

Irinotecan Pathway Genotype Analysis to Predict Pharmacokinetics¹

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ABSTRACT

Purpose: The purpose was to explore the relationships between irinotecan disposition and allelic variants of genes coding for adenosine triphosphate binding cassette transporters and enzymes of putative relevance for irinotecan.

Experimental Design: Irinotecan was administered to 65 cancer patients as a 90-min infusion (dose, 200–350 mg/m²), and pharmacokinetic data were obtained during the first cycle. All patients were genotyped for variants in genes encoding *MDR1* P-glycoprotein (ABCB1), multidrug resistance-associated proteins MRP-1 (ABCC1) and MRP-2 (canalicular multispecific organic anion transporter; ABCC2), breast cancer resistance protein (ABCG2), carboxylesterases (CES1, CES2), cytochrome P450 isozymes (CYP3A4, CYP3A5), UDP glucuronosyltransferase (UGT1A1), and a DNA-repair enzyme (XRCC1), which was included as a nonmechanistic control.

Results: Eighteen genetic variants were found in nine genes of putative importance for irinotecan disposition. The homozygous T allele of the *ABCB1* 1236C>T polymorphism was associated with significantly increased exposure to irinotecan ($P = 0.038$) and its active metabolite SN-38 ($P = 0.031$). Pharmacokinetic parameters were not related to any of the other multiple variant genotypes, possibly because of the low allele frequency. The extent of SN-38 glucuronidation was slightly impaired in homozygous variants of

*UGT1A1**28, although differences were not statistically significant ($P = 0.22$).

Conclusions: It is concluded that genotyping for *ABCB1* 1236C>T may be one of the factors assisting with dose optimization of irinotecan chemotherapy in cancer patients. Additional investigation is required to confirm these findings in a larger population and to assess relationships between irinotecan disposition and the rare variant genotypes, especially in other ethnic groups.

INTRODUCTION

The topoisomerase I inhibitor irinotecan has a major role in the management of metastatic colorectal cancer and has been approved either in combination with 5-fluorouracil and folinic acid in the first line treatment setting or as monotherapy in the second line setting (1). In clinical use, irinotecan is subject to very substantial interindividual variability in pharmacokinetic behavior, treatment efficacy, and the occurrence of unpredictable, sometimes severe toxic side effects that might be life threatening in some patients (2). Potential causes for such variability in drug effects include the pathogenesis and severity of the disease being treated, the occurrence of unintended drug interactions, and impairment of hepatic and renal function or both (3, 4). Despite the potential importance of these clinical variables in determining drug effects, it is now recognized that inherited differences in the metabolism and excretion into the feces and urine can have an even greater impact on the efficacy and toxicity of drugs (5).

The metabolism of irinotecan is very complex, and involves several Phase I and II metabolizing enzymes (Fig. 1). In humans, the ester bond of irinotecan is cleaved by CESs⁴ to form the primary pharmacologically active metabolite SN-38 (6), which is further conjugated by UGT isoforms to form an inactive β -glucuronic acid conjugate, SN-38G (7). Another prominent pathway of irinotecan metabolism consists of a CYP3A4-mediated oxidation of the bipiperidine side chain attached to the core structure, which results in the formation of a major metabolite identified as APC (8). The pharmacological behavior of irinotecan is additionally complicated by the fact that its elimination pathways are partially mediated by membrane-localized, energy-dependent outward drug pumps that facilitate cellular efflux mechanisms (Fig. 1). These proteins belong to the superfamily of ABC transporters and include *MDR1* P-glycoprotein (ABCB1; Ref. 9), multidrug resistance-

