Role of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms in irinotecan-induced neutropenia in Asian cancer patients

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The objectives of the present study were (i) to study the pharmacogenetics of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A in three distinct healthy Asian populations (Chinese, Malays and Indians), and (ii) to investigate the polygenic influence of these polymorphic variants in irinotecan-induced neutropenia in Asian cancer patients. Pharmacokinetic and pharmacogenetic analyses were done after administration of irinotecan as a 90-min intravenous infusion of 375 mg/m^2 once every 3 weeks ($n = 45$). Genotypic–phenotypic correlates showed a non-significant influence of *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms on the pharmacokinetics of SN-38 ($P > 0.05$), as well as severity of neutropenia ($P > 0.05$). Significantly higher exposure levels to SN-38 ($P = 0.018$), lower relative extent of glucuronidation (REG; $P = 0.006$) and higher biliary index (BI; $P = 0.003$) were found in cancer patients homozygous for the *UGT1A1*6* allele compared with patients harboring the reference genotype. The mean absolute neutrophil count (ANC) was 85% lower and the prevalence of grade 4 neutropenia (ANC ≤ 500/μL) was 27% in patients homozygous for *UGT1A1*6* compared with the reference group. Furthermore, the presence of the *UGT1A1*6* allele was associated with an approximately 3-fold increased risk of developing severe grade 4 neutropenia compared with patients harboring the reference genotype. These exploratory findings suggest that homozygosity for *UGT1A1*6* allele may be associated with altered SN-38 disposition and may increase the risk of severe neutropenia in Asian cancer patients, particularly in the Chinese cancer patients who comprised 80% ($n = 36$) of the patient population in the present study. (*Cancer Sci* 2007; 98: 1461–1467)

Irinotecan is a topoisomerase I inhibitor that is used for the treatment of several malignancies including colorectal, lung and gastric cancers.[1–4] It is converted by carboxylesterase I/II to SN-38, which has 100-fold greater antitumor activity than the parent compound.[5] SN-38 is in turn glucuronidated to SN-38G by the uridine glucuronosyltransferase (UGT) family of enzymes.[6,7] The disposition of irinotecan is complex and exhibits large variations in cancer patients of different ethnic backgrounds. The factors responsible for the wide interpatient variability are several-fold and include both genetic and non-genetic causes. The genetic causes include polymorphisms in genes encoding the various drug transporters and drug metabolizing enzymes along the irinotecan biochemical pathway and can greatly impact on the pharmacokinetics and pharmacodynamics of irinotecan.[8,9]

Diarrhea and neutropenia are two well-characterized toxicities associated with irinotecan treatment and the frequency of occurrence of these toxicities is dependent on the schedule of administration as well as on the effectiveness of SN-38 detoxification by glucuronidation reactions mediated by several hepatic (UGT1A1, UGT1A6 and UGT1A9) and extrahepatic (UGT1A7) glucuronosyl transferases.[10,11] Among these UGT1A enzymes, the UGT1A1 protein has the highest capacity to glucuronidate SN-38.

Selective functional genetic polymorphisms in the various *UGT1A* isoforms have been shown to influence the severity of irinotecan-induced gastrointestinal and hematologic toxicities in cancer patients of different ethnic origins. Homozygosity for the microsatellite tandem repeat polymorphism in the TATA box region of *UGT1A1* promoter (-53(TA)$_{6>7}$; *UGT1A1*28*) is associated with decreased UGT1A1 expression and activity and can critically impact the glucuronidation of SN-38 in Caucasian cancer patients, in whom its allelic frequency is higher than in other ethnic populations.[12,13] The *UGT1A1*28* is a rare allele in Asians,[14] and a recent study in Korean cancer patients has suggested the *UGT1A1*6* (c.211G>A) allele to be more predictive of neutropenia.[15] This finding differs from that of Ando *et al*., who found no influence of the *UGT1A1*6* polymorphism in Japanese cancer patients treated with irinotecan.[10] These results suggest that the impact of the *UGT1A1*6* allele may differ among Asians from different geographic locations and it is important to delineate the molecular determinants of irinotecan-induced neutropenia in other Asian ethnic groups as well.

Apart from the influence of *UGT1A1*6* and *UGT1A1*28* on irinotecan-induced neutropenia, a recent *in vitro* study by Imai *et al*.[16] suggested that the non-synonymous *ABCG2* c.421C>A (Q141K) polymorphism in exon 5 exhibited low-level drug resistance and increased the sensitivity of normal cells to SN-38 compared with cell lines transfected with *ABCG2* gene harboring the reference genotype.[16] A clinical study in European Caucasian cancer patients receiving irinotecan at a fixed dose of 600 mg once every 3 weeks, however, a suggested limited role of the c.421C>A polymorphism in influencing the disposition of irinotecan and SN-38.[16,17] Furthermore the allele frequency distribution of the c.421C>A polymorphism exhibited wide ethnic variations,[17] suggesting that perhaps the functional effect of this polymorphism, and hence its effect on the disposition of irinotecan and its metabolites, may vary in different ethnic groups receiving different dosages and schedules of irinotecan.

