

Fig. 1 Linkage disequilibrium analysis for *UGT1A7*, *UGT1A9* and *UGT1A1* single nucleotide polymorphisms r^2 (*lower red*) and *D'* (*upper blue*) values are shown in each *square*. r^2-values; *open squares*, 0–0.25; *pink squares*, 0.25–0.5; *red squares*, 0.5–1.0: *D'*-values; *open squares*, 0–0.6; *light blue squares*, 0.6–0.8; *dark blue squares*, 0.8–1.0. *UGT1A7*2* causes N129K and R131K. *UGT1A7*3* causes N129K, R131K, and W208R

Table 2 Haplotype structures of the *UGT1A7*, *UGT1A9*, and *UGT1A1* genes in Japanese patients with cancer

Haplotype	*UGT1A7*		*UGT1A9*	*UGT1A1*	Frequency (%) (95%CI)
	−57	Exon1	−118(*T*)9 or 10		
I	*T*	**1*	10	**1*	56.7 (53.0–60.4)
II	*G*	**3*	9	**6*	15.0 (12.4–18.3)
III	*T*	**2*	9	**1*	8.64 (6.67–10.8)
IV	*G*	**3*	9	**28*	5.05 (3.31–6.73)
V	*T*	**3*	9	**6*	3.31 (2.04–4.80)
VI	*T*	**1*	10	**28*	2.18 (1.09–3.59)
VII	*G*	**3*	10	**6*	1.87 (0.827–2.93)
VIII	*G*	**3*	9	**1*	1.85 (0.924–3.05)
IX	*T*	**2*	9	**28*	1.45 (0.563–2.58)
X	*T*	**3*	9	**28*	0.834 (0.338–1.75)
XI	*G*	**2*	9	**6*	0.684 (0.318–1.46)
XII	*T*	**3*	9	**1*	0.624 (0.306–1.27)
XIII	*T*	**2*	9	**6*	0.613 (0.302–1.24)
XIV	*T*	**3*	10	**6*	0.595 (0.298–1.19)
XV	*G*	**2*	10	**6*	0.510 (0.108–1.16)

*UGT1A1*27* and *UGT1A9*5* were not included in this analysis because these loci were not in Hardy–Weinberg equilibrium

Table 3 Demographic characteristics of patients participating in the pharmacokinetic study

		Number
Age (year)[a]	62 (42–85)	52
Sex	Male	32
	Female	20
Performance status	0	33
	1	17
	2	2
Creatinine (mg/dl)[a]	0.66 (0.42–1.15)	52
Total bilirubin (mg/dl)[a]	0.5 (0.2–1.1)	52
Tumor type	Colon	27
	Stomach	14
	Ovarian	4
	Others	7
Type and dose of irinotecan therapy (mg/m^2)[a]	Monotherapy 100 (50–150)	12
	IFL 100 (50–150)	3
	FOLFIRI 180 (150–180)	22
	IP 80 (60–100)	15
Toxicity	Grade 4 neutropenia	5
	Grade 3 neutropenia	4
	Grade 3 diarrhea	0

[a] The values are expressed as the median with the range in parentheses

received the same dose of irinotecan in the second cycle of treatment (FOLFIRI, 180 mg/m^2) as the first cycle. The $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratio during the second cycle was also high (2.73). The relation between the $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios and diplotype configurations of I/I, I/II, and II/II was examined (Fig. 3). The Kruskal–Wallis test for these three data sets (three diplotypes) yielded *P*-value of 0.027. The $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios in the three patients who were homozygous for the haplotype II were significantly higher than those in 20 patients with I/I diplotype ($P = 0.011$). The diplotype configurations II/II occurred at frequency of 3.6% in Japanese patients with cancer (Table 4).

Discussion

Our study showed that genetic linkage of *UGT1A7* and *UGT1A9* polymorphisms to *UGT1A1*6*, related to low catalytic and transcriptional activities of UGTs, was associated with the pharmacokinetics of irinotecan and with reduced glucuronosyltransferase activity for SN-38. Given that UGT1A7 and UGT1A9 might be involved in the glucuronidation of SN-38 in vivo, the lower glucuronidation capacity for SN-38 in patients homozygous for haplotype II was probably caused not only by *UGT1A1*6*, but also by polymorphisms in the *UGT1A7* and *UGT1A9* gene.

