Cancer Chemother Pharmacol (2010) 66:95–105
DOI 10.1007/s00280-009-1138-y

ORIGINAL ARTICLE

Additive effects of drug transporter genetic polymorphisms on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients

Kimie Sai · Yoshiro Saito · Keiko Maekawa · Su-Ryang Kim · Nahoko Kaniwa · Tomoko Nishimaki-Mogami · Jun-ichi Sawada · Kuniaki Shirao · Tetsuya Hamaguchi · Noboru Yamamoto · Hideo Kunitoh · Yuichiro Ohe · Yasuhide Yamada · Tomohide Tamura · Teruhiko Yoshida · Yasuhiro Matsumura · Atsushi Ohtsu · Nagahiro Saijo · Hironobu Minami

Received: 13 April 2009 / Accepted: 8 September 2009 / Published online: 22 September 2009

Abstract
Purpose Effects of genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in addition to "*UGT1A1*28* or **6*" on irinotecan pharmacokinetics/pharmacodynamics in Japanese cancer patients were investigated.
Methods Associations between transporter haplotypes/variations along with *UGT1A1*28* or **6* and SN-38 area under the time–concentration curve (AUC) or neutropenia were examined in irinotecan monotherapy (55 patients) and irinotecan–cisplatin-combination therapy (62 patients).
Results Higher SN-38 AUC values were observed in *ABCB1* 2677G>T (A893S) (**2* group) for both regimens. Associations of grade 3/4 neutropenia were observed with *ABCC2* −1774delG (**1A*), *ABCG2* 421C>A (Q141K) and IVS12 + 49G>T (*#IIB*) and *SLCO1B1* 521T>C (V174A) (**15 · 17*) in the irinotecan monotherapy, while they were

K. Sai (✉) · Y. Saito · K. Maekawa · T. Nishimaki-Mogami · J. Sawada
Division of Functional Biochemistry and Genomics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
e-mail: sai@nihs.go.jp

S.-R. Kim
Project Team for Pharmacogenetics, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

N. Kaniwa
Division of Medicinal Safety Science, National Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan

K. Shirao · T. Hamaguchi · N. Yamamoto · H. Kunitoh · Y. Ohe · Y. Yamada · T. Tamura
Division of Internal Medicine, National Cancer Center Hospital, 5-1-5 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

Present Address:
K. Shirao
Department of Medical Oncology, OITA University Faculty of Medicine, 1-1 Idaigaoka, Hasama-machi, Yufu 879-5593, Japan

T. Yoshida
Genomics Division, National Cancer Center Research Institute, 5-1-5 Tsukiji, Chuo-ku, Tokyo 104-0045, Japan

Y. Matsumura
Investigative Treatment Division, Research Center for Innovative Oncology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

A. Ohtsu
Division of GI Oncology/Digestive Endoscopy, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

N. Saijo
National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

H. Minami
Division of Oncology/Hematology, National Cancer Center Hospital East, 6-5-1 Kashiwanoha, Kashiwa, Chiba 277-8577, Japan

Present Address:
H. Minami
Medical Oncology, Department of Medicine, Kobe University Hospital and Graduate School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650-0017, Japan

evident only in homozygotes of *ABCB1*2*, *ABCG2#IIB*, *SLCO1B1*15 · 17* in the cisplatin-combination therapy. With combinations of haplotypes/variations of two or more genes, neutropenia incidence increased, but their prediction power for grade 3/4 neutropenia is still unsatisfactory.
Conclusions Certain transporter genotypes additively increased irinotecan-induced neutropenia, but their clinical importance should be further elucidated.

Keywords Irinotecan · Transporter · Genetic polymorphism · Haplotype

Introduction

Irinotecan, an anticancer prodrug, is widely used for treating a broad range of carcinomas including colorectal and lung cancers. However, unexpected severe diarrhea and neutropenia are important clinical side effects from irinotecan treatment. The active metabolite SN-38 (7-ethyl-10-hydroxycamptothecin), a topoisomerase I inhibitor, is generated by hydrolysis of the parent compound by carboxylesterases [1], and is subsequently glucuronidated by uridine diphosphate glucuronosyltransferases (UGTs), such as UGT1A1, UGT1A7, and UGT1A9, to form an inactive metabolite, SN-38 glucuronide (SN-38G) [2–4]. Irinotecan is also inactivated by CYP3A4 to produce 7-ethyl-10-[4-*N*-(5-aminopentanoic acid)-1-piperidino]carbonyloxycamptothecin (APC) and 7-ethyl-10-(4-amino-1-piperidino)carbonyloxycamptothecin (NPC) [5]. Irinotecan and its metabolites are excreted into the bile and urine via the action of ATP-binding cassette (ABC) transporters, such as P-glycoprotein (P-gp/ABCB1), multiple resistance-associated protein 2 (MRP2/ABCC2), and breast cancer resistance protein (BCRP/ABCG2) [6]. Transport of SN-38 from the plasma into the liver is mediated by the organic anion transporting polypeptide C (OATP-C/SLCO1B1) [7]. Most of the previous pharmacogenetic studies on irinotecan have focused on *UGT1A1* polymorphisms and have shown clinical relevance of *UGT1A1*28*, a repeat polymorphism in the TATA box [−54_−39A(TA)$_6$TAA>A(TA)$_7$TAA or −40_−39ins TA], to severe toxicities [8–10]. Based on these findings, in 2005, the Food and Drug Administration (FDA) of the United States approved an amendment for the label of Camptosar (irinotecan HCl) (NDA 20-571/S-024/S-027/S-028) and the clinical use of a genetic diagnostic kit for the **28* allele. In parallel with this advance in the USA, clinical relevance to severe neutropenia of *UGT1A1*6* [211G>A (G71R)], another low-activity allele detected specifically in East-Asians, as well as **28* was demonstrated in several studies on Asian patients [11–14]. Accordingly, in June 2008, the Ministry of Health, Labor and Welfare of Japan approved changes to irinotecan labels (Campto and Topotecin) by adding a caution for the risk of severe toxicities in patients either homozygous or compound heterozygous for *UGT1A1*28* and **6* (**28/*28*, **6/*6*, **28/*6*) and the clinical use of a diagnostic kit for *UGT1A1*28* and **6*. Severe toxicities, however, are found in patients without **6/*6*, **28/*28*, and **28/*6*; therefore, other factors responsible for irinotecan toxicities should be identified.

Several clinical studies have suggested polymorphisms of the drug transporter genes, such as *ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1*, might affect irinotecan pharmacokinetics (PK)/pharmacodynamics (PD) in Caucasian and Asian patients. However, the results obtained from different ethnic populations with various irinotencan regimens are still controversial, and the genetic markers examined also differ [13, 15–26]. We previously identified a number of haplotypes/variations of transporter genes, including *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* in Japanese [12, 26–29], but their clinical significance, either alone or in combination, in irinotecan therapy has not yet been examined.

This study aimed to identify the genetic polymorphisms/variations of *ABCB1*, *ABCC2*, *ABCG2*, and *SLCO1B1* which can affect irinotecan PK/PD in Japanese cancer patients. We carefully stratified the patients considering the irinotecan regimen (irinotecan monotherapy or combination therapy with cisplatin) and *UGT1A1* genotype (*UGT1A1 *6* or **28*), and examined additive effects of transporter haplotypes/variations on the area under the time–concentration curves (AUC) of the toxic metabolite SN-38 and on the risk of severe neutropenia.

Patients and methods

Patients

The patients used in this study were the same as those described in a previous paper [12], where details on the eligibility criteria for irinotecan therapy, patient profiles, and irinotecan regimens were described. In this study, 55 patients with irinotecan monotherapy (100 mg/m^2 weekly or 150 mg/m^2 biweekly) and 62 patients with combination therapy of irinotecan (60 mg/m^2 weekly or 70 mg/m^2 biweekly) and cisplatin (60 or 80 mg/m^2, respectively) were included. This study was approved by the ethics committees of the National Cancer Center and the National Institute of Health Sciences, and written informed consent was obtained from all participants.