The objective of the present study was twofold: first, to investigate the genotypic and allelic frequencies of *UGT1A1*6* and *ABCG2* c.421C>A polymorphisms in three distinct healthy Asian populations, namely, the Chinese, Malay and Indian populations, which have not been reported previously; and second, to investigate the multigenic influence of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms on irinotecan-induced neutropenia in a cohort of Asian cancer patients receiving irinotecan as a 3-weekly regimen.

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Materials and Methods

Study patients. A total of 45 cancer patients were enrolled at the National University Hospital, Singapore. Patients were considered to be suitable for participation in the study if they satisfied the following inclusion criteria: age range of between 26 and 75 years; histologically confirmed diagnosis of the particular tumor type for which irinotecan is indicated; a performance status of between 0 and 2 on the Eastern Cooperative Oncology Group (ECOG) scale; no prior use of irinotecan; a life expectancy of at least 3 months; adequate hematological (total white blood cell count ≥3500/μL; absolute neutrophil count [ANC] ≥1500/μL; platelet count ≥100 000/μL; and hemoglobin level ≥9 g/dL), hepatic (total bilirubin levels ≤2.0 mg/dL; aspartate aminotransferase [AST] and alanine aminotransferase [ALT] levels ≤2.5 times the upper limit of normal) and renal (creatinine level ≤1.5 g/dL or 133 μmol/L and 24-h creatinine clearance ≥40 mL/min) functions. The following exclusion criteria were included in the protocol: serious infectious diseases or other severe complications such as pre-existing cardiac disease, uncontrolled diabetes, bleeding or colitis; active concurrent malignancies; symptomatic brain metastases; lactating or pregnant women, or patient's planning for pregnancy; or other medical problems severe enough to prevent compliance with the protocol. None of the patients were receiving drugs, dietary supplements and/or herbal preparations known to interact with irinotecan or affect the expression and/or function of proteins relevant to irinotecan disposition.

All study participants gave approved informed consent prior to enrollment in the study and the protocol was approved by the ethics committee of the National University Hospital, Singapore.

Drug administration. Irinotecan was supplied by Aventis Pharma as 5-mL vials containing 100 mg of the drug. The cancer patients received single agent irinotecan at 375 mg/m^2 once every 3 weeks as a 90-min intravenous infusion. All patients received intravenous ondansetron 8 mg and intravenous dexamethasone 8 mg as pre-medication schedules before administration of their chemotherapy. Treatment with irinotecan was withheld in subsequent cycles if the patients on either regimen experienced unacceptable toxicity such as absolute neutrophil count ≤1000/μL, platelet count ≤100 000/μL, or diarrhea of grade 3 or 4.

Pharmacokinetic study. Pharmacokinetic blood samplings were done on day 1 of cycle 1 and blood (3 mL) was obtained from the opposite arm of each patient at the following times: immediately before infusion; 15, 30, 45 and 90 min after start of infusion; and at 2, 2.5, 3, 3.5, 4, 6, 8, 12 and 24 h after the end of infusion. The blood samples were immediately centrifuged and the plasma thus obtained was stored at –20°C until analysis. Plasma levels of irinotecan and SN-38 were determined using reversed-phase high-performance liquid chromatography with fluorescence detection as described previously.[9] Briefly, plasma was extracted using a single protein precipitation step. The extracts were chromatographed on a C18 reversed-phase analytical column using a mobile phase composed of 30% acetonitrile and 70% potassium dihydrogen phosphate (0.045 M) containing 10 mM sodium heptanesulfonic acid (pH 3.5). Irinotecan, SN-38 and the internal standard (camptothecin) were determined using a fluorescence detector with excitation wavelength at 373 nm and emission at 428 nm for irinotecan and 380 nm and 540 nm for SN-38. The concentrations of irinotecan and SN-38 were determined from peak area ratios of either compound to the internal standard. SN-38G concentrations were determined as the increase in SN-38 concentration following incubation with β-glucuronidase. The limits of quantification for irinotecan, SN-38 and SN-38G were 3, 0.6 and 6 ng/mL, respectively. The calibration curves were linear over the concentration ranges tested, that is, 3–10 000 ng/mL for irinotecan ($r^2 = 0.9996$), 0.6–2000 ng/mL for SN-38 ($r^2 = 0.9996$) and 6–20 000 ng/mL for SN-38G ($r^2 = 0.9997$) in plasma. The coefficients of variation for the intraday and interday reproducibility were 7.0% and 6.4% for irinotecan, 4.4% and 3.0% for SN-38, and 2.5% and 2.0% for SN-38G, respectively.