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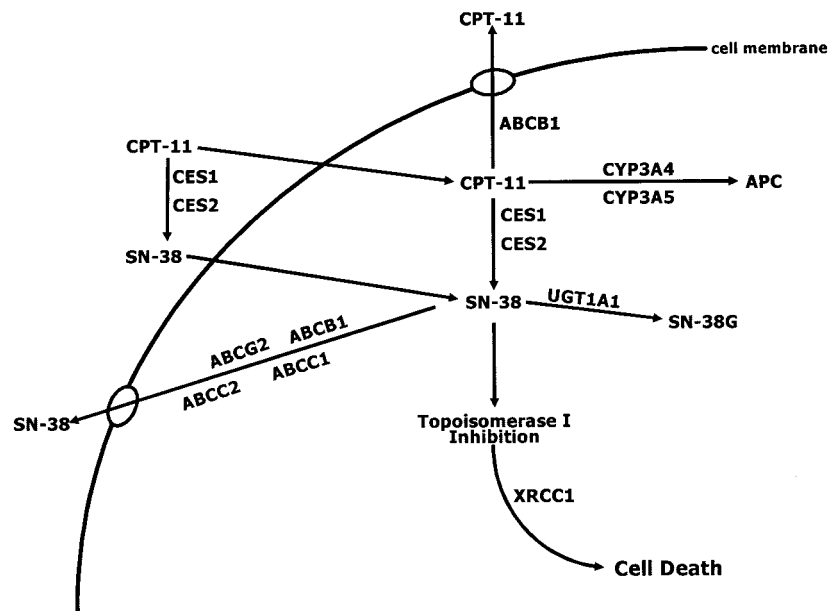
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⁴ The abbreviations used are: CES, carboxylesterase; UGT, uridine diphosphate glucuronosyltransferase; ABC, adenosine triphosphate binding cassette; ALT, alanine aminotransferase; APC, 7-ethyl-10-[4-N-(5-aminopentanoic-acid)-1-piperidino]-carbonyloxycamptothecin; AST, aspartate aminotransferase; AUC, area under the plasma concentration-time curve; CL, clearance; CYP, cytochrome P450; NONMEM, nonlinear mixed effects modeling; REG, relative extent of glucuronidation; SN-38, 7-ethyl-10-hydroxycamptothecin; SNP, single nucleotide polymorphism; SN-38G, SN-38 glucuronide.

Fig. 1 Pathway of genes with a putative role in regulating irinotecan disposition.



associated protein 1 (ABCC1; Ref. 10), and its homologue multidrug resistance-associated protein 2 [also referred to as canalicular multispecific organic anion transporter or ABCG2; Ref. (11)], and breast cancer resistance protein [also referred to MXR or ABCG2; Ref. (12)]. The aim of this study was to link genetic polymorphisms in transporters and enzymes involved in irinotecan elimination to interindividual differences in measures of drug exposure and to provide a stronger scientific basis for optimizing irinotecan therapy on the basis of each patient's genetic constitution.

PATIENTS AND METHODS

Patients and Treatment. Patients with a histologically confirmed diagnosis of a malignant solid tumor for which there was no effective standard regimen and irinotecan was a reasonable treatment option were treated with a 90-min i.v. infusion of irinotecan. The drug was given once every 3 weeks until progression of disease or dose-limiting toxicities appeared and was given as a single agent at a dose of 350 mg/m² or as part of a combination chemotherapy regimen with cisplatin at doses ranging between 200 and 300 mg/m². All patients were treated between January 1997 and June 2001 at the Erasmus MC-Daniel den Hoed Cancer Center (Rotterdam, the Netherlands). Inclusion criteria included the following: (a) adequate hematopoietic function (neutrophil count, >2.0 × 10⁹/liter and platelet count, >100 × 10⁹/liter); and (b) normal renal and hepatic functions (serum creatinine concentration, <135 μmol/liter and/or creatinine CL, >60 ml/min; serum AST and ALT concentrations, less than three times the upper limit of normal, and less than five times the upper limit of normal in case of liver metastasis). None of the patients received other drugs, dietary supplements or herbal preparations known to interfere with irinotecan pharmacokinetics. The clinical protocols, including blood sampling for the purpose of pharmacokinetic and pharmacogenetic analyses,

were approved by the Erasmus MC Ethics Board, and all patients provided written informed consent before study entry.