Analyses on genetic polymorphisms and PK/PD

Patients' data on genetic variations and haplotypes of *UGT1A1*, *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were

previously obtained [12, 26–29]. Regarding *ABCG2*, combination haplotypes were newly defined using the previously reported haplotypes from three linkage disequilibrium (LD) blocks [28]. Patients' PK data on the area under the concentration–time curve (AUC) and toxicities were previously obtained [12].

Association analyses

Associations of transporter genotypes with AUC/dose values for irinotecan, SN-38 and SN-38G, absolute neutrophil count (ANC) nadir, and incidence of grade 3 diarrhea or grade 3/4 neutropenia were investigated. For SN-38 AUC/dose and neutropenia, the patients were stratified by the presence of *UGT1A1*6* or **28* (*UGT+*). Statistical significance (two-sided, $P < 0.1$) was determined by the Mann–Whitney (MW) test or Jonckheere–Terpstra (JT) test for AUC/dose, and by Fisher's exact test and chi-square test (for trend) for incidence of grade 3 and 4 toxicities, using Prism version 4.0 (GraphPad Prism Software Inc., San Diego, CA, USA) and StatXact version 6.0 (Cytel Inc., Cambridge, MA). Multiplicity adjustment was not applied to bivariate analysis, and contributions of the candidate genetic markers to SN-38 AUC/dose values and ANC nadir were further determined by multiple regression analysis after logarithmic transformation of the AUC/dose values and ANC nadir counts. The variables examined were age, sex, body surface area, history of smoking or drinking, performance status, serum biochemistry (GOT, ALP, creatinine) at baseline, the ANC at baseline (for neutropenia), and genetic markers including *UGT1A1*6* or **28* (*UGT+*) and the transporter haplotypes. The variables in the final models were selected by the forward and backward stepwise procedure at a significance level of 0.20 using JMP version 7.0.0 (SAS Institute Inc., Cary, NC, USA).

Results

Definition of major transporter haplotypes and their selected markers

For screening transporter gene polymorphisms affecting irinotecan PK/PD, major haplotypes and their tagging single nucleotide polymorphisms (SNPs) from *ABCB1*, *ABCC2*, *ABCG2* and *SLCO1B1* were selected (Table 1) according to their frequencies (more than 5%) and/or from preliminary results obtained from all patients treated with irinotecan.

For *ABCB1* block 1[26], the haplotype group *BJL*, which consists of **1B* (having −1789G>A), **1J* (having −1789G>A and −371A>G) and **1L* (having −1789G>A and −145C>G), was selected because an association of the marker SNP −1789G>A with lower expression levels of P-gp has been reported [30]. *ABCB1* block 2 **2* was originally defined as haplotypes containing three SNPs, 1236C>T, 2677G>T (A893S) and 3435C>T [31]. Since the **9* haplotype with 1236C>T, 2677G>T (A893S) without 3435C>T [16] showed the same trend for PK/PD as **2* (data not shown), the current study classified the

Table 1 List of major transporter haplotypes and their markers analyzed for Japanese cancer patients

Gene	Haplotype	Tagging SNP	Abbreviation used in this paper	Haplotype frequency	
				Monotherapy ($N = 110$)[a]	With cisplatin ($N = 124$)[a]
ABCB1	*BJL*[b] (block 1)	−1789G>A		0.182	0.210
	**2* group[c] (block 2)	2677G>T(A893S)	*B*	0.382	0.379
	**10* group[d] (block 2)	2677G>A(A893T)		0.182	0.169
	**1b* (block 3)	IVS27-182G>T		0.200	0.169
ABCC2	**1A*	−1774delG	*C*	0.373	0.371
	**1C/G*	3972C>T(I1324I)		0.218	0.266
ABCG2	#*IIB* [**1a–*2–*1b*][e]	421C>A(Q141K), IVS12 + 49G>T	*G*	0.200	0.274
	#*IIIC* [**1b–*3–*1c*][e]	34G>A(V12M), IVS9-30A>T		0.164	0.097
SLCO1B1	**1b*	388A>G(N130D)		0.373	0.573
	**15 · 17*	521T>C(V174A)	*S*	0.191	0.153

[a] Number of chromosome

[b] *BJL* consists of **1B* (having −1789G>A), **1J* (having −1789G>A and −371A>G) and **1L* (having −1789G>A and −145C>G) previously defined [26]

[c] **2* Group includes **2*, **9*, **12* and **14* haplotypes previously defined [26]

[d] **10* Group includes **10* and **13* haplotypes previously defined [26]

[e] Combination of *ABCG2* haplotypes of three blocks [block (−1)–block 1–block 2] previously defined [28]

haplotypes with 2677G>T (A893S), *2, *9, *12 and *14 [26], as the *2 group (*2 in this paper). Similarly, the *10 group was classified as haplotypes with 2677G>A (A893T), i.e., *10 and *13, since no differences in PK/PD parameters were observed between these haplotypes. The *4, *6, and *8 haplotypes in block 2 [16, 26] showed no significant effect in the current analysis (data not shown). The *ABCB1* block 3 *1b haplotype containing IVS27-182G>T was selected because our previous study showed it was associated with an increased renal clearance of SN-38 [16].

Based on reports showing possible functional alterations of −1774delG [32] and 3972C>T (I1324I) [18, 24], *ABCC2* haplotypes containing those variations were classified as *1A and "*1C and *1G (*1C/G)", respectively, according to our previous definition: *1A, −1774delG; *1C, −24C>T and 3972C>T; *1G, 3972C>T [27]. *ABCC2*2* [1246G>A (V417I)] and *1H [2934G>A (S978S)] [27] showed no statistically significant effects (data not shown).

The *ABCG2* combinatorial haplotypes were newly defined as combinations of haplotypes across the three blocks [block (−1)–block 1–block 2] previously reported [28]. Major combinations in 177 patients were the wild type $^{\#}$*IA* (frequency = 0.291), $^{\#}$*IIB* [containing 421C>A (Q141K) and IVS12 + 49G>T] (0.251) and $^{\#}$*IIIC* [containing 34G>A (V12M) and IVS9-30A>T] (0.107). Note that $^{\#}$*IIB* and $^{\#}$*IIIC* are subgroups of block 1 *2 [421C>A (Q141K)] and block 1*3 [34G>A (V12M)], respectively [28].

The *SLCO1B1* haplotypes used were the major haplotypes *1b [containing 388A>G (N130D) without 521T>C (V174A)] [33] and *15 · 17 [containing 521T>C (V174A)], the functional relevance of which has been reported [34].

Association of transporter genotypes with AUC values

Since we previously found that some PK parameters, including AUC/dose, Cmax/dose and $t_{1/2}$ for irinotecan and/or its metabolites, as well as incidence of grade 3/4 toxicities were affected by irinotecan regimen [12], the following analyses were conducted using the two groups of patients; i.e., those treated with irinotecan monotherapy (100–150 mg/m^2 for initial dosage) or by combination therapy with cisplatin (60–70 mg/m^2 for initial dose of irinotecan). Since SN-38 AUC levels were largely dependent on the *UGT1A1* genotype "*6 or *28" [12], the associations of transporter genotypes with SN-38 AUC values were analyzed within the groups stratified by the marker *UGT1A1* "*6 or *28" (*UGT*+); i.e., *UGT*−/−, *UGT*+/− and *UGT*+/+. Since the SN-38 AUC/dose level of one patient with haplotypes *ABCB1*2* [2677G>T (A893S)] and *14 [2677G>T (A893S) and 1345G>A (E448K)] showed an outlying value (indicated as "*a*" in Fig. 1), this patient was excluded from the statistical analysis. In this study, we preliminarily found that effect of each transporter genotype on irinotecan PK/PD was generally small. However, it was hypothesized that multiple transporter genotypes might act additively as described below. Accordingly, we adopted a statistical significance level of $P = 0.1$ (two-sided) to pick up candidate polymorphisms for further evaluation of their combined effects.