Pharmacokinetic analysis. Pharmacokinetic parameters for total irinotecan, SN-38 and SN-38G were determined using non-compartmental methods with a non-linear regression program, WinNonLin version 2.1 (Pharsight Inc, Mountain View, CA, USA). Peak plasma concentrations (C_{max}) and time to peak concentration (t_{max}) were identified from individual subject concentration time curves. Area under the plasma concentration time curve from time zero to the time (t) of the last detectable concentration ($AUC_{0\to t}$) was calculated using the trapezoidal rule. The area was extrapolated to infinity ($AUC_{0\text{-inf}}$) by adding $C_t/\lambda z$ to $AUC_{0\to t}$, where C_t was the last detectable plasma concentration. The apparent elimination rate constant (λ_z) was estimated by least-squares regression of values in the terminal log-linear region of the plasma irinotecan, SN-38 and SN-38G concentration time curves, whereas half-life ($t_{1/2}$) was calculated as $\ln(2)/\lambda_z$. Total clearance (CL) of irinotecan was calculated as the ratio of dose to $AUC_{0\text{-inf}}$. The following AUC ratios were calculated: the relative extent of conversion (REC) of irinotecan to SN-38 ($AUC_{SN\text{-}38}/AUC_{irinotecan}$), the relative extent of glucuronidation (REG) of SN-38 to SN-38G ($AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$) and the biliary index (BI; $AUC_{SN\text{-}38}/AUC_{SN\text{-}38G} \times AUC_{irinotecan}$).[11]

***UGT1A1*6, UGT1A1*28* and *ABCG2* c.421C>A genotyping.** A total of 269 healthy subjects (90 Chinese, 85 Malays and 94 Indians) and 45 cancer patients were genotyped for the *UGT1A1*6* polymorphism. Genotyping for *UGT1A1*28* in a healthy Asian population has been described previously,[14] and was similarly performed in the cancer patients ($n = 45$) in this study. A total of 285 healthy subjects (94 Chinese, 97 Malays and 94 Indians) and 45 cancer patients were genotyped for the *ABCG2* c.421C>A polymorphism. The healthy subjects in each ethnic group were carefully screened for their ethnic background before being enrolled into the study. Individual subjects self-reported their race and ethnic group, including their parents and grandparents of two generations. An additional confirmatory screening process involved confirming the race and ethnic group of each subject against that listed in their National Registration Identity Cards.

Genotyping for *UGT1A1*6* was carried out using 5′-CTC-CACCTTCTTTATCTC-3′ and 5′-GCATAGCAGAGTCCTTTT-3′ as the forward and reverse primers, respectively. The primer sequences were created based on published sequences (accession no: NM000463). The polymerase chain reaction (PCR) amplifications were carried out in a 20-μL reaction mixture under the following conditions: denaturation at 94°C for 5 min, followed by amplification using 36 cycles of 94°C for 30 s, 49.7°C for 30 s and elongation at 72°C for 1 min, followed by a final extension at 72°C for 10 min. The amplified PCR fragments were purified using QIAquick PCR purification kit followed by direct sequencing of the PCR fragments as outlined above.

The PCR primer sequences for the *ABCG2* c.421C>A polymorphism was created based on published sequences (accession no: NM004827). The sequences of the forward and reverse primers were 5′-ATGATGTTGTGATGGGCACTC-3′ and 5′-CAGACCTAACTCTTGAATGACCCT-3′, respectively. The PCR amplifications were carried out in an MJ Research PTC-100 thermal cycler (MJ Research Inc. Waltham, MA, USA) with the following reagents in a 20 μL reaction mixture: 50 ng genomic DNA, 0.25 μM of each primer, 10 × PCR buffer containing 10 mM Tris and 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each of dNTPs and 1 U *Taq* DNA polymerase. The PCR conditions consisted of an initial denaturation at 94°C for 2 min, followed by amplification using 33 cycles of 94°C for 30 s, 58°C for 30 s and elongation at 72°C for 30 s, followed by a final extension at 72°C for 5 min. The amplified reaction products were purified

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using QIAquick PCR purification kit followed by digestion with *Mse*I (c.421C>A) for a minimum of 2 h at 37°C. The digested PCR products (131 bp) were analyzed by electrophoretic separation on 15% polyacrylamide gel, followed by direct visualization under UV light. Genotype verifications were carried out by direct sequencing of the PCR product using the CEQ Dye Terminator Cycle Sequencing Quick Start Kit and a CEQ 2000 automated sequencer (Beckman Coulter, Fullerton, CA, USA).

Statistical analysis. The chi-squared test was used to assess the Hardy–Weinberg equilibrium. The inter-ethnic differences of genotype and allelic frequencies for the *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphism was performed using Fisher's exact test. All pharmacokinetic parameters are expressed as mean ± SD, unless otherwise stated. The statistical differences between the pharmacokinetic parameters among the *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C> genotype groups were determined by applying the Kruskal–Wallis and Mann–Whitney *U*-tests. The minimum level of statistical significance was set at $P = 0.05$ for overall comparisons between the different genotype groups. The Bonferroni correction was applied for multiple pairwise comparisons. Analysis of covariance was performed to confirm that statistical significance was not confounded by other measured variables. Factors included genotype (*UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A), ethnicity, sex, age, dose, weight, body surface area and schedule of irinotecan administration. Non-significant factors were dropped from the models. Bonferroni adjustment was used to adjust the significance level for each parameter included in each model. All statistical analyses were done using Stata (STATA Statistical Software release 7.0, Stata Corporation, College Station, TX, USA).