Pharmacokinetic Data Analysis. In view of the small intrasubject variability in irinotecan pharmacokinetic parameters (2), blood samples of ~5 ml were only collected during the first cycle of treatment. The sampling was performed at the following time points: immediately before infusion; at 30 min after the start of the infusion; 5 min before the end of infusion; and at 10, 20, and 30 min and 1, 1.5, 2, 4, 5, 8.5, 24, 32, and 48 h after the end of infusion. In 37 of 65 patients, additional blood samples were taken at 56, 196 (day 8), 360 (day 15), and 500 h (day 21) after the end of infusion. Blood samples were handled as outlined (13), and concentrations of irinotecan, SN-38 APC, and SN-38G were determined in all patients by reversed-phase high-performance liquid chromatography with fluorescence detection as described in detail elsewhere (14). Concentrations of SN-38G and APC in plasma were measured in only 53 and 12 patients, respectively, because of limited sample supply that precluded an additional analysis on the same material.

Previously developed population pharmacokinetic models were used to predict the pharmacokinetic parameters of the lactone and carboxylate forms of the analytes (15). The considered parameters included CL, volume of distribution in the central compartment, and the dose-normalized accumulated AUC. The latter parameter was simulated for irinotecan and its metabolites in all patients from time 0 to 100 h after start of infusion (AUC_{0-100 h}) for a 90-min i.v. infusion and a standard dose of 350 mg/m². This data analysis was performed using NONMEM version VI (S. L. Beal and L. B. Sheiner, San Francisco, CA). Metabolic ratios were calculated as the AUC ratio of SN-38 to irinotecan (relative extent of conversion), the AUC ratio of SN-38G to SN-38 (REG), and the AUC ratio of APC to irinotecan (relative extent of metabolism). To allow for a comparative analysis with literature data (16), the REG in each

Table 1 PCR primers, conditions, Pyrosequencing primers and restriction enzymes

Polymorphism	Forward primer 5'-3'	Reverse primer 5'-3'	Annealing temp (°C)	Product size (bp)	Pyrosequencing primer 5'-3'	RFLP Restriction enzyme
ABCBI 1236 C>T	TGTGTCTGTGAATTGCCCTTGAAG ^a	CCTCTGCATCAGCTGGACTGT	65	148	GCACCTTCAGGTTTCAG	N/a ^b
ABCBI 2677 G>T/A	ATGGTTGGCAACTAACACACTGTTA ^a	AGCAGTAGGGAGTAACAAAATAACA	50	207	TCATCTATTAGTTTGGACTCACCTTC	N/a
ABCBI 3435 C>T	GAGCCCACTCTGTTTACTG	GCATGTATGTTGGCCCTCCTT ^a	60	250	GGTGGTGTACAGGAAGA	N/a
ABCCJ 14008 G>A	GAGGCCACTTTGGGGCA ^a	TCTTGAAGCGGAGTCTGTG	62	214	ATAAGCCCGAGGTCA	N/a
ABCCJ 462 C>T	GGTAAATGAGGCTCGGTGG	CCCCCTCACTTTGTCCATCTC ^a	64	197	CACCTTCTCCATCCC	N/a
ABCCJ 34215 C>G	GGGTGCATGTCCCACTT	CGTCTCGAGCCAGCAGCT ^a	64	257	GTCTCACACATGTGCACT	N/a
ABCC2 156231 A>G	GCAAGATCCAGGGACTTGTG	CATCGTACAGTACACGAAAGGTGAAA	55	100	N/a	HhaI
ABCG2 623 T>C	CAGTTTATCCCTGGTGTCTGG	AAAGGACAGCAITTTGCTGTGCT	55	136	N/a	MboII
CESJ 1440 A>T	TGGACTTAAATGGGGACGTA	GCCATATGGAATCAGCCTTT	55	200	N/a	HaeIII
CESJ 1525 A>C	CCCAAGACGGTATAGGAGA	CCAGGAGAGGACAAAATTGC	55	180	N/a	MseI
CES2 1647 C>T	TGAATGAATGGCGAAGTGAA	GCCCAGAGTGACCCCTCATAC	60	354	N/a	XhoI
CYP3A4 -392 A>G	AGGACAGCCATAGAGCAAAG	ATCAATGTTACTGGGGAGTCC ^a	55	116	CCATAGAGACAAAGGGCA	N/a
CYP3A4 15713 T>C	AACAATCCACAAGACCCCTT	ATCTTCAAATGTACTACAAATCACTGA ^a	55	212	TTTGGATCCATTCTTTC	N/a
CYP3A4 23172 T>C	CCACGATGTACACCCACG	ATTAGGGTGTGACACAGCAAGA	60	160	N/a	NlaIII
CYP3A5 22893 G>A	CCCACGATGTACACCCACG ^a	ATTAGGGTGTGACACAGCAAGA	65	176	CCAAAACAGGGAAGAGA	N/a
CYP3A5 30597 G>A	TCTTTGGGGCTACAGCATG ^a	AAAGAATAATAGCCACATCTTATTGAGAG	62	153	AGAAAACCAAATTTTAGGAA	N/a
UGT1A1 (TA) _n	GTCACGTTGACACAGTCAAAC ^a	TTTGTCTGCCAGAGGTT	50	100	AGGTTGGCCCTCTCCTA	N/a
UGT1A1 1456 T>G	TCTCCAGCCCTTCAAAGGAC	ATTTCCCAACCCACTTCTCAA	60	291	N/a	BSrI
XRCCJ 26304 C>T	CTTCTCCCTGCTCTCCAC	CTACCCCTCTCCCTCAGAAAC	66	485	N/a	PvuII
XRCCJ 27466 G>A	CCTGGATTGCTGGGTCTG	AGCCACTCAGCACCACTACC	60	640	N/a	PvuI
XRCCJ 28152 G>A	GCCCCCTCAGATCACACCTAA	TCCCCGCTCCTCTCAGTAGTTC	60	593	N/a	NciI