Figure 1 shows the association of transporter genotypes with SN-38 AUC values in the irinotecan monotherapy. In all patients (ALL), higher values of the SN-38 AUC/dose were observed in the *ABCB1*2/*2* [1.64-fold of −/−, $P = 0.095$ (MW test)] (Fig. 1b) and *ABCG2*$^{\#}$*IIB* [1.24-fold of −/−, $P = 0.078$ (MW test)] genotypes (Fig. 1e) and lower values were observed in the *ABCB1*1b* (block 3) [0.78-fold of −/−, $P = 0.008$ (MW test)] (Fig. 1c) genotype. In *UGT*−/− patients, an increase in SN-38 AUC/dose was observed in the *ABCB1 BJL* [1.22-fold of −/−, $P = 0.073$ (MW test)] (Fig. 1a) and *ABCG2*$^{\#}$*IIB* [1.21-fold of −/−, $P = 0.082$, (MW test)] genotypes (Fig. 1e). In *UGT* (+/− and +/+) patients, an increase in SN-38 AUC/dose in *SLCO1B1*15 · 17* (*S*) [1.59-fold of −/−, $P = 0.036$ (MW test)] was also observed (Fig. 1f). Multiple regression analysis for the SN-38 AUC/dose (logarithm-transformed values) in the irinotecan monotherapy revealed significant associations of *ABCB1*2/*2* (coefficient = 0.212 ± 0.075, $P = 0.007$), along with *UGT*+/− (0.113 ± 0.054, $P = 0.040$) and *UGT*+/+ (0.225 ± 0.088, $P = 0.014$) in the final model [R^2 = 0.226, Intercept = 0.281 (log 10^{-3}h m^2/L), $N = 53$].

Regarding other compounds, *ABCB1*2/*2* also showed higher irinotecan AUC/dose (1.27-fold) [66.2 (48.2–82.4) [median (25th–75th percentiles)] for *2/*2 vs. 52.2 (40.6–61.9) for −/− and *2/−; $P = 0.063$ (MW test)] and SN-38G AUC/dose (1.62-fold) [18.0 (14.6–27.7) for *2/*2 vs. 11.1 (7.7–14.2) for −/− and *2/−; $P = 0.002$ (MW test)]. Conversely, lower irinotecan AUC/dose for *ABCB1*10/*10* (0.79-fold) [54.8 (44.4–65.7) for −/− vs. 43.3 (40.6–54.1) for *10/*10; $P = 0.062$ (JT test)] was detected.

For the combination therapy with cisplatin, an increase of the SN-38 AUC/dose for *ABCB1*2/*2* (1.43-fold) in *non-UGT*+/+ patients (*UGT*−/− and *UGT*+/−) ($N = 55$) [3.57 (2.72–4.19) for *2/*2 vs. 2.51 (1.99–3.28) for −/− and *2/−; $P = 0.032$ (MW test)], and a decrease for *ABCB1*1b* (0.80-fold) in *UGT*−/− patients ($N = 35$) [2.03 (1.72–2.33) for *1b/− and *1b/*1b* vs. 2.55 (2.02–3.31) for −/−; $P = 0.026$ (MW test)] were observed. Multivariate analysis, however, showed no significant contributions of these transporter haplotypes to the SN-38 AUC/dose values.

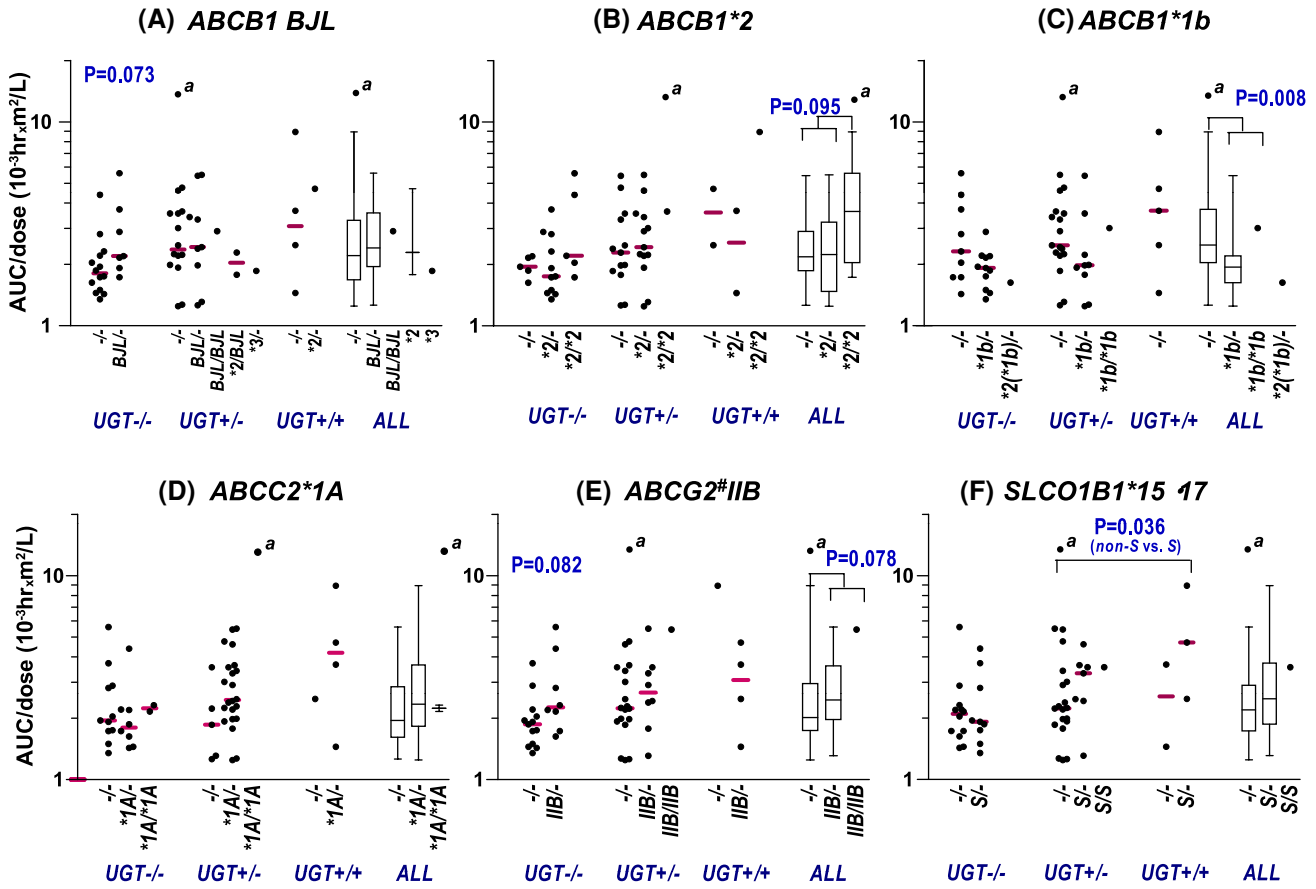

Fig. 1 Effects of transporter genotypes on SN-38 AUC/dose in irinotecan monotherapy ($N = 54$). *a* Excluded from statistical analysis. The *bars* represent the medians. *UGT+* = *UGT1A1*6* or **28*. **a** *BJL* contains −1789G>A, *2 (block 1) = 325G>A (E109K), **3* (block 1) = 304G>A (G102R); **b** *2 (block 2) contains 2677G>T (A893S); **c** **1b* (block 3) = IVS27-182G>T, *2 (block 3) = 3751G>A (V1251I); **d** **1A* contains −1774delG; **e** *IIB* contains 421C>A (Q141K) and IVS12 + 49G>T; **f** *S* = *SLCO1B1*15 · 17* containing 521T>C (V174A)

Effects of transporter genotypes on toxicities in irinotecan monotherapy

Since 80 and 100% of *UGT+/+* patients showed grade 3/4 neutropenia in the irinotecan monotherapy and combination therapy with cisplatin, respectively, neutropenia incidence was analyzed only in the *non-UGT+/+* population. Two patients were excluded from the analysis; one patient who showed an outlier SN-38 value (indicated as "*a*" in Fig. 1) and a second patient from the cisplatin-combination therapy group who discontinued irinotecan therapy.