Results

Patient characteristics. Table 1 summarizes the demographics of the cancer patients. The Chinese and Malay cancer patients comprised approximately 98% of the total patient population in each group. Patients with colorectal, non-small cell lung and genitourinary cancers comprised 94% of the total tumor types that received irinotecan treatment.

Table 1. Asian cancer patient demographics

Characteristics	No. of patients (%)	Median (range)
Total no. of patients	45 (100)	
Sex		
Male	25 (56)	
Female	20 (44)	
Race		
Chinese	36 (80.0)	
Malay	8 (17.8)	
Indian	0 (0)	
Others	1 (2.2)	
Age (years)		55 (26–75)
Weight (kg)		59 (35.6–89.5)
Body surface area (m^2)		1.6 (1.3–2.0)
Tumor types		
Nasopharyngeal carcinoma	1 (2)	
Gastrointestinal	35 (78)	
Genitourinary	2 (4)	
Non-small-cell lung cancer	5 (11)	
Miscellaneous	2 (4)	

***UGT1A1*6, UGT1A1*28* and *ABCG2* c.421C>A polymorphisms in Asian healthy subjects and cancer patients.** Table 2 summarizes the genotype and allele frequencies of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms in healthy Asian subjects belonging to three distinct ethnic groups, namely, Chinese, Malay and Indian populations, as well as Asian cancer patients. There were no significant deviations from Hardy–Weinberg equilibrium in

Table 2. *UGT1A1*6, UGT1A1*28* and *ABCG2* c.421C>A genotype frequencies in Asian healthy subjects and cancer patients

Populations	*n*	Genotype frequency, *n* (%)			Allele frequency	
		*UGT1A1*6*			G	A
		GG	GA	AA		
Healthy Asians						
Chinese	90	64 (71.1)	25 (27.8)	1 (1.1)	0.85	0.15
Malays	85	78 (91.8)	6 (7.1)	1 (1.2)	0.95	0.05
Indians	94	89 (94.7)	4 (4.3)	1 (1.1)	0.97	0.03
Pooled healthy Asians	269	231 (85.9)	35 (13.0)	3 (1.1)	0.92	0.08
Asian cancer patients	45	35 (77.8)	8 (11.1)	2 (4.4)	0.87	0.13
		*UGT1A1*28*				
		6/6	6/7	7/7	6s	7s
Healthy Asians						
Chinese	89	63 (70.8)	24 (27.0)	2 (2.2)	0.84	0.16
Malays	93	62 (66.7)	27 (29.0)	4 (4.3)	0.81	0.19
Indians	84	36 (42.9)	37 (44.0)	11 (13.1)	0.65	0.35
Pooled healthy Asians	266	161 (60.5)	88 (33.1)	17 (6.4)	0.77	0.23
Asian cancer patients	45	30 (66.7)	15 (33.3)	0 (0)	0.83	0.17
		ABCG2 c.421C>A				
		CC	CA	AA	C	A
Healthy Asians						
Chinese	94	49 (52.1)	38 (40.4)	7 (7.4)	0.72	0.28
Malays	97	54 (55.7)	33 (34.0)	10 (10.3)	0.73	0.27
Indians	94	69 (73.4)	21 (22.3)	4 (4.3)	0.85	0.15
Pooled healthy Asians	285	172 (60.4)	92 (32.3)	21 (7.4)	0.76	0.24
Asian cancer patients	45	22 (48.9)	23 (51.1)	0 (0)	0.74	0.26

Table 3. Summary of irinotecan pharmacokinetic parameters in Asian cancer patients ($n = 45$)

Pharmacokinetic parameters	Mean ± SD
Irinotecan	
C_{max}/dose/BSA (/m^5)	5.6 ± 1.9
$AUC_{0\text{-}inf}$/dose/BSA (h/m^5)	42.3 ± 19.0
$t_{1/2}$ (h)	7.0 ± 1.5
CL/BSA (L/h/m^2)	0.4 ± 3.4
V_{ss}/BSA (L/m^2)	70.0 ± 17.4
SN-38	
C_{max}/dose/BSA (10^{-2}/m^5)	14.4 ± 7.0
$AUC_{0\text{-}inf}$/dose/BSA (h/m^5)	1.2 ± 0.64
$t_{1/2}$ (h)	13.4 ± 8.4
SN-38G	
C_{max}/dose/BSA (m^{-5})	26.6 ± 12.8
$AUC_{0\text{-}inf}$/dose/BSA (h/m^5)	4.2 ± 2.5
$t_{1/2}$ (h)	15.3 ± 7.6
AUC ratios	
REC (10^{-2})	3.1 ± 1.5
REG	3.9 ± 2.2
BI (10^2 ng h/mL)	106.1 ± 58.4
TMR	0.13 ± 0.04