^a Biotinylated.^b N/a, not applicable.

individual patient was also calculated on the basis of the linear trapezoidal-rule AUC values from time 0 to 24 h after start of infusion, without extrapolation to infinity, with uniform weighting using noncompartmental analysis in WinNonLin version 3.0 (Pharsight Corp., Mountain View, CA).

Pharmacogenetic Data Analysis. Genomic DNA was extracted from 1 ml of whole blood or plasma using the Gentra PureGene Blood kit (Gentra, Minneapolis, MN) and the QIAamp DNA Blood midi kit (Qiagen, Inc., Valencia, CA), respectively, following the manufacturers instructions, and was reconstituted in a buffer containing 10 mM Tris (pH 7.6) and 1 mM EDTA. SNPs and other genetic variations were identified from the literature or were mined using the publicly available SNP databases (17).

Variations in the *ABCB1* (nucleotide 1236C>T, 2677G>T/A, and 3435C>T), *ABCC1* (14008G>A, 462C>T, and 34215C>G), *ABCC2* (156231A>G), *ABCG2* (623T>C), *CES1* (1440A>T, 1525A>C), *CES2* (1647C>T), *CYP3A4* (*CYP3A4*1B*, *CYP3A4*2*, and *CYP3A4*3*), *CYP3A5* (*CYP3A5*3C* and *CYP3A5*6*), *UGT1A1* (*UGT1A1*7* and *UGT1A1*28*), and *XRCC1* (26304C>T, 27466G>A, and 28152G>A) genes were analyzed by PCR-RFLP or by Pyrosequencing (Table 1). Variations in the *XRCC1* gene were included as negative controls. PCR for *ABCB1* 3435C>T and *UGT1A1*28* was carried out as described previously (18, 19). All other PCR primers were designed using Primer Express version 1.5 (ABI, Foster City, CA), and the Pyrosequencing primers were designed using the Pyrosequencing SNP Primer Design Version 1.01 software.⁵ RFLP sites were determined using Rebase.⁶ The PCR primers, conditions, and restriction enzymes (NEB, Beverly, MA) used in the current study are listed in Table 1. PCR was carried out using AmpliTaq Gold PCR master mix (ABI), 5 pmol of each primer, and 10–50 ng of DNA isolated from whole blood or DNA from 1 μ l of undiluted plasma. Pyrosequencing was carried out as previously described (20) using the Pyrosequencing AB PSQ96 instrument and software (Uppsala, Sweden). RFLP results were analyzed by electrophoresis using 4% agarose (Promega Corporation, Madison, WI). The genotype was called variant if it differed from the Refseq consensus sequence for the SNP position.⁷ Genotype frequency analysis of Hardy-Weinberg equilibrium was carried out using Clump version 1.9 (21). Linkage disequilibrium between different pairs of SNPs was determined in terms of the classical statistic D' . The absolute value for D' ($|D'|$) of 1 denotes complete linkage disequilibrium, whereas a value of 0 denotes complete linkage equilibrium (22).