In terms of incidence of grade 3/4 neutropenia in irinotecan monotherapy (Table 2), *ABCC2*1A*-dependent increases [0, 25.8 and 50.0% for −/−, **1A/−* and **1A/*1A*, respectively; $P = 0.014$ (chi-square test for trend)] were observed in *UGT* (−/− and +/−) patients. Higher incidence with *ABCG2#IIB* was also found in *UGT* (−/− and +/−) patients [9.5% for −/− and 35.3% for *#IIB/−* and *#IIB/#IIB*, respectively; $P = 0.049$ (Fisher's exact test)], and with *SLCO1B1*15 · 17*(*S*) in the *UGT+/−* patients [15.0, 28.6 and 100% for −/−, *S/−* and *S/S*, respectively; $P = 0.076$ (chi-square test for trend)].

Multiple regression analysis for the ANC nadir (logarithm-transformed values) was conducted. The final model [$R^2 = 0.466$, Intercept = 1.088 (log counts/μL), $N = 52$] revealed associations of *ABCC2*1A/*1A* (coefficient = -0.339 ± 0.088, $P = 0.0004$), *ABCG2#IIB* (-0.131 ± 0.067, $P = 0.057$) and *SLCO1B1*15 · 17* (-0.136 ± 0.066, $P = 0.046$) in addition to *UGT+/−* (-0.134 ± 0.073, $P = 0.074$) and *UGT+/+* (-0.238 ± 0.117, $P = 0.047$) and ANC at baseline (0.541 ± 0.226, $P = 0.021$), but association of *ABCB1*2/*2* was not significant (-0.158 ± 0.095, $P = 0.104$).

Although total incidence of grade 3 diarrhea was low (11%), an *ABCB1*2*-dependent increase was observed [0, 15.4 and 28.6% for −/−, **2/−* and **2/*2*, respectively; $P = 0.022$ (chi-square test for trend)]. Note that all patients who experienced grade 3 diarrhea had neither the *ABCC2*1C/G* nor *ABCG2#IIIC* genotypes.

Table 2 Effects of transporter genotypes on incidences of grade 3/4 neutropenia in Japanese patients treated with irinotecan monotherapy

Gene	Genotype	*UGT*−/−				*UGT*+/−				*UGT* (−/−, +/−)			
		No./total	%	*P* value		No./total	%	*P* value		No./total	%	*P* value	
				Exact[a]	Trend[b]			Exact[a]	Trend[b]			Exact[a]	Trend[b]
ABCB1	*BJL* (block 1)[c]												
	−/−	3/14	21.4	>0.1		4/15	26.7	>0.1	>0.1	7/29	24.1	>0.1	>0.1
	+/−	0/7	0.0			2/9	22.2			2/16	12.5		
	+/+					0/1	0.0			0/1	0.0		
	*2 group (block 2)												
	−/−	1/5	20.0	>0.1[d]	>0.1	5/14	35.7	>0.1[d]	>0.1	6/19	31.6	>0.1[d]	>0.1
	+/−	1/11	9.1			0/13	0.0			1/24	4.2		
	+/+	1/5	20.0			1/1	100			2/6	33.3		
	1b (block 3)[e]												
	−/−	2/9	22.2	>0.1		4/18	22.2	>0.1	>0.1	6/27	22.2	>0.1	>0.1
	+/−	0/11	0.0			2/9	22.2			2/20	10.0		
	+/+					0/1	0.0			0/1	0.0		
ABCC2	*1A*												
	−/−	0/11	0.0	>0.1	0.031	0/5	0.0	>0.1		0/16	0.0	0.022	0.014
	+/−	2/8	25.0			6/23	26.1			8/31	25.8		
	+/+	1/2	50.0							1/2	50.0		
ABCG2	#*IIB*												
	−/−	0/13	0.0	0.042		3/19	15.8	>0.1	>0.1	3/32	9.4	0.049	0.057
	+/−	3/8	37.5			3/8	37.5			6/16	37.5		
	+/+					0/1	0.0			0/1	0.0		
SLCO1B1	*15 · 17												
	−/−	2/12	16.7	>0.1		3/20	15.0	>0.1	0.076	5/32	15.6	>0.1	>0.1
	+/−	1/9	11.1			2/7	28.6			3/16	18.8		
	+/+					1/1	100			1/1	100		

[a] Fisher's exact test for (−/−) versus (+/− and +/+)

[b] Chi-square test for trend

[c] Three patients bearing *2 (block 1) or *3 (block 1) were excluded

[d] Fisher's exact test for (−/− and +/−) versus (+/+)

[e] One patient bearing *2 (block 3) was excluded

Effects on toxicities in combination therapy with cisplatin

Since only four patients (6.0%) experienced grade 3 diarrhea from the cisplatin-combination therapy, association analysis for diarrhea was not done.

Grade 3/4 neutropenia incidence was higher with *ABCB1*2* [47.1, 63.3 and 85.7% for −/−, *2/− and *2/*2, respectively; $P = 0.073$ (chi-square test for trend)] in *UGT* (−/− and +/−) patients. In *UGT*−/− patients, a higher incidence was also observed with *ABCG2*#*IIB* [55.6, 83.3 and 100% for −/−, #*IIB*/− and #*IIB*/#*IIB*, respectively; $P = 0.075$ (chi-square test for trend)]. Conversely, the incidence was lower with *ABCG2*#*IIIC* [71.4% for −/−, and 25% for #*IIIC*/− and #*IIIC*/#*IIIC*, respectively; $P = 0.006$ (Fisher's exact test)] in *UGT* (−/− and +/−) patients. Notably, all patients homozygous for *ABCG2*#*IIB* ($N = 5$) or *SLCO1B1*15 · 17* ($N = 1$) experienced grade 3/4 neutropenia. The effect of *ABCC2*1A* on neutropenia was not consistent among the *UGT* genotypes in contrast to the results from the monotherapy. Multiple regression analysis was not applied to the neutropenia parameters in the cisplatin-combination therapy because, as described in the next section, contributions of minor variations could not be ignored.

Minor genetic variations possibly related to grade 4 neutropenia

We have detected a number of rare non-synonymous variations of the transporter genes to which statistical analysis could not be applied. Since grade 4 neutropenia

Table 3 Minor genetic variations detected in non-*UGT*+/+ patients who experienced grade 4 neutropenia

ID	Gene	Genetic variation	
		Nucleotide change (amino acid substitution)	Haplotype[a]
b1	*ABCB1*	304G>C (G102R)	*Block 1 *3*
b2(B)[b]		1804G>A (D602N)	*Block 2 *12*
b3(B)[b]		1342G>A (E448K)	*Block 2 *14*
b4		3043A>G (T1015A)	*Block 2 *16*
b5		3751G>A (V1251I)	*Block 3 *2*
c1	*ABCC2*	1177C>T (R393W)	*7
g1	*ABCG2*	376C>T (Q126X)	*Block 1 *4*
g2		1465T>C (F489L)	*Block 2 *2*
g3		1723C>T (R575X)	*Block 2 *5*
s1(S)[c]	*SLCO1B1*	1007C>G (P336R)	
s2		311T>A (M104K)	
u1	*UGT1A1*	−3279T>G, 1941C>G	*#60-#IB* (+/+)

[a] Defined in previous papers for *ABCB1* [26], *ABCC2* [27], *ABCG2* [28] and *UGT1A1* [35]

[b] Linked with *ABCB1*2* (*B*)

[c] Linked with *SLCO1B1*15 · 17* (*S*)

occurred in *non-UGT*+/+ patients at rates of 8.0% (4/50) in the irinotecan monotherapy and 20% (11/55) in the cisplatin-combination therapy, we investigated possible contributions of these minor transporter variations and another low-activity *UGT*-haplotype, *UGT1A1#60-#IB* [35], to severe neutropenia.