$AUC_{0\text{-}inf}$/dose/BSA, area under plasma concentration-time curve from time zero to infinity normalized by dose and body surface area; BI, biliary index (AUC ratio of irinotecan to the relative extent of glucuronidation); BSA, body surface area; CL/BSA, plasma clearance normalized by body surface area; C_{max}/dose/BSA, peak plasma concentration normalized by dose and body surface area; REC, relative extent of conversion (AUC ratio of SN-38 to irinotecan); REG, relative extent of glucuronidation (AUC ratio of SN-38G to SN-38); $t_{1/2}$, half-life; TMR, total metabolic ratio (AUC ratio of the sum of SN-38 + SN-38G to irinotecan); V_{ss}/BSA, volume of distribution at steady-state normalized by body surface area.

the genotype frequencies of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms in both healthy subjects and cancer patients.

The allelic frequency of *UGT1A1*6* was found to be three- to five-fold higher in the healthy Chinese population compared with the healthy Malay and Indian populations. These interethnic differences in the distribution of *UGT1A1*6* allelic frequency were significantly different between the Chinese and Malay ($P = 0.001$) and Chinese and Indian ($P < 0.001$) populations. The genotype and allele frequencies of *UGT1A1*6* polymorphism in the cancer patients, which mainly comprised patients belonging to Chinese ethnicity (80.0%; Table 1) were similar to the healthy Chinese population. The *UGT1A1*28/*28* genotype was not detected in the cancer patients.

Fisher's exact test showed the variation in genotype frequencies between the three healthy ethnic groups to be statistically significant for the *ABCG2* c.421C>A polymorphism ($P = 0.022$). The homozygous c.421AA genotype was lowest in the Indians (4.3%) compared with the Chinese and Malay healthy populations (7.4% and 10.3%, respectively). Pairwise ethnic comparisons also revealed the Indian population to be significantly different from both the Chinese ($P = 0.010$) and Malay ($P = 0.031$), populations. There was no difference in genotype frequency distributions between the Chinese and Malay populations ($P = 0.596$). The majority of the cancer patients belonged to the Chinese ethnic group (Table 1) and the allele frequencies of the *ABCG2* c.421C>A polymorphism compared well with the frequencies found in the healthy Chinese population (Table 2). There were no patients homozygous for the c.421AA genotype.

Effect of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms on irinotecan disposition in Asian cancer patients. Table 3 summarizes the pharmacokinetic parameters of the cancer patients. Table 4 depicts the influence of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms on pharmacokinetics of irinotecan and its metabolites in cancer patients receiving irinotecan. With regards to the impact of *UGT1A1*6* polymorphism on irinotecan pharmacokinetics, the SN-38 exposure level was significantly higher (Fig. 1; $P = 0.018$) and REG was significantly lower (Fig. 2; $P = 0.006$) in patients homozygous for the *UGT1A1*6* allele compared with patients harboring the reference genotype. Patients homozygous for the *UGT1A1*6* allele also had approximately 61% higher BI values compared with patients with the reference genotype ($P = 0.003$). No significant differences in SN-38 pharmacokinetics were found in the cancer patients with regards to *UGT1A1*28* ($P = 0.700$; Table 4) or *ABCG2* c.421C>A ($P = 0.677$; Table 4) genotypic status.

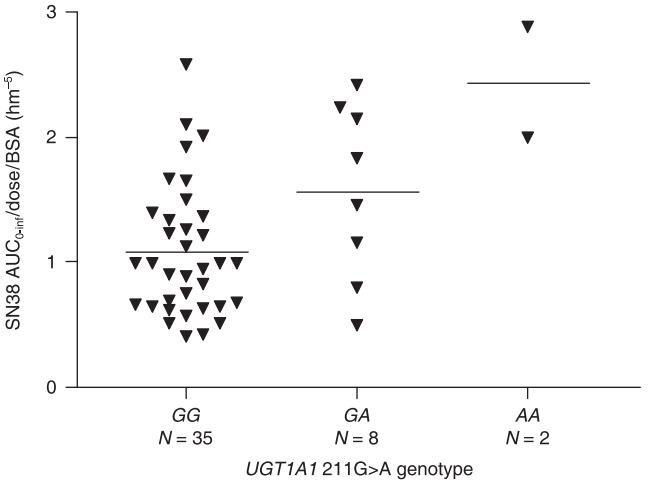

Fig. 1. Plot of area under the plasma concentration-time from zero to infinity normalized by dose and body surface area of SN-38 in relation to *UGT1A1* 211G>A genotypes in Asian cancer patients ($n = 45$). Bars represent the median values. $AUC_{0\text{-}inf}$/dose/BSA, area under plasma concentration time curve from time zero to infinity normalized by dose and body surface area; BSA, body surface area.