Previous studies have shown that the gene product of *UGT1A7*3* has about 50% lower catalytic activity for SN-38 than that of *UGT1A7*1* [6, 14]. The $-57T > G$ polymorphism in the putative TATA box of *UGT1A7* reduces promoter activity to 30% [16]. However, an in vivo role of UGT1A7 might be less likely, because this enzyme is not expressed in liver or intestine [8–13]. Yamanaka et al. [17] have demonstrated that −118(*T*)9 polymorphism is related to the 2.6-fold lower transcriptional activity of the *UGT1A9*

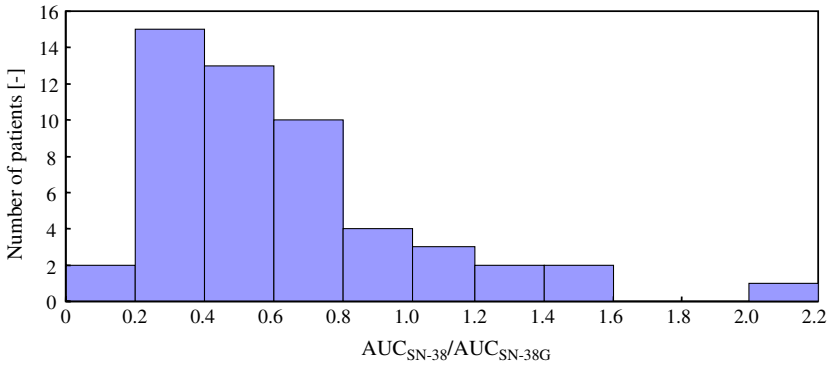

Fig. 2 Distribution of AUC_{SN-38}/AUC_{SN-38G} ratios. The median value of the AUC_{SN-38}/AUC_{SN-38G} ratios was 0.5

Table 4 Diplotype configurations in *UGT1A7*, *UGT1A9*, and *UGT1A1* in Japanese patients with cancer

Haplotype[a]/ haplotype	All patients		Fifty-two patients with pharmacokinetic data
	n	Frequency (%)	*n*
I/I	25	29.7	20 (1; 1.39)[b]
I/II	14	16.6	11 (2; 1.16 and 1.24)
I/III	10	11.8	6
I/IV	6	7.1	1
I/V	4	4.8	1
I/VI	1	1.2	
I/VII	2	2.4	1
I/VIII	2	2.4	2
I/IX	3	3.6	1
I/X	1	1.2	
I/XII	1	1.2	
I/XIII	1	1.2	1
I/XIV	1	1.2	
II/II	3	3.6	3 (3; 1.40, 1.10, and 1.11)
II/III	2	2.4	2
II/VI	2	2.4	1 (1; 1.04)
II/XV	1	1.2	
III/V	1	1.2	
III/XI	1	1.2	1 (1; 1.42)
IV/V	1	1.2	
IV/VII	1	1.2	1 (1; 2.16)
IV/VIII	1	1.2	
Total	84		52

[a] The haplotype numbers are identical to those shown in Table 2

[b] Numbers in parenthesis represent the number of patient(s) showing AUC_{SN-38}/AUC_{SN-38G} ratio(s) higher than 1.0 and the ratio(s)

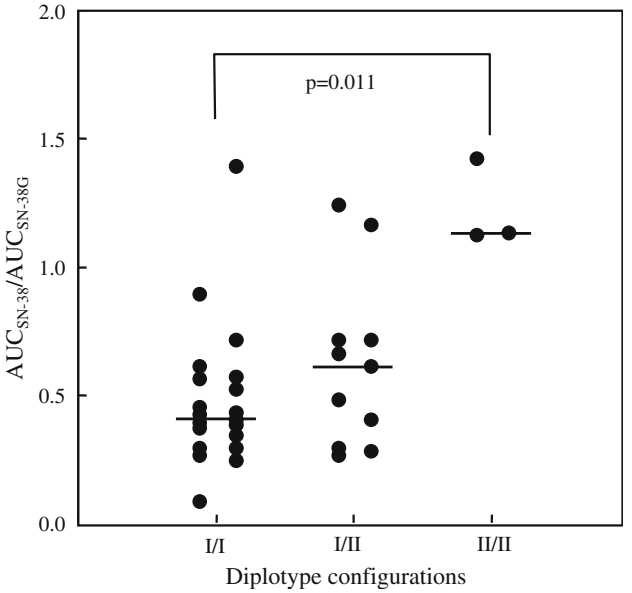

Fig. 3 Relationship between plots of the AUC for SN-38 versus the ratio of the AUC for SN-38 and the diplotype configurations. Numbers of patients for respective diplotype configurations I/I, I/II, and II/II were 20, 11, and 3. *Lines indicate* median-value

gene. On the other hand, the no association between −118(*T*)9 polymorphism and UGT1A9 protein level has been reported [24]. Innocenti et al. [25] have demonstrated that SN-38 glucuronidation rate was higher in patients heterozygous for −118(*T*)9 than in patients with homozygous −118(*T*)10. Taking these results into account, an alternative hypothesis is rose.