Statistical Considerations. All pharmacokinetic data are presented as mean values \pm SD, unless stated otherwise. To relate pharmacokinetic parameters with each polymorphism, a nonparametric Kruskal-Wallis test was used after a logarithmic transformation for data with a skewed distribution. These statistical calculations were performed using SPSS version 9.0 (Paris, France) with an *a priori* cutoff of $P < 0.05$. To relate

Table 2 Patient demographics

	No. of patients	Median (range)
Total no. entered (males/females)	65 (32:33)	
Age (yr)		53 (34–75)
Body surface area (m ²)		1.86 (1.29–2.36)
Performance score		1 (0–1)
Ethnicity		
European Caucasian	63	
Asian	2	
Tumor types		
Gastrointestinal	49	
(Adeno-) carcinoma of unknown primary	7	
Miscellaneous	9 ^a	
Clinical chemistry		
AST/ALT < 3 \times ULN ^b	60	
AST/ALT 3 to 5 \times ULN	5	
Bilirubin < ULN	62	
Bilirubin 1 to 1.5 \times ULN	3	
Creat < ULN/creat CL > LLN	59	
Creat < 135 μ mol/l/creat CL > 60 ml/min	6	
Irinotecan dose		
200–230 mg/m ²	6	
250–300 mg/m ²	8	
350 mg/m ²	51	
Concomitant medication		
Cisplatin	3	
None	62	

^a Pancreas (five), lung (two), cervix (two).

^b ULN, upper limit of normal; Creat, creatinine; LLN, lower limit of normal.

REG with the *UGT1A1*28* polymorphism, a nonparametric-trend analysis was conducted using Stata version 7.0 (Stata Corp., College Station, TX), as described previously (16).

RESULTS

Patients and Pharmacokinetics. A total of 65 adult cancer patients (32 males and 33 females) with a median age of 53 years was enrolled onto this study (Table 2). The majority of patients was European Caucasian, the most prominent disease type was a gastrointestinal malignancy, and 62 of 65 individuals received single agent irinotecan (51 at a dose level of 350 mg/m²). The observed plasma concentration-time profiles of irinotecan and its metabolites SN-38, SN-38G, and APC were well predicted by previously defined NONMEM models (15), as indicated by goodness-of-fit plots (data not shown). The individual and mean pharmacokinetic parameters of irinotecan and its metabolites are consistent with previous findings from patients on a similar regimen (2), showing extensive glucuronidation of SN-38 with wide interindividual variability (Table 3). All AUC ratios found in this group of patients were highly variable, with up to 6-, 22-, and 15-fold difference between the lowest and highest values for relative extent of conversion, REG, and relative extent of metabolism, respectively (Table 3).

Genotyping. Seventeen SNPs and 1 dinucleotide repeat were analyzed in nine genes of putative relevance for irinotecan disposition (Fig. 1), and 3 SNPs in one gene likely to act downstream of topoisomerase I inhibition (*i.e.*, *XRCC1*; Ref. 23). *XRCC1* was included as a control gene of unlikely signif-

⁵ Internet address: <http://www.pyrosequencing.com>.

⁶ Internet address: <http://rebase.neb.com>.

⁷ Internet address: <http://www.ncbi.nlm.nih.gov/LocusLink/refseq.html>.

Table 3 Summary of pharmacokinetic parameters ($n = 65$)