Influence of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms on the toxicity profile in Asian cancer patients. The common Toxicity Criteria version 2.0 was used for assessment of patient's toxicity profile (Table 5). Severe grade 4 diarrhea was absent in the cancer patients. Of the six patients who experienced grade 3 diarrhea, one patient harbored the homozygous *UGT1A1*6* allele, one was a heterozygote (*UGT1A1*1/*6*) and four harbored the homozygous reference genotype. With regards to *UGT1A1*28* genotypic status, there were two heterozygotes (*UGT1A1*1/*28*) and four harbored the reference genotype. With regards to the *ABCG2* c.421C>A genotypic status, five patients were heterozygotes and one harbored the reference genotype.

The prevalence of grade 4 neutropenia (ANC ≤ 500/μL) was 27% (12 of 45 patients). The mean ANC nadir values were independent of the *ABCG2* ($P = 0.700$) and *UGT1A1*28* ($P = 0.300$) genotype status. None of the patients with grade 4 neutropenia harbored the homozygous *UGT1A1*28* or c.421C>A allele. With regards to the *UGT1A1*6* genotype status, eight of the patients with grade 4 neutropenia harbored the reference genotype, two patients were heterozygous and two patients were homozygous for the *UGT1A1*6* allele. The mean ANC value in patients homozygous for the *UGT1A1*6* allele was 85% lower compared with patients with the reference genotype. Also, their median SN-38 AUC was approximately 60% higher than in patients harboring the reference genotype (median $AUC_{0\text{-}\infty}$/dose/BSA: 2.45 [range: 2.0–2.9] *vs* 0.99 [range: 0.41–2.60], h m^{-5}; $P = 0.018$) and REG values were approximately 70% lower compared with the reference genotypic group (median REG: 1.11 [range: 0.91–1.3] *vs* 3.7 [range: 1.0–9.0]; $P = 0.006$).

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Table 4. Effect of *UGT1A1*6*, *UGT1A1*28* and *ABCG2* c.421C>A genotypes on pharmacokinetics of irinotecan and its metabolites in Asian cancer patients (median ± range)

Pharmacokinetic parameters	Genotype frequency, *n* (%)			Pairwise *P*-values			Overall *P*-values
	*UGT1A1*6*						
	GG ($n = 35$)	GA ($n = 8$)	AA ($n = 2$)	GG *vs* GA	GG *vs* AA	GA *vs* AA	
$AUC_{0\text{-inf}}$/dose/BSA (h/m^5)							
Irinotecan	36.0 (17.5–96.5)	33.7 (22.8–80.2)	39.9 (32.4–47.5)	0.748	0.973	0.889	0.936
SN-38	0.99 (0.41–2.6)	1.64 (0.50–2.4)	2.4 (2.0–2.9)	0.072	0.018	0.267	0.025
SN-38G	4.0 (1.1–11.1)	3.3 (1.9–6.1)	2.7 (1.8–3.6)	0.571	0.468	0.406	0.587
AUC ratios							
REC (10^{-2})	2.4 (0.8–5.4)	3.4 (2.2–9.7)	6.1 (6.1–6.2)	0.031	0.003	0.178	0.008
REG	3.7 (1.0–9.0)	2.4 (1.1–3.9)	1.1 (0.91–1.3)	0.007	0.006	0.089	0.003
BI (10^2 ng h/mL)	80.1 (29.0–226.5)	125.2 (48.0–216.8)	237.9 (232.7–243.1)	0.168	0.003	0.044	0.025
	*UGT1A1*28*						
	6/6 ($n = 30$)	6/7 ($n = 15$)		6/6 *vs* 6/7			
$AUC_{0\text{-inf}}$/dose/BSA (h/m^5)							
Irinotecan	35.3 (20.8–96.5)	34.6 (17.5–57.1)		0.630			
SN-38	1.1 (0.49–2.6)	1.1 (0.42–2.9)		0.700			
SN-38G	4.2 (1.1–11.1)	3.3 (1.6–7.8)		0.613			
AUC ratios							
REC (10^{-2})	2.4 (0.80–9.7)	3.1 (1.6–6.1)		0.386			
REG	3.1 (0.91–9.0)	3.3 (1.3–6.3)		0.981			
BI (10^2 ng h mL^{-1})	81.8 (29.0–232.7)	107.7 (41.9–243.1)		0.962			
	ABCG2 c.421C>A						
	CC ($n = 22$)	CA ($n = 23$)		CC *vs* CA			
$AUC_{0\text{-inf}}$/dose/BSA (h/m^5)							
Irinotecan	32.5 (20.8–96.5)	41.6 (17.5–81.8)		0.305			
SN-38	1.1 (0.41–2.6)	0.99 (0.42–2.9)		0.677			
SN-38G	2.8 (1.1–8.3)	4.8 (1.4–11.1)		0.037			
AUC ratios							
REC (10^{-2})	2.8 (1.1–9.7)	2.4 (0.80–6.1)		0.245			
REG	2.6 (0.91–6.5)	4.2 (1.3–9.0)		0.007			
BI (10^2 ng h/mL)	109.3 (41.9–232.7)	72.5 (28.9–243.1)		0.037			

