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Genetic Polymorphisms of the UDP-Glucuronosyltransferase 1A7 Gene and Irinotecan Toxicity in Japanese Cancer Patients

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Irinotecan often causes unpredictably severe, occasionally fatal, toxicity involving leukopenia or diarrhea. It is converted by carboxyesterase to an active metabolite, SN-38, which is further conjugated and detoxified to SN-38-glucuronide by UDP-glucuronosyltransferase (UGT). We genotyped the *UGT1A7* gene by direct sequencing analysis and polymerase chain reaction-restriction fragment length polymorphism in 118 cancer patients and 108 healthy subjects. All the patients had received irinotecan-containing chemotherapy and were evaluated to see whether the variant *UGT1A7* genotype would increase the likelihood of severe toxicity of irinotecan consisting of grade 4 leukopenia and/or grade 3 or more diarrhea. Among the 26 patients with severe toxicity, the allele frequencies were 61.5% for *UGT1A7*1*, 15.4% for *UGT1A7*2*, and 23.1% for *UGT1A7*3*. On the other hand, the frequencies were 63.6% for *UGT1A7*1*, 15.8% for *UGT1A7*2*, and 20.7% for *UGT1A7*3* among the 92 patients without severe toxicity. None of the 118 patients had *UGT1A7*4*. Neither univariate analysis (odds ratio, 1.13; 95% confidential interval, 0.46–2.75) nor multivariate logistic regression analysis (odds ratio, 0.74; 95% confidential interval, 0.26–2.07) found any significant association between carrying at least one of the variant alleles and the occurrence of severe toxicity. The distribution of *UGT1A7* genotypes in 108 healthy subjects was not significantly different from that in the patients ($P=0.99$ and 0.86 for those with and without severe toxicity, respectively), but significantly less than that in Caucasians reported previously ($P<0.001$). The results suggested that determination of *UGT1A7* genotypes would not be useful for predicting severe toxicity of irinotecan.

Key words: UDP-glucuronosyltransferase 1A7 — Irinotecan — SN-38 — Genetic polymorphisms

Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperi dino]-carbonyloxycamptothecin, CPT-11) is a camptothecin analogue with strong antitumor activity that inhibits topoisomerase I, which is now commonly used in the treatment of patients with colorectal or lung cancers.[1, 2] Irinotecan is hydrolyzed by carboxylesterase to form SN-38 (7-ethyl-10-hydroxycamptothecin), which has a 100- to 1000-fold higher antitumor activity than the parent drug.[3] SN-38 is conjugated by UDP-glucuronosyltransferase (UGT) 1A1 in the liver to yield SN-38 glucuronide, which has 1/100 the antitumor activity of SN-38.[4] The SN-38 glucuronide is excreted in the small intestine via bile, where bacterial glucuronidase resolves the glucuronide into SN-38 and glucuronic acid.[5] A part of the SN-38 is reabsorbed from the intestine into body, resulting in enterohepatic circulation of SN-38.[6]

Irinotecan often causes unpredictably severe, occasionally fatal, toxicity, involving leukopenia or diarrhea. An inter-individual difference in drug sensitivity would be caused by a difference in drug disposition after irinotecan administration.[6, 7] We have recently suggested that genetic polymorphisms of *UGT1A1* could explain some of the inter-individual differences in the pharmacokinetics and pharmacodynamics of irinotecan.[8, 9] The metabolic ratios (SN-38/SN-38 glucuronide) in a patient homozygous for *UGT1A1*28*, having a 2-base pair (bp) insertion (TA) in the TATA box in the promoter region, were uncharacteristically higher than those in other patients.[8] Furthermore, a case control study of 118 Japanese patients, who had received irinotecan for cancer, revealed that genotypes either heterozygous or homozygous for *UGT1A1*28* would be a significant risk factor for severe irinotecan toxicity.[9] Thus, we considered that patients with variant genotypes of the *UGT1A1* gene would be at higher risk for severe toxicity due to a relatively increased bioavailability of active unconjugated SN-38.