Parameter	Mean \pm SD	Range
Irinotecan		
CL lactone (liter/h)	73.0 \pm 19.9	31.1–109
AUC lactone ($\mu\text{g}\cdot\text{h}/\text{ml}$)	5.73 \pm 0.856	4.05–8.04
AUC total ($\mu\text{g}\cdot\text{h}/\text{ml}$)	20.5 \pm 7.07	10.4–46.1
SN-38		
CL(m) ^a lactone (liter/h)	1,260 \pm 690	300–3,330
AUC total ($\mu\text{g}\cdot\text{h}/\text{ml}$)	0.637 \pm 0.336	0.216–1.75
SN-38G		
AUC total ($\mu\text{g}\cdot\text{h}/\text{ml}$) ^b	4.46 \pm 4.67	0.962–30.0
APC		
AUC total ($\mu\text{g}\cdot\text{h}/\text{ml}$) ^c	4.57 \pm 4.02	1.12–14.0
AUC ratios		
SN-38/CPT-11	0.032 \pm 0.015	0.014–0.088
SN-38G/SN-38 ^b	7.60 \pm 5.88	1.70–37.5
APC/CPT-11 ^c	0.21 \pm 0.15	0.035–0.53

^a CL(m), metabolic clearance.

^b $n = 53$.

^c $n = 12$; fewer patients were studied because of a lack of sufficient material for repeat analyses.

icance to irinotecan disposition. In 7 of the tested SNPs (*i.e.*, *ABCC1* 462C>T, *ABCC2* 156231A>G, *ABCG2* 623T>C, *CES1* 1440A>T, *CYP3A4**2, *CYP3A5**6, and *UGT1A1**7), no variants were observed. In the other polymorphisms studied, frequencies of the rarest alleles ranged from 0.01 to 0.47 (Table 4). The *ABCB1* 2677G>T/A contains a tri-allelic SNP, with G at nucleotide 2677 found in the wild-type sequence, and with A or T at that position being the two possible variants. All genotype frequencies were found to be in Hardy-Weinberg equilibrium. Complete genetic linkage was not seen among polymorphisms from within the same gene. SNPs in the *ABCB1* gene (1236C>T, 2677G>T/A, and 3435C>T) have been previously described to be in linkage with each other (24). In this study, *ABCB1* 3435C>T and *ABCB1* 1236C>T had a D' of 0.4, *ABCB1* 3435C>T and *ABCB1* 2677G>T/A had a D' of -0.5 , and *ABCB1* 1236C>T and *ABCB1* 2677G>T/A had a D' of -0.6 . Overall, 35% linkage was observed among the 3 *ABCB1* SNPs. The 26% allele frequency for *UGT1A1**28, a seven copy dinucleotide repeat polymorphism in the promotor region, was somewhat lower than the 38–40% reported in some previous studies (25).

Genotype-Phenotype Associations. For the nine polymorphisms in five genes in which variant alleles were observed, only one of the *ABCB1* SNPs was associated with differences in irinotecan and SN-38 levels (Table 5). The systemic exposure to both irinotecan and SN-38, based on total drug concentrations, was significantly higher for patients with two variant alleles in *ABCB1* 1236 C>T (*i.e.*, the TT allele) when compared with the patients with only one or no variant alleles. The mean AUC values for irinotecan and SN-38 were 25.5 ± 8.21 versus 19.8 ± 6.81 $\mu\text{g}\cdot\text{h}/\text{ml}$ ($P = 0.038$) and 889 ± 445 versus 604 ± 293 $\text{ng}\cdot\text{h}/\text{ml}$ ($P = 0.031$), respectively. Likewise, the metabolic CL of the lactone form of SN-38 was significantly different among homozygous variants of this SNP and wild-type or heterozygous carriers ($P = 0.015$; Table 5; Fig. 2). No statistically significant association was observed among the other variants in ABC transporters and any of the studied pharmacokinetic parameters (Table 5).

Comparison of pharmacokinetic data in patients wild-type versus heterozygous for *CYP3A4**3 suggested that the CL of irinotecan lactone might be reduced and that the AUC might be increased in the heterozygotes, although these differences were not statistically significant, presumably as a result of the small sample size ($P = 0.059$ and $P = 0.070$, respectively). Statistically significant differences were also not observed in pharmacokinetic parameters, including REG (Table 6), among variants in *UGT1A1**28 (wild type > heterozygous variant > homozygous variant, $P > 0.22$).