Abbreviations: N, number of subjects; BSA, body surface area; C_{max}/dose/BSA, peak plasma concentration normalized by dose and body surface area; $AUC_{0\text{-inf}}$/dose/BSA, area under plasma concentration-time curve from time zero to infinity normalized by dose and body surface area; REC, relative extent of conversion (AUC ratio of SN-38 to irinotecan); REG, relative extent of glucuronidation (AUC ratio of SN-38G to SN-38); BI, biliary index (AUC ratio of irinotecan to the relative extent of glucuronidation); $P < 0.05$ considered statistically significant.

Table 5. Adverse events in Asian cancer patients receiving irinotecan ($n = 45$)

Adverse event	Toxicity grade, *n* (%)		
	Grade 0–2	Grade 3	Grade 4
Neutropenia	29 (64.4)	4 (8.9)	12 (26.7)
Leukocytopenia	31 (68.9)	8 (17.8)	6 (13.3)
Thrombocytopenia	41 (91.1)	4 (8.9)	0
Nausea	43 (95.6)	2 (4.4)	0
Diarrhea	39 (86.7)	6 (13.3)	0

Patients with at least one *UGT1A1*6* allele had an approximately 3-fold increased risk of developing grade 4 neutropenia compared with patients carrying the reference genotype (odds ratio [OR], 3.2; 95% CI, 0.69–15.04).

Influence of covariate factors on pharmacokinetics of irinotecan, SN-38 and SN-38G. Univariate analysis showed that BSA was the most significant factor in the analysis of covariance models for area under the curve (AUC) of irinotecan ($P = 0.0001$), SN-38 ($P = 0.020$) and SN-38G ($P = 0.001$). The three-weekly schedule of administration was significantly related to higher AUC of irinotecan ($P < 0.001$), lower AUC of SN-38G ($P < 0.0001$) and lower REG ($P < 0.0001$) in the cancer patients. Both univariate and multivariate analysis showed that *UGT1A1*28* and *ABCG2* c.421C>A genotype status were not significant factors in analysis of covariance models for AUC of irinotecan, SN-38 and SN-38G. *UGT1A1*6* genotypic status was significantly correlated to SN-38 AUC ($P < 0.001$) and REC ($P < 0.0003$).

Discussion

Several recently published studies have shown the disposition of irinotecan and its metabolites to differ among different ethnic groups.[11,13–15] These interethnic variations have been attributed to a genetic basis, resulting from the occurrence of functionally relevant single nucleotide polymorphism (SNP) in genes encoding

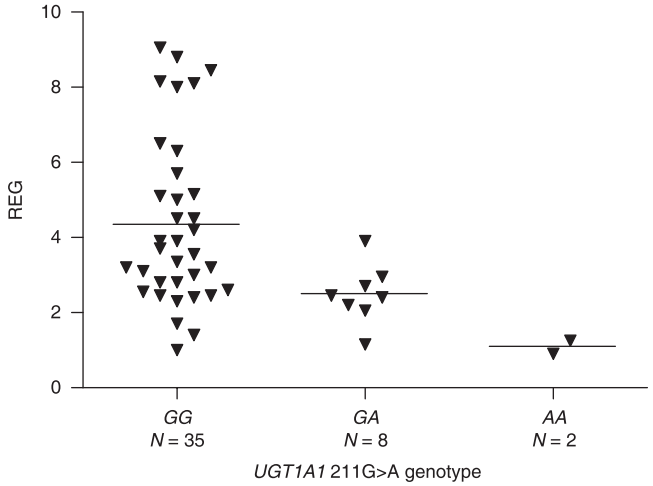

Fig. 2. Plot of relative extent of glucuronidation (REG; area under the curve ratio of SN-38G to SN-38) in relation to *UGT1A1* 211G>A genotypes in Asian cancer patients ($n = 45$). Bars represent the median values.

the various drug metabolizing enzymes and drug transporters involved in the biochemical pathway of irinotecan. The frequency of functional SNP in candidate genes involved in the irinotecan biochemical pathway varies between ethnic groups, resulting in inter-ethnic differences in phenotypic characteristics.[8–15,17] Because SN-38 is the main cytotoxic metabolite responsible for the pharmacodynamic activity of irinotecan, it is important to delineate the causative factors affecting SN-38 disposition in cancer patients belonging to different ethnic populations. In the present study, the polygenic influence of the functional *UGT1A1*6, UGT1A1*28* and *ABCG2* c.421C>A polymorphisms were evaluated in a cohort of Asian cancer patients to further understand the mechanistic basis of inter-ethnic variation in irinotecan-induced neutropenia.