Besides UGT1A1, another UGT isoform, UGT1A7, has been recently reported to glucuronidate SN-38 at a more than 9-fold higher level than that by UGT1A1 *in vitro*, using the human UGT1 enzymes expressed transiently in COS-1 cells.[10] UGT1A7 is expressed in the gastrointestine and lung, but not in the liver.[11, 12] Since it has been

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reported that SN-38 concentrations in intestinal tissues, as well as in liver, were high after irinotecan administration,[13] it can be speculated that SN-38 is conjugated to SN-38 glucuronide again by UGT1A7 after reabsorption into the intestinal tissues. Furthermore, an inverse relationship between SN-38 glucuronidation and diarrhea was reported in cancer patients treated with irinotecan.[14] In mouse models, a dose-dependent relationship between diarrhea and accumulation of SN-38 in the intestine has been noted after intraperitoneal administration of irinotecan.[15] Thus, we hypothesized that the *UGT1A7* polymorphisms would also affect the occurrence of severe toxicity by irinotecan, especially in respect to diarrhea, through modifying the enzyme activity in gastrointestinal tissues. Single nucleotide polymorphisms (SNPs) of the *UGT1A7* gene are known to be linked to the *in vitro* enzymatic activity: *UGT1A7*1* ($N^{129}R^{131}W^{208}$ as the reference sequence), *UGT1A7*2* ($K^{129}K^{131}W^{208}$), *UGT1A7*3* ($K^{129}K^{131}R^{208}$), and *UGT1A7*4* ($N^{129}R^{131}R^{208}$).[16] *UGT1A7*2* comprises two transversions and one transition (T387G, C391A and G392A), which produce the amino acid substitutions Asn129Lys and Arg131Lys, respectively. *UGT1A7*3* comprises two transversions and two transitions (T387G, C391A, G392A and T622C), producing Asn129Lys, Arg131Lys and Trp208Arg, respectively. *UGT1A7*4* has a T622C transition producing a Trp208Arg substitution. When these four UGT1A7 variants were expressed in HEK cells and their *in vitro* enzymatic activities toward benzo(*a*)pyrene metabolites were examined, the membrane from the UGT1A7*3-expressing cells exhibited a 5.8-fold lower relative V_{max} compared to that of UGT1A7*1, whereas UGT1A7*2 and UGT1A7*4 had a 2.6- and 2.8-fold lower relative V_{max} than UGT1A7*1, respectively. While the previous population study revealed that more than 85% of Caucasians had at least one of the three variant alleles,[16] the frequency of *UGT1A7* allele in Asians has not yet been reported. We hypothesized that *UGT1A7* polymorphisms would also affect inter-patient or inter-ethnic variations in sensitivity to drugs or carcinogens.

The purpose of this study is to evaluate the influence of genetic polymorphisms of *UGT1A7* gene on risk for severe irinotecan toxicity in cancer patients treated with irinotecan, as well as the frequency of the *UGT1A7* allele in the healthy Japanese population.

MATERIALS AND METHODS

Subjects We genotyped the *UGT1A7* gene in 118 cancer patients and 108 healthy subjects (31 females and 77 males; median age 49 years). We retrospectively reviewed the clinical records including patient characteristics (age,

Table I. Baseline Characteristics of Patients

	Leukopenia (grade 4) and/or diarrhea (grade 3 or worse)[a]		
	Experienced (N=26)	Not experienced (N=92)	P
Gender (men/women)	14/12	66/26	0.085[b]
Median age (range, years)	60 (38–76)	61 (41–75)	>0.2[c]
Performance status			>0.2[b]
0	8 (31%)	31 (34%)	
1	15 (58%)	51 (55%)	
≥2	3 (12%)	10 (11%)	
Primary disease			>0.2[b]
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[b]
Previous treatment			>0.2[b]
None	12 (46%)	36 (39%)	
Systemic chemotherapy	12 (46%)	47 (51%)	
Surgery	8 (31%)	34 (37%)	
Radiotherapy	3 (12%)	17 (18%)	
Complications			>0.2[b]
Diabetes	2 (8%)	8 (9%)	
Liver diseases	3 (12%)	6 (7%)	

a) Japan Society for Cancer Therapy criteria.
b) χ^2 test.
c) Mann-Whitney *U* test.