Among the rare variations detected, eleven heterozygous transporter genetic variations and one *UGT1A1#60-#IB* homozygote were found in *non-UGT*+/+ patients who experienced grade 4 neutropenia (Table 3). These variations include an amino acid substitution leading to reduced in vitro activity, *ABCG2* 1465T>C (F489L) [36], and the stop codons, *ABCG2* 376C>T (Q126X) and 1723C>T (R575X) [28].

Additive effects of transporter gene haplotypes on neutropenia

Since multiple transporters are involved in irinotecan PK/PD, severity of toxicity might depend on the number and combinations of the low-activity variants, each of which does not effectively affect PD. To examine this possibility, we surveyed relationships between ANC nadirs and combinations of haplotypes associated with grade 3/4 neutropenia ($P < 0.1$) and the minor variations associated with grade 4 neutropenia (listed in the previous section); the data for selected haplotypes/variations are depicted in Fig. 2. For the combination therapy with cisplatin (Fig. 2b), homozygous *SLCO1B1*15 · 17* was included, but *ABCC2*1A* was excluded since its effect in the cisplatin-combination therapy was not consistent among the *UGT* genotypes.

In the irinotecan monotherapy, ANC nadirs in most patients with either one or more of *ABCG2#IIB*, *SLCO1B1*15 · 17* and the minor variations were lower than the median ANC nadirs of both *UGT*−/− and *UGT*+/− patients without them (None) (Fig. 2a). In particular, the effects were more evident in patients bearing two or more of the selected haplotypes/variations (including the *UGT*+). Among the patients who experienced grade 3 or 4 neutropenia, 80% of patients had two or more candidate haplotypes/variations in the *UGT* (−/− and +/−) group (Fig. 2a).

In *UGT*+/− patients with the cisplatin-combination therapy, ANC nadirs of the patients with *ABCB1*2/*2*, *ABCG2#IIB/#IIB*, *SLCO1B1*15 · 17/*15 · 17* or any minor variations, and their combinations were lower than the median values of patients without these markers (None), except for one patient with *ABCB1*2/*2* and *SLCO1B1*15 · 17* (*B/B* + *S*/−) (Fig. 2b). Also, in *UGT*−/− and *UGT*+/− patients, the effects were more evident in the patients with two or more of the selected haplotypes/variations. Among the patients who experienced grade 4 neutropenia, 82% of patients had two or more candidate haplotypes/variations in the *UGT* (−/− and +/−) group (Fig. 2b).

It was noted that the additive effect of *g1* [*ABCG2* 376C>T (Q126X)] was not observed in the heterozygotes (*g1*/−), but was evident in the compound heterozygotes with another *ABCG2* genetic polymorphism, *#IIB*, (*G/g1*) (Fig. 2a, b).

Regarding the combined effects of the above transporter genotypes on SN-38 AUC values, higher levels were observed in patients with the candidate haplotypes/variations of two or more genes in the monotherapy, but this trend was not always evident in the cisplatin-combination therapy patients (data not shown).

Discussion

In this study, we showed possible additive effects of transporter and *UGT1A1* genotypes on irinotecan PK and PD. Since multiple transporters are involved in irinotecan PK, it is likely that a functional alteration of one of the responsible transporters can be compensated by other transporters; thus, changes in PK/PD parameters by transporter genotypes may not always be large. However, the overall elimination rate of irinotecan or its metabolites might be altered under the conditions of simultaneously reduced activities of multiple transporters, higher irinotecan doses, or reduced UGT activity.

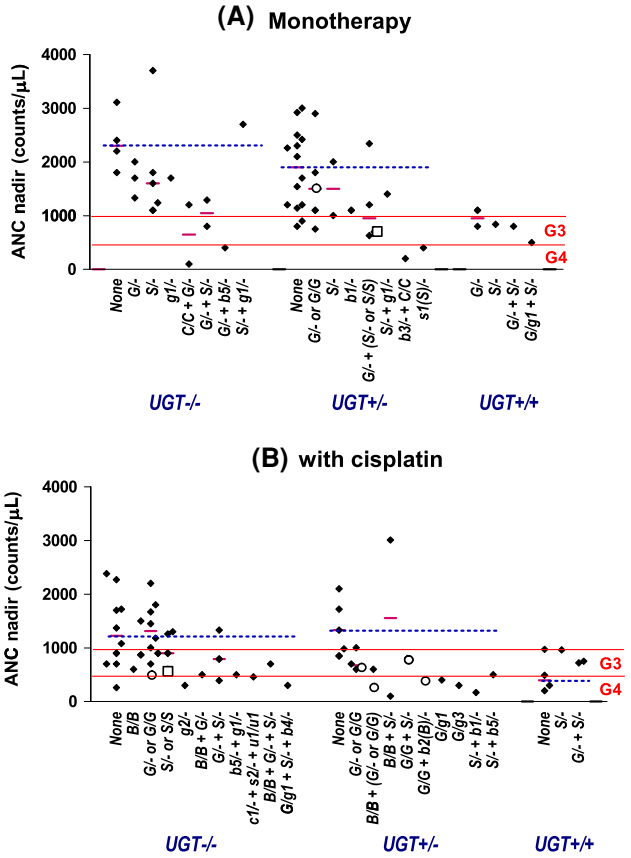

Fig. 2 Additive effects of transporter haplotypes/variations on ANC nadirs in irinotecan monotherapy (**a**) and combination therapy with cisplatin (**b**). *UGT+* = *UGT1A1*6* or **28*; *B* = *ABCB1*2*; *C* = *ABCC2*1A*; *G* = *ABCG2#IIB* (*open circle*, *#IIB/#IIB*); *S* = *SLCO1B1*15 · 17* (*open square*, **15 · 17/*15 · 17*); *b1–u1* = minor variations listed in Table 3. **a** *None* = *non-*(*C*, *G*, *S* or *minors*), **b** *None* = *non-*(*B*, *G*, *S* or *minors*). The *bar* in each genotype represents the median. The *dotted lines* in each *UGT* genotype show the median values of patients without any selected transporter polymorphisms/variations (*None*). The *lines* (G3 and G4) represent the border of grade 3 and 4 neutropenia

In the irinotecan monotherapy, the increasing effect of *ABCB1*2/*2* (block 2) on SN-38 AUC/dose was evident while contributions of *ABCB1 BJL* (block 1), *ABCB1*1b* (block 3), *ABCG2#IIB* and *SLCO1B1*15 · 17* were not significant in the multivariate analysis. For neutropenia, additive effects were suggested for *ABCC2*1A/*1A*, *ABCG2#IIB*, *SLCO1B1*15 · 17*, and possibly some minor genetic variations in addition to *UGT1A1*6* or **28* (Fig. 2a). The association of *ABCB1*2* (block 2) with grade 3 diarrhea was also observed.

In the combination therapy with cisplatin, an increase in the SN-38 AUC/dose by *ABCB1*2* and for a decrease by *ABCB1*1b* were observed, but the multivariate analysis did not show their significant contributions. Regarding neutropenia, additive effects of *ABCB1*2/*2*, *ABCG2#IIB/#IIB*, and possibly, *SLCO1B1*15 · 17/*15 · 17* and some minor variations were suggested (Fig. 2b).