Baseline screening in healthy Asian populations showed that the prevalence of *UGT1A1*6* allele was approximately three- to five-fold higher in Chinese populations compared with Malay and Indian populations. These findings contrast with the frequency distribution of the homozygous *UGT1A1*28* allele in the Asian population, which is found to be higher in the Indian population compared with the Chinese and Malay populations.[14] The *ABCG2* c.421A allele was highly variable and in concordance with reported values in the Han Chinese population in the study of de Jong *et al*.[17] The frequency of the *ABCG2* c.421A polymorphic variant in the Indian population was similar to the Caucasian populations but higher than those reported in sub-Saharan African populations.[15,17] These inter-ethnic pharmacogenetic findings further support the view that the frequency distribution of the functional *ABCG2* c.421C>A polymorphism is probably ethnically dependent.

The findings of the present study suggest that the presence of the *UGT1A1*6* allele is probably an important factor influencing the risk of severe neutropenia in Asian cancer patients. Among the 12 grade 4 neutropenic patients, cancer patients harboring the *UGT1A1*6* allele had higher exposure levels to SN-38 and lower REG compared with patients harboring the reference genotype, thereby further supporting the hypothesis that the presence of the *UGT1A1*6* allele may be an important risk factor for severe neutropenia in Asian cancer patients. These findings are in concordance with recent studies conducted in Korean and Japanese cancer patients,[15,18] and suggest that the *UGT1A1*6* polymorphic variant is perhaps a more important biomarker polymorphism for irinotecan-induced severe neutropenia in Asian cancer patients compared with Caucasian cancer patients.[13] The low number of patients harboring the *UGT1A1*6* polymorphism should be considered to be a limitation of the present study and the findings should be taken as exploratory until they are validated in a larger sample. Also, due to the low frequency of occurrence of the *UGT1A1*28/*28* variant in the present study, the functional impact of this polymorphism cannot be completely ruled out in the Asian cancer patients. Also, it is difficult to generalize the findings of this study to the Indian population, which has previously been shown to harbor a higher frequency of patients homozygous for the *UGT1A1*28* allele.[14]

Genotypic–phenotypic analysis showed a lack of significant influence of the *ABCG2* c.421C>A polymorphism on the pharmacokinetics of SN-38. A trend towards statistical significance was observed for SN-38G ($P = 0.037$) while REG was highly significant in the Asian cancer patients receiving the three-weekly irinotecan regimen ($P = 0.007$). These findings, though similar to the observations reported by de Jong *et al*.[17] in a cohort of European Caucasian cancer patients, were somewhat inconsistent with the *in vitro* findings of Imai *et al*.,[16] which showed that the *ABCG2* c.421A allele was associated with decreased ABCG2 protein expression and decreased drug resistance to ABCG2 substrates such as SN-38. There are several possible explanations that could have accounted for these observations. Firstly, the lack of a significant relationship between *ABCG2* c.421C>A polymorphism and SN-38 pharmacokinetics could be due to efficient detoxification of cytotoxic SN-38 by hepatic uridine glucuronosyl transferase 1 A (UGT1A) enzymes in the Asian cancer patients in the present study. This hypothesis is supported by the fact that of the 23 patients who harbored the c.421A allele, none of them harbored the variant *UGT1A1*28* promoter TATA box polymorphism. With regards to the *UGT1A1*6* polymorphism, 91% (21 of 23 patients) harbored the reference genotype and 4% (one each of 23 patients) harbored the heterozygous and homozygous *UGT1A1*6* allele, respectively. These findings may also account for the significantly higher REG values in these patients compared with the reference group. Secondly, and probably more importantly, due to the involvement of several hepatic and extrahepatic ABC transporters in the disposition of SN-38, it is possible that the effect of the *ABCG2* variant allele on the pharmacokinetics of SN-38 may be blunted by functional polymorphic proteins belonging to the other ABC subfamilies that are involved in the transport of SN-38.

In conclusion, the findings of this exploratory study suggest that the presence of the *UGT1A1*6* allele may predispose susceptible patients to higher systemic exposures to SN-38 and lower relative rates of glucuronidation. The risk is probably higher in cancer patients harboring the homozygous *UGT1A1*6* allele due to the poor glucuronidator phenotype of this allele, which is independent of the *UGT1A1*28* glucuronidator status. These findings should be validated in a larger population of Asian cancer patients stratified by their respective ethnic groups. The present study showed that the *UGT1A1*28* and *ABCG2* c.421C>A polymorphisms did not impact significantly on the pharmacokinetics or pharmacodynamics of SN-38 in Asian cancer patients receiving a three-weekly irinotecan regimen. The most likely cause for this lack of genotypic–phenotypic relationships is probably due to SN-38 pharmacokinetics not being selectively influenced by ABCG2, as well as the low frequency of occurrence of the poor glucuronidating *UGT1A1*28* polymorphic variant in Asian cancer patients.

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