Table II. Information on Irinotecan Chemotherapy

	Leukopenia (grade 4) and/or diarrhea (grade 3 or worse)[a]		
	Experienced (N=26)	Not experienced (N=92)	P
Regimens			0.015[b]
Irinotecan alone	3 (12%)	32 (35%)	
Irinotecan & platinum	13 (50%)	45 (49%)	
Irinotecan & other	10 (38%)	15 (16%)	
Concurrent radiotherapy	1 (4%)	8 (9%)	>0.2[c]
Intended schedule			0.059[b]
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 (mg/m^2)			>0.2[b]
<60	9 (35%)	18 (20%)	
60	8 (31%)	34 (37%)	
>60	9 (35%)	40 (43%)	
Total actual dosage (mg/m^2)			0.010[b]
<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[b]
0	5 (19%)	61 (66%)	
1–14	11 (42%)	13 (14%)	
≥15	10 (38%)	18 (20%)	
Use of loperamide hydrochloride (days)			0.002[b]
0	4 (15%)	51 (55%)	
1–7	15 (58%)	28 (30%)	
≥8	7 (27%)	13 (14%)	

a) Japan Society for Cancer Therapy criteria.
b) χ^2 test.
c) Fisher's exact test.

gender, primary disease, 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 following irinotecan infusion (Tables I and II). The chemotherapy regimens were categorized into 3 groups; irinotecan alone, irinotecan plus platinum (cisplatin or carboplatin), and irinotecan plus other agents (paclitaxel, docetaxel, etoposide, mitomycin C or 5-fluorouracil). 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. Since the dose-limiting toxicity of irinotecan results in leukopenia and diarrhea,[2] we defined "severe toxicity" in this research as leukopenia of grade 4 ($<0.9\times10^9$/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.[17] Then, we identified 26 patients who had experienced severe toxicity and 92 patients who did not. All the patients gave informed consent in writing for their peripheral blood to be used for the research. All healthy subjects, who were unrelated and were not found to have any malignant diseases by medical checkup at Nagoya University Hospital, were consecutively enrolled after having given their informed consent in writing for their blood to be used in the genetic research. The study was approved by the Ethical Committees of Nagoya University School of Medicine and the participating institutes.

Genotyping Genomic DNA was prepared from whole blood (100–200 μl) using a QIAamp Blood Kit (QIAGEN GmbH, Hilden, Germany). We distinguished the following variant *UGT1A7* alleles from the reference allele *UGT1A7*1* by direct sequencing analyses: *UGT1A7*2*,

*UGT1A7*3*, and *UGT1A7*4*.[18] Polymerase chain reaction (PCR) amplification was performed using a forward primer, 5′-TGCCGATGCTCGCTGGACG-3′, and a reverse primer, 5′-CCAATGAAGATCATATTGGGC-3′ (nucleotides 279 to 818 in GenBank HSU39570). The amplification reaction mixture (50 μl) contained 1 μl of DNA in 0.2 mmol/liter of each deoxynucleoside triphosphate, 50 mmol/liter KCl, 10 mmol/liter Tris-HCl (pH 8.3), 1.5 mmol/liter $MgCl_2$, 0.5 μmol/liter of each primer, and 1.3 unit of *Taq* polymerase (Takara Shuzo Co., Ltd., Otsu). The PCR condition was: 95°C for 5 min followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 40 s (PCR Thermal Cycler MP, Takara Shuzo Co., Ltd.).

The possible genotypes for individuals heterozygous for the variant sequences at the three codons 129, 131 and 208 are *UGT1A7*1*/*UGT1A7*3* or *UGT1A7*2*/*UGT1A7*4*. Hemi-nested PCR-restriction fragment length polymorphism (RFLP) assay was performed to determine the haplotype of these individuals. We used a forward primer 5′-CAAATTGCAGGAGTTTGTTTAATGACCG-3′ (nucleotide 365 to 392), which matches *UGT1A7*1* and *UGT1A7*4* (reference sequence at codon 131), but not *UGT1A7*2* and *UGT1A7*3*, with the same reverse primer used in the first PCR. One microliter of the 1000-fold-diluted product of the first PCR was subjected to the hemi-nested PCR. The PCR condition was: 95°C for 5 min followed by 25 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 40 s. Only DNA from *UGT1A7*1* or *UGT1A7*4* gave a 454-bp fragment, which was subsequently digested with *Rsa*I (Toyobo Co., Tokyo) for 1 h at 37°C. Restriction fragments were analyzed by 4% agarose gel electrophoresis and ethidium bromide staining. DNA from *UGT1A7*1* gave an undigested fragment (genotyped as *UGT1A7*1*/*UGT1A7*3*), whereas that from *UGT1A7*4* was digested into 256- and 198-bp fragments (genotyped as *UGT1A7*2*/*UGT1A7*4*).