Thus, in both regimens, the associations of *ABCB1*2* (block 2) with higher SN-38 AUC/dose levels and toxicities (diarrhea or neutropenia), and additive effects of *ABCG2#IIB* and *SLCO1B1*15 · 17* with *UGT1A1*6* or **28* on neutropenia were observed. The current study also suggests that combination genotypes with two or more genes could have a greater effect on neutrophil count reduction than a single gene, indicating a quantitative property of multiple genetic factors affecting phenotype. These findings could partly explain a large interindividual variation in irinotecan toxicities within each *UGT* genotype.

In this study, influences of the transporter genotypes on SN-38 AUC/dose did not always correlate to an influence on neutropenia as observed in the combination therapy with cisplatin and in the case of *ABCB1*2* (block 2) in the monotherapy. Although weak negative correlations were observed between the SN-38 AUC level and ANC nadir, the SN-38 AUC values of patients who exhibited grade 3/4 neutropenia (ANC nadir $< 1{,}000$ counts/μL) were fairly diverse, especially in the combination therapy with cisplatin (Fig. 3). It is likely that the extent of toxicities depends not only on systemic exposure levels of the active metabolite for which hepatic UGT activity is a large contributor, but also on the elimination from the target cells (neutrophil progenitor cells or enterocytes) where transporter function might be more critical.

Our previous study showed the association of *ABCB1* block 2 **2* [1236C>T, 2677G>T (A893S) and 3435C>T] with lower renal clearance of irinotecan and its metabolites [16]. The current data obtained in the irinotecan monotherapy also suggest higher AUC/dose for irinotecan, SN-38G, and SN-38 with *ABCB1*2/*2*. Since a high affinity of P-gp for irinotecan is known, lower elimination rate of irinotecan could also result in higher plasma levels of its metabolites. Other studies have also suggested associations of the haplotype 1236T–2677T (corresponding to our **2* group in this study) with a reduced excretion rate of P-gp substrates [37] and SN-38 [25], and associations of the haplotype 2677T–3435T (corresponding to our **2* group in this study) with paclitaxel-induced neutropenia [38].

For *ABCC2*, *ABCC2* −1774delG, a tagging SNP of **1A*, was reported to be associated with low promoter activity and cholestatic or mixed-type hepatitis [32]. Patients with *ABCC2*1A/*1A* together with *ABCB1*2/*2* or *ABCG2#IIB* showed higher values of SN-38 AUC (Fig. 1) and neutropenia in the monotherapy (Fig. 2a), but these trends were not evident in the *UGT−/−* patients treated with cisplatin-combination therapy (data not shown). Thus, the effects of *ABCC2* might be dependent on combinations with other genetic and non-genetic factors. Conflicting clinical outcomes of *ABCC2* 3972C>T, a marker of **1C/G*, were reported to cause higher AUC of irinotecan and its

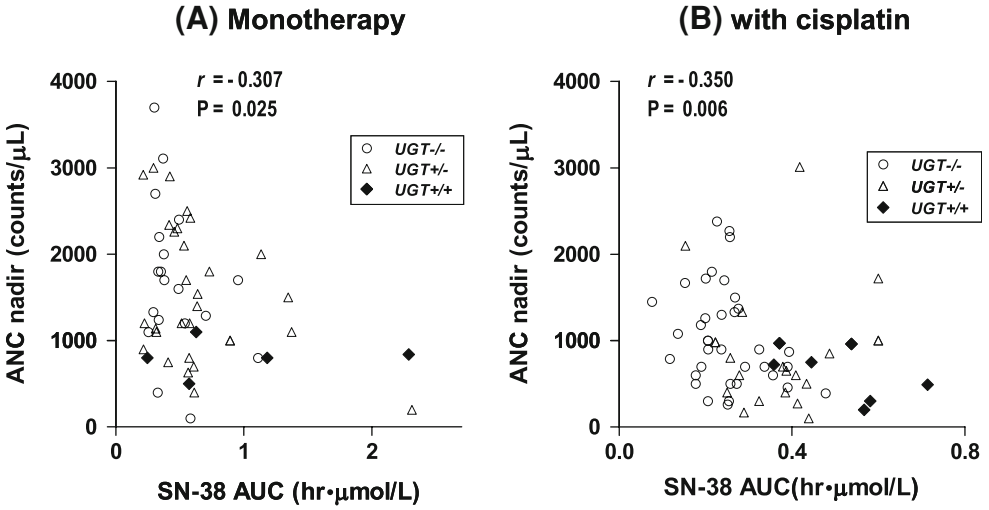

Fig. 3 Correlations between SN-38 AUC and ANC nadir in patients in irinotecan monotherapy (**a**) and combination therapy with cisplatin (**b**). *r* Spearman's rank correlation coefficient

metabolites in Caucasians treated with irinotecan monotherapy [18] and to lower the incidence of grade 3 diarrhea in Koreans treated with a combination therapy of irinotecan and cisplatin [24]. In the current study, no significant association of *ABCC2*1C/G* on PK/PD was observed in the monotherapy. Although a high incidence of grand 3/4 neutropenia was observed in patients with *ABCC2*1C/G* in the combination therapy with cisplatin, most patients also had *ABCG2*$^{\#}$*IIB* (data not shown); thus, the effect of *ABCC2*1C/G* remains obscure.

For *ABCG2*, the current study examined the association with the combinatorial haplotypes consisting of the three previously defined block haplotypes [28]. *ABCG2*$^{\#}$*IIB* contains the non-synonymous SNP 421C>A (Q141K), which was detected at higher frequencies in Asians and was reported to cause reduced expression of BCRP in vitro [36, 39–41]. In clinical studies, the association of 421C>A (Q141K) with higher plasma levels of diflomotecan was shown in Caucasians [42]. However, an association of this SNP with irinotecan PK/PD had not been shown [19, 24]. An association of 421C>A (Q141K) alone with irinotecan PK/PD was not significant in our hands (data not shown), but $^{\#}$*IIB* containing both 421C>A (Q141K) and IVS12 + 49G>T showed a moderate association with neutropenia. It is unclear whether the additional SNP IVS12 + 49G>T itself or another unknown linked SNP is causative for the reduced function. *ABCG2*$^{\#}$*IIIC* contains a non-synonymous SNP 34G>A (V12M) which has no influence on BCRP expression or activity in vitro [36, 39–41]. Our study showed no influence of *ABCG2*$^{\#}$*IIIC* on the SN-38 AUC/dose levels and neutropenia in the irinotecan monotherapy (data not shown), but did show a decreasing trend in grade 3/4 neutropenia in the combination therapy with cisplatin. In contrast, a report on Korean patients suggested the association of *ABCG2* 34G>A (V12M) with a higher incidence of grade 3 diarrhea in a combination therapy of irinotecan and cisplatin [24].

Among *SLCO1B1* polymorphisms, 521T>C (V174A), a tagging SNP of **15 · 17*, was demonstrated to reduce in vitro SN-38 influx [7], and clinical studies in Asians also showed its relevance to a higher SN-38 AUC and severe neutropenia in combination therapy of irinotecan with cisplatin [22–24]. Our results support these previous findings. Note that our **15 · 17* mainly consists of **17* [containing −11187G>A, 521T>C (V174A) and 388A>G (N130D)].

Taken together, the clinical data on transporter genotypes show variability among the studies. The reasons for these conflicting findings might be partly attributed to the ethnic differences in transporter genotypes and the regimens used. In addition, non-genetic factors, such as disease status and inflammation [43, 44], hepatic or renal function [45], and co-administered or pre-administered drugs, may also influence the clinical outcome.