(1) UGT1A1 has a role in SN-38 glucuronidation in vivo, whereas UGT1A7 and UGT1A9 do not.
(2) The polymorphisms in the *UGT1A7* and *UGT1A9* gene are linked to *UGT1A1*6* because of the close proximity of these loci (<100 kb) (GeneBank, AF297093).
(3) The reduced glucuronidation capacity for SN-38 in patients homozygous for haplotype II is principally caused by *UGT1A1*6*.

If patients homozygous for polymorphism in *UGT1A7* and *UGT1A9* together with *UGT1A1*1* (e.g., haplotype VIII) could be identified, it would theoretically be possible to evaluate the roles of UGT1A7 and UGT1A9 by comparing the in vivo pharmacokinetics of irinotecan in these patients

with the pharmacokinetics in patients homozygous for haplotype II. However, patients homozygous for haplotype VIII were not found in the present study. Therefore, from the results of the present study, it is difficult to assess the in vivo roles of UGT1A7 and UGT1A9 in SN-38 glucuronidation on the basis of the phenotypic effects of haplotype II. Further studies should need.

In whites, the allele frequencies of $-57T>G$ and **3* in the *UGT1A7* gene and of $-118(T)9$ in the *UGT1A9* gene are higher than those in Japanese (39, 36, and 61%, respectively) [16, 17, 23, 25, 26]. The frequency of *UGT1A1*6* in whites is estimated to be lower than that in Asians, including Japanese [2, 25, 27], whereas the frequency of the *UGT1A1*28* allele is higher in whites than in Asians [2, 28]. These findings suggest that the genetic linkages of these polymorphisms in *UGT1A7* and *UGT1A9* to *UGT1A1*6*, frequently seen in Japanese patients with cancer, probably occur at lower frequencies in whites.

In a patient homozygous for haplotype II showing the $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratio of 1.40, grade 3 neutropenia developed during the second cycle of treatment with irinotecan, given in the same dose as the first cycle (FOLFIRI, 180 mg/m^2). The other patient with the diplotype II/II with the $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratio of 1.11 suffered from grade 4 neutropenia 10 days after the first cycle of irinotecan treatment (monotherapy, 100 mg/m^2). Therefore, the third cycle of irinotecan treatment for the patient was discontinued. These results might indicate that the configuration of diplotype (II/II) might be important for predicting not only lower SN-38 glucuronidation capacity, but also the risk of irinotecan-related toxicity in Japanese patients with cancer.

The diplotype configuration in a patient showing the highest $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratio (2.16) consisted of haplotype VII and haplotype IV (Table 4). The structure of haplotype VII was nearly consistent with that of haplotype II, except for -118(*T*)10 of *UGT1A9*, indicating genetic linkages of $-57T>G$ and **3* of *UGT1A7* to *UGT1A1*6*. The polymorphisms of *UGT1A7* and *UGT1A9* in haplotype IV were similar to those seen in haplotype II and were linked to *UGT1A1*28*. The higher $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratio seen in the patient may be related to this diplotype configuration. Furthermore, the patient suffered from the grade 4 neutropenia at the first treatment of irinotecan (150 mg/m^2) with FOLFIRI regimen. Therefore, the dose of irinotecan was reduced to 100 mg/m^2 at the second treatment. Although the $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ seen in the patient at the second treatment was still high (1.56), the severe toxicity was not observed.

Among nine patients showing $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G}$ ratios higher than 1.0, 6 patients did not suffer from irinotecan-related sever toxicity. According to our data, dose of irinotecan was not necessarily correlated to the sever toxicity in these of nine patients. Factors determining the sensitivity to the irinotecan-related toxicity need to be examined.

Recently, the novel *UGT1A9* intronic *c.* $855+399C>T$ polymorphism has been reported to appear as a predictor of SN-38 glucuronidation levels in the liver [29]. The most of the liver samples used in this study were from Caucasian, and did not include those from Japanese. Therefore, it is of interest to know the impact of this polymorphism on the irinotecan pharmacokinetics in Japanese patients with cancer.

In genotype-pharmacokinetic association analysis, Han et al. [30] had recently reported that *UGT1A1*6/*6* ($n=6$), *UGT1A7*3/*3* ($n=6$), and $-118(T)9/9$ in the *UGT1A9* ($n=11$) were associated with significantly lower glucuronosyltransferase activity in Korean patients, despite they did not examine the effects of $-57T>G$ variant in *UGT1A7* gene. The results obtained by Han et al. and by us indicated that the combination of genotypes of *UGT1A7*, *UGT1A9* and *UGT1A1* might be important to predict atypical irinotecan pharmacokinetics and reduced glucuronosyltransferase activity in Asian patients with cancer.

In conclusion, our study showed that genetic linkages of *UGT1A7* and *UGT1A9* polymorphisms to *UGT1A1*6*, related to low catalytic and transcriptional activities of UGTs, is associated with the pharmacokinetics of irinotecan and reduced glucuronosyltransferase activity for SN-38.

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