Statistical analysis The correlation or association between potential variables was assessed using the χ^2 test or Fisher's exact test for categorical variables, or the Mann-Whitney *U* test for continuous ones. A crude odds ratio with a 95% confidence interval (CI) was calculated to analyze the association between carrying at least one of the variant *UGT1A7* alleles and severe irinotecan toxicity. Multivariate logistic regression analysis was used to assess the association when controlling for other variables. Possible variables that seemed to be associated with severe toxicity ($P<0.1$) were evaluated by stepwise procedures to be included in the final model: *UGT1A1*28*, female gender, and chemotherapy regimen, which were recognized as important covariates to explain the severe toxicity in the previous study.[9] We performed these analyses using SAS ver. 6.12 software (SAS Institute Inc., Cary, NC). A difference was considered statistically significant when the two-tailed *P* value was under 0.05.

RESULTS

Among the 26 patients with severe toxicity, the allele frequencies were 61.5% for *UGT1A7*1*, 15.4% for *UGT1A7*2*, and 23.1% for *UGT1A7*3*. On the other hand, 63.6% for *UGT1A7*1*, 15.8% for *UGT1A7*2*, and 20.7% for *UGT1A7*3* were found among the 92 patients without severe toxicity. None had *UGT1A7*4* among the total of 118 patients. Distributions of *UGT1A7* genotypes among the patients who experienced severe toxicity and those who did not were apparently comparable (Table III). Univariate analysis showed no significant association between carrying at least one of the variant alleles and the occurrence of severe toxicity (crude odds ratio, 1.13; 95% CI, 0.46–2.75). The relationship remained non-significant after controlling for the other factors (Table IV). Among the 26 patients with severe toxicity, 3 and 19 patients experienced grade 4 and grade 3 diarrhea, respectively, and there was no significant association between the variant alleles and the incidence of such diarrhea (crude odds ratio, 1.27; 95%CI, 0.50–3.46). Genotypes of the 3 individuals who experienced grade 4 diarrhea were *UGT1A7*1*/*UGT1A7*3*, *UGT1A7*2*/*UGT1A7*3* and *UGT1A7*3*/*UGT1A7*3*.

The allelic frequencies in 108 healthy subjects were 59.2% for *UGT1A7*1*, 15.3% for *UGT1A7*2* and 25.5% for *UGT1A7*3*. The *UGT1A7*4* allele was not found in this healthy population. The patients heterozygous for the variant sequences at the three codons 129, 131 and 208 were all genotyped as *UGT1A7*1*/*UGT1A7*3* in both the patients and the healthy population. The distribution of the *UGT1A7* genotypes in the healthy subjects was not significantly different from that in the patients ($P=0.99$ and 0.86 for those with and without severe toxicity, respectively), although it was significantly less than that in the Caucasians reported previously ($P<0.001$) (Table V).[16]

DISCUSSION

In the present study, we analyzed the association between *UGT1A7* polymorphisms and severe toxicity from irinotecan treatment in Japanese cancer patients, and established the frequency of *UGT1A7* alleles in the Japa-

Table III. Distribution of *UGT1A7* Genotypes and Irinotecan Toxicity[a)]

Toxicity[b)]	*N*	**1/*1*	**1/*2*	**1/*3*	**2/*2*	**2/*3*	**3/*3*
Yes	26	38%	15%	31%	4%	8%	4%
No	92	41%	21%	24%	3%	4%	7%

a) None had *UGT1A7*4*.
b) Leukopenia of grade 4 and/or diarrhea of grade 3 or worse (the Japan Society for Cancer Therapy criteria).

Table IV. Results of Multivariate Logistic Regression Analysis to Identify Predictors of Toxicity

Term	β[a)]	SE	χ^2	*P* value	Odds ratio (95%CI)
Intercept	−3.047	0.695			
*UGT1A1*28*[b)]	1.972	0.568	12.04	0.0005	7.18 (2.36−21.87)
Regimen[c)]	1.234	0.505	5.97	0.0146	3.43 (1.28−9.24)
Gender	0.917	0.513	3.19	0.0740	2.50 (0.92−6.84)
UGT1A7[d)]	−0.301	0.525	0.33	0.5661	0.74 (0.26−2.07)

a) β=coefficient.
b) *UGT1A1*28* has a two-extra-nucleotide insertion (TA) within the TATA box, altering the sequence from $(TA)_6TAA$ to $(TA)_7TAA$.
c) Regimen of irinotecan plus other anticancer drugs apart from platinums.
d) *UGT1A7*1* apart from *UGT1A7*2*, *UGT1A7*3*, and *UGT1A7*4*.