The current study suggests combined effects of multiple haplotypes/variations on neutropenia. From clinical aspects of irinotecan therapy, the benefit of additional genotyping of transporters to predict severe toxicities should be clarified. Regarding grade 3 and 4 neutropenia, positive prediction values for two or more candidate genotypes including *UGT* (+) (Fig. 2) were 46 and 89% in the monotherapy and the cisplatin-combination therapy, respectively, which are low compared with *UGT+/+* (80 and 100%, respectively). Regarding grade 4 neutropenia, positive predictive values for these candidate genotypes were 15 and 41% in the monotherapy and the cisplatin-combination therapy, respectively, while for *UGT+/+*, they were 0 and 43%, respectively. Further studies using a

larger population size are needed to further elucidate the roles of these candidate markers.

In conclusion, the current study suggests there are additive effects for several transporter genotypes on the SN-38 AUC level and the reduction of neutrophil counts in irinotecan therapy. The clinical benefits of additional genotyping of these candidate markers should be further delineated.

Acknowledgments This study was supported in part by the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation, and by the Program for the Promotion of Studies in Health Sciences of the Ministry of Health, Labor and Welfare of Japan. We thank Yakult Honsha Co., Ltd (Tokyo, Japan) for providing analytical standards of irinotecan and its metabolites. We also thank Ms. Chie Sudo for her administrative assistance.

References

1. Slatter JG, Su P, Sams JP, Schaaf LJ, Wienkers LC (1997) Bioactivation of the anticancer agent CPT-11 to SN-38 by human hepatic microsomal carboxylesterases and the in vitro assessment of potential drug interactions. Drug Metab Dispos 25:1157–1164
2. Iyer L, King CD, Whitington PF, Green MD, Roy SK, Tephly TR, Coffman BL, Ratain MJ (1998) Genetic predisposition to the metabolism of irinotecan (CPT-11). Role of uridine diphosphate glucuronosyltransferase isoform 1A1 in the glucuronidation of its active metabolite (SN-38) in human liver microsomes. J Clin Invest 15:847–854
3. Ciotti M, Basu N, Brangi M, Owens IS (1999) Glucuronidation of 7-ethyl-10-hydroxycamptothecin (SN-38) by the human UDP-glucuronosyltransferases encoded at the UGT1 locus. Biochem Biophys Res Commun 260:199–202
4. Gagne JF, Montminy V, Belanger P, Journault K, Gaucher G, Guillemette C (2002) Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). Mol Pharmacol 62:608–617
5. Haaz MC, Rivory L, Riché C, Vernillet L, Robert J (1998) Metabolism of irinotecan (CPT-11) by human hepatic microsomes: participation of cytochrome P-450 3A and drug interactions. Cancer Res 58:468–472
6. Sparreboom A, Danesi R, Ando Y, Chan J, Figg WD (2003) Pharmacogenomics of ABC transporters and its role in cancer chemotherapy. Drug Resist Updat 6:71–84
7. Nozawa T, Minami H, Sugiura S, Tsuji A, Tamai I (2005) Role of organic anion transporter OATP1B1 (OATP-C) in hepatic uptake of irinotecan and its active metabolite, 7-ethyl-10-hydroxycamptothecin: in vitro evidence and effect of single nucleotide polymorphisms. Drug Metab Dispos 33:434–439
8. Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, Hasegawa Y (2000) Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 60:6921–6926
9. Iyer L, Das S, Janisch L, Wen M, Ramirez J, Karrison T, Fleming GF, Vokes EE, Schilsky RL, Ratain MJ (2002) UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. Pharmacogenomics J 2:43–47
10. Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramírez J, Rudin CM, Vokes EE, Ratain MJ (2004) Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. J Clin Oncol 22:1382–1388
11. Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, Jang IJ, Lee DH, Lee JS (2006) Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. J Clin Oncol 24:2237–2244
12. Minami H, Sai K, Saeki M, Saito Y, Ozawa S, Suzuki K, Kaniwa N, Sawada J, Hamaguchi T, Yamamoto N, Shirao K, Yamada Y, Ohmatsu H, Kubota K, Yoshida T, Ohtsu A, Saijo N (2007) Irinotecan pharmacokinetics/pharmacodynamics and UGT1A genetic polymorphisms in Japanese: Roles of UGT1A1*6 and *28. Pharmacogenet Genomics 17:497–504
13. Jada SR, Lim R, Wong CI, Shu X, Lee SC, Zhou Q, Goh BC, Chowbay B (2007) Role of UGT1A1*6, UGT1A1*28 and ABCG2 c.421C>A polymorphisms in irinotecan-induced neutropenia in Asian cancer patients. Cancer Sci 98:1461–1467
14. Sai K, Saito Y, Sakamoto H, Shirao K, Kurose K, Saeki M, Ozawa S, Kaniwa N, Hirohashi S, Saijo N, Sawada J, Yoshida T (2008) Importance of UDP-glucuronosyltransferase 1A1*6 for irinotecan toxicities in Japanese cancer patients. Cancer Lett 261:165–171
15. Mathijssen RH, Marsh S, Karlsson MO, Xie R, Baker SD, Verweij J, Sparreboom A, McLeod HL (2003) Irinotecan pathway genotype analysis to predict pharmacokinetics. Clin Cancer Res 9:3246–3253
16. Sai K, Kaniwa N, Itoda M, Saito Y, Hasegawa R, Komamura K, Ueno K, Kamakura S, Kitakaze M, Shirao K, Minami H, Ohtsu A, Yoshida T, Saijo N, Kitamura Y, Kamatani N, Ozawa S, Sawada J (2003) Haplotype analysis of ABCB1/MDR1 blocks in a Japanese population reveals genotype-dependent renal clearance of irinotecan. Pharmacogenetics 13:741–757
17. Zhou Q, Sparreboom A, Tan EH, Cheung YB, Lee A, Poon D, Lee EJ, Chowbay B (2005) Pharmacogenetic profiling across the irinotecan pathway in Asian patients with cancer. Br J Clin Pharmacol 59:415–424
18. Innocenti F, Undevia SD, Chen PX, Das S, Ramirez J, Dolan ME, Relling MV, Kroetz DL, Ratain MJ (2004) Pharmacogenetic analysis of interindividual irinotecan (CPT-11) pharmacokinetic (PK) variability: evidence for a functional variant of ABCC2. In: 2004 ASCO annual meeting proceedings (post-meeting edition), vol 22, No 14S, abstract no: 2010
19. de Jong FA, Marsh S, Mathijssen RH, King C, Verweij J, Sparreboom A, McLeod HL (2004) ABCG2 pharmacogenetics: ethnic differences in allele frequency and assessment of influence on irinotecan disposition. Clin Cancer Res 10:5889–5894
20. de Jong FA, Scott-Horton TJ, Kroetz DL, McLeod H, Friberg LE, Mathijssen RH, Verweij J, Marsh S, Sparreboom A (2007) Irinotecan-induced diarrhea: functional significance of the polymorphic ABCC2 transporter protein. Clin Pharmacol Ther 81:42–49
21. Xiang X, Jada SR, Li HH, Fan L, Tham LS, Wong CI, Lee SC, Lim R, Zhou QY, Goh BC, Tan EH, Chowbay B (2006) Pharmacogenetics of SLCO1B1 gene and the impact of *1b and *15 haplotypes on irinotecan disposition in Asian cancer patients. Pharmacogenet Genomics 16:683–691
22. Takane H, Miyata M, Burioka N, Kurai J, Fukuoka Y, Suyama H, Shigeoka Y, Otsubo K, Ieiri I, Shimizu E (2007) Severe toxicities after irinotecan-based chemotherapy in a patient with lung cancer: a homozygote for the SLCO1B1*15 allele. Ther Drug Monit 29:666–668
23. Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, Kim HT, Lee JS (2008) Influence of the organic anion-transporting polypeptide 1B1 (OATP1B1) polymorphisms on irinotecan-pharmacokinetics and clinical outcome of patients with advanced non-small cell lung cancer. Lung Cancer 59:69–75