Table V. Genotypes of *UGT1A7* in Healthy Japanese and Caucasian Populations

Variant	Japanese			Caucasians[a)]		
UGT1A7 genotypes	*N*	(*N*=108) Frequency (%)	(95%CI)	*N*	(N=144) Frequency (%) (Guillmette *et al.*)	(95%CI)
**1/*1*	39	36.1%	(27.1−45.9)	21	14.6%	(9.3−21.4)
**1/*2*	19	17.6%	(10.9−26.1)	30	19.5%	(14.5−28.4)
**1/*3* or **2/*4*	31	28.7%	(20.4−38.2)	30	20.8%	(14.5−28.4)
**1/*4*	0	0%	(0.0−3.4)	1	0.7%	(0.0−3.8)
**2/*2*	3	2.7%	(0.6−7.9)	9	6.3%	(2.9−11.5)
**2/*3*	8	7.4%	(3.3−14.1)	28	19.4%	(13.3−26.9)
**3/*3*	8	7.4%	(3.3−14.1)	22	15.3%	(9.8−22.2)
**3/*4*	0	0%	(0.0−3.4)	2	1.4%	(0.2−4.9)
**4/*4*	0	0%	(0.0−3.4)	1	0.7%	(0.0−3.8)

a) Caucasian population has been reported by Guillmette *et al.*, *Pharmacogenetics* (2000).

nese population. We did not find a significant association between the *UGT1A7* genotypes and the severe leukopenia and/or diarrhea caused by irinotecan, though UGT1A7 has been reported to glucuronidate SN-38 more efficiently than UGT1A1 *in vitro*.[10)] The reason for this discrepancy is unclear, but it might be because UGT1A1 is expressed in the liver, the primary organ for detoxifying intravenous irinotecan, while UGT1A7 is not. This finding suggests that UGT1A7 would play only a minor role in SN-38 glucuronidation *in vivo*.

In addition, we found the variant *UGT1A7* alleles are rare in the Japanese population compared with those reported in Caucasians.[16)] Additionally, the *UGT1A7*4* allele, whose frequency in Caucasians has been reported as 0.017, has not been found in Japanese. This is the first report investigating inter-ethnic differences in the frequency of the variant *UGT1A7* alleles. Such *UGT1A7* polymorphisms are potentially a cause of inter-patient or inter-ethnic variations in sensitivity to drugs or carcinogens that are metabolized by UGT1A7.

Genetic polymorphisms of drug-metabolizing enzymes might also be an important factor in cancer susceptibility. So far, several phase I drug-metabolizing enzymes, such as cytochrome P450 (CYP1A1, CYP2D6 or CYP2E1), and phase II enzymes (*N*-acetyltransferase or glutathione *S*-transferase) have been reported to be associated with an increased risk of cancers.[19−21)] Glucuronidation by UGTs is regarded as an important pathway to detoxify toxic and or carcinogenic compounds.[22)] Differences in the activities of these enzymes might cause variations in the toxic or carcinogenic effects of drugs or environmental pollutants. In *in vitro* expression systems, the *UGT1A7* polymorphisms exhibited significant variations in glucuronidation activity towards 3-, 7-, and 9-hydroxybenzo[*a*]pyrene, which is a strong carcinogen in cigarette smoke.[16)] On the other hand, the *UGT1A*-deficient Gunn rat was more susceptible to DNA adduct formation after exposure to benzo[*a*]pyrene than the UGT1A-intact rat.[23)] Significant associations of *UGT1A7* genotypes with risks of hepatocellular[24)] or oro-laryngeal cancers[25)] have recently been reported. Although the distributions of the *UGT1A7* polymorphisms did not differ among cancer patients and the healthy subjects in this study, further investigation into the effects of polymorphism on cancer susceptibility is necessary. Since

UGT1A7 is also expressed in the lung,[16] we are planning to investigate the association with lung cancer.

Inter-individual variations in cancer chemotherapy are often due to genetic alterations in drug-metabolizing enzymes, for example, thiopurine *S*-methyltransferase and dihydropyrimidine dehydrogenase.[26–28] This study, however, did not suggest any potential clinical utility of the determination of the *UGT1A7* genotypes for predicting severe toxicity by irinotecan, and we found no difference in the genotype distribution between Japanese cancer patients and healthy subjects.

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