24. Han JY, Lim HS, Park YH, Lee SY, Lee JS (2009) Integrated pharmacogenetic prediction of irinotecan pharmacokinetics and toxicity in patients with advanced non-small cell lung cancer. Lung Cancer 63:115–120
25. Michael M, Thompson M, Hicks RJ, Mitchell PL, Ellis A, Milner AD, Di Iulio J, Scott AM, Gurtler V, Hoskins JM, Clarke SJ, Tebbut NC, Foo K, Jefford M, Zalcberg JR (2006) Relationship of hepatic functional imaging to irinotecan pharmacokinetics and genetic parameters of drug elimination. J Clin Oncol 24:4228–4235
26. Sai K, Itoda M, Saito Y, Kurose K, Katori N, Kaniwa N, Komamura K, Kotake T, Morishita H, Tomoike H, Kamakura S, Kitakaze M, Tamura T, Yamamoto N, Kunitoh H, Yamada Y, Ohe Y, Shimada Y, Shirao K, Minami H, Ohtsu A, Yoshida T, Saijo N, Kamatani N, Ozawa S, Sawada J (2006) Genetic variations and haplotype structures of the ABCB1 gene in a Japanese population: an expanded haplotype block covering the distal promoter region, and associated ethnic differences. Ann Hum Genet 70:605–622
27. Sai K, Saito Y, Itoda M, Fukushima-Uesaka H, Nishimaki-Mogami T, Ozawa S, Maekawa K, Kurose K, Kaniwa N, Kawamoto M, Kamatani N, Shirao K, Hamaguchi T, Yamamoto N, Kunitoh H, Ohe Y, Yamada Y, Tamura T, Yoshida T, Minami H, Matsumura Y, Ohtsu A, Saijo N, Sawada J (2008) Genetic variations and haplotypes of ABCC2 encoding MRP2 in a Japanese population. Drug Metab Pharmacokinet 23:139–147
28. Maekawa K, Itoda M, Sai K, Saito Y, Kaniwa N, Shirao K, Hamaguchi T, Kunitoh H, Yamamoto N, Tamura T, Minami H, Kubota K, Ohtsu A, Yoshida T, Saijo N, Kamatani N, Ozawa S, Sawada J (2006) Genetic variation and haplotype structure of the ABC transporter gene ABCG2 in a Japanese population. Drug Metab Pharmacokinet 21:109–121
29. Kim SR, Saito Y, Sai K, Kurose K, Maekawa K, Kaniwa N, Ozawa S, Kamatani N, Shirao K, Yamamoto N, Hamaguchi T, Kunitoh H, Ohe Y, Yamada Y, Tamura T, Yoshida T, Minami H, Ohtsu A, Saijo N, Sawada J (2007) Genetic variations and frequencies of major haplotypes in SLCO1B1 encoding the transporter OATP1B1 in Japanese subjects: SLCO1B1*17 is more prevalent than *15. Drug Metab Pharmacokinet 22:456–461
30. Takane H, Kobayashi D, Hirota T, Kigawa J, Terakawa N, Otsubo K, Ieiri I (2004) Haplotype-oriented genetic analysis and functional assessment of promoter variants in the MDR1 (ABCB1) gene. J Pharmacol Exp Ther 311:1179–1187
31. Kim RB, Leake BF, Choo EF, Dresser GK, Kubba SV, Schwarz UI, Taylor A, Xie HG, McKinsey J, Zhou S, Lan LB, Schuetz JD, Schuetz EG, Wilkinson GR (2001) Identification of functionally variant MDR1 alleles among European Americans and African Americans. Clin Pharmacol Ther 70:189–199
32. Choi JH, Ahn BM, Yi J, Lee JH, Lee JH, Nam SW, Chon CY, Han KH, Ahn SH, Jang IJ, Cho JY, Suh Y, Cho MO, Lee JE, Kim KH, Lee MG (2007) MRP2 haplotypes confer differential susceptibility to toxic liver injury. Pharmacogenet Genomics 17:403–415
33. Tirona RG, Leake BF, Merino G, Kim RB (2001) Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. J Biol Chem 276:35669–35675
34. Niemi M, Schaeffeler E, Lang T, Fromm MF, Neuvonen M, Kyrklund C, Backman JT, Kerb R, Schwab M, Neuvonen PJ, Eichelbaum M, Kivistö KT (2004) High plasma pravastatin concentrations are associated with single nucleotide polymorphisms and haplotypes of organic anion transporting polypeptide-C (OATP-C, SLCO1B1). Pharmacogenetics 14:429–440
35. Saeki M, Saito Y, Sai K, Maekawa K, Kaniwa N, Sawada J, Kawamoto M, Saito A, Kamatani N (2007) A combinatorial haplotype of the UDP-glucuronosyltransferase 1A1 gene (#60-#IB) increases total bilirubin concentrations in Japanese volunteers. Clin Chem 53:356–358
36. Tamura A, Wakabayashi K, Onishi Y, Takeda M, Ikegami Y, Sawada S, Tsuji M, Matsuda Y, Ishikawa T (2007) Re-evaluation and functional classification of non-synonymous single nucleotide polymorphisms of the human ATP-binding cassette transporter ABCG2. Cancer Sci 98:231–239
37. Wong M, Evans S, Rivory LP, Hoskins JM, Mann GJ, Farlow D, Clarke CL, Balleine RL, Gurney H (2005) Hepatic technetium Tc 99m-labeled sestamibi elimination rate and ABCB1 (MDR1) genotype as indicators of ABCB1 (P-glycoprotein) activity in patients with cancer. Clin Pharmacol Ther 77:33–42
38. Sissung TM, Mross K, Steinberg SM, Behringer D, Figg WD, Sparreboom A, Mielke S (2006) Association of ABCB1 genotypes with paclitaxel-mediated peripheral neuropathy and neutropenia. Eur J Cancer 42:2893–2896
39. Imai Y, Nakane M, Kage K, Tsukahara S, Ishikawa E, Tsuruo T, Miki Y, Sugimoto Y (2002) C421A polymorphism in the human breast cancer resistance protein gene is associated with low expression of Q141K protein and low-level drug resistance. Mol Cancer Ther 1:611–616
40. Kondo C, Suzuki H, Itoda M, Ozawa S, Sawada J, Kobayashi D, Ieiri I, Mine K, Ohtsubo K, Sugiyama Y (2004) Functional analysis of SNPs variants of BCRP/ABCG2. Pharm Res 21: 1895–1903
41. Mizuarai S, Aozasa N, Kotani H (2004) Single nucleotide polymorphisms result in impaired membrane localization and reduced ATPase activity in multidrug transporter ABCG2. Int J Cancer 109:238–246
42. Sparreboom A, Gelderblom H, Marsh S, Ahluwalia R, Obach R, Principe P, Twelves C, Verweij J, McLeod HL (2004) Diflomotecan pharmacokinetics in relation to ABCG2 421C>A genotype. Clin Pharmacol Ther 76:38–44
43. Teng S, Piquette-Miller M (2008) Regulation of transporters by nuclear hormone receptors: implications during inflammation. Mol Pharm 5:67–76
44. Englund G, Jacobson A, Rorsman F, Artursson P, Kindmark A, Rönnblom A (2007) Efflux transporters in ulcerative colitis: decreased expression of BCRP (ABCG2) and Pgp (ABCB1). Inflamm Bowel Dis 13:291–297
45. de Jong F, van der Bol J, Mathijssen R, van Gelder T, Wiemer E, Sparreboom A, Verweij J (2008) Renal function as a predictor of irinotecan-induced neutropenia. Clin Pharmacol Ther 84:254–262