DISCUSSION

The desire for better tools to individualize chemotherapy has led to new ways of evaluating patients. The observation that most chemotherapy agents have a high degree of variability in drug disposition has prompted the use of genetics to try and identify the mechanistic basis for this variation. Previous investigations have shown that pharmacogenetic testing may contribute to the individualization of drug treatment and hence may have an increasing impact on enhanced drug safety and efficacy (5). In this study, exploratory relationships were assessed between disposition characteristics of irinotecan and 21 allelic variants of 10 genes coding for various ABC transporters and drug-metabolizing enzymes in a group of 65 cancer patients.

The most relevant finding of this study was an apparent association between the presence of the homozygous T allele of *ABCB1* 1236C>T (located at exon 12) and increased exposure to both irinotecan and SN-38. This appears to be the first *in vivo* observation that this allelic variant may be functionally polymorphic in that it alters the activity of the encoded protein in relation to the wild-type and heterozygous sequences. *ABCB1* 1236C>T is a synonymous cSNP located in codon 411 of the P-glycoprotein. It is unlikely that this variation directly affects the expression of P-glycoprotein. However, it may have an indirect effect such as altering RNA stability. A comprehensive analysis of all DNA variations in this gene is warranted to uncover the basis of the association between P-glycoprotein and irinotecan and SN-38 exposure.

Previously, Hoffmeyer *et al.* (26) found an association between *ABCB1* 3435C>T at exon 26 and increased exposure to the P-glycoprotein substrate drug digoxin after oral administration. Although this SNP is also a silent polymorphism, intestinal P-glycoprotein expression was significantly decreased, leading to increased absorption of the drug. More recently, a study was published that confirmed the functional importance of the *ABCB1* 3435C>T polymorphism, although in this case, the variant allele was associated with lower plasma concentrations of the oral antiretroviral drugs nelfinavir and efavirenz (27). To explain this paradox, it was hypothesized that low levels of P-glycoprotein expression might be compensated for by overexpression of other ABC transporter proteins with affinity for these antiretroviral drugs and/or the induction of *CYP3A* isoforms (27). In our study, neither the *ABCB1* 3435C>T nor the *ABCB1* 2677G>T/A SNP was associated with altered plasma concentrations of irinotecan and/or its metabolites. The latter mutation leads to the replacement of Ala to Ser or Thr but does not appear to result directly in an altered expression of P-glycoprotein (28).

Table 4 Genotype and Allele frequencies for the studied genes

Polymorphism ^a	Nomenclature	Description ^b	Genotype frequencies ^c			Allele frequencies (95% CI) ^d		
			Wt	Het	Var	p	q	r
<i>ABCB1</i> 1236 C>T	n/a ^e	G411G	23	15	8	0.66 (0.52–0.78)	0.34 (0.22–0.48)	
<i>ABCB1</i> 3435 C>T	n/a	E1143E	16	35	8	0.57 (0.44–0.69)	0.43 (0.31–0.56)	
<i>ABCB1</i> 2677 G>T/A	n/a	A893S or T	12	23/4	13/1	0.48 (0.35–0.61)	0.47 (0.34–0.60)	0.05 (0.02–0.14)
<i>ABCC1</i> 14008 G>A	n/a	intron 27	32	27	5	0.71 (0.59–0.81)	0.29 (0.19–0.41)	
<i>ABCC1</i> 462 C>T	n/a	P154P	65	0	0	1.00	0.00	
<i>ABCC1</i> 34215 C>G	n/a	intron 18	0	20	40	0.17 (0.1–0.28)	0.83 (0.72–0.90)	
<i>ABCC2</i> 156231 A>G	n/a	intron 3	65	0	0	1.00	0.00	
<i>ABCG2</i> 623T>C	n/a	F208S	63	0	0	1.00	0.00	
<i>CES1</i> 1440 A>T	n/a	L480F	64	0	0	1.00	0.00	
<i>CES1</i> 1525 A>C	n/a	N509H	60	1	0	0.99 (0.92–1)	0.01 (0–0.08)	
<i>CES2</i> 1647 C>T	n/a	L549L	56	1	0	0.99 (0.92–1)	0.01 (0–0.08)	
<i>CYP3A4</i> -392 A>G	<i>CYP3A4</i> *1B	Promoter	46	3	0	0.97 (0.88–0.99)	0.03 (0.01–0.12)	
<i>CYP3A4</i> 15713 T>C	<i>CYP3A4</i> *2	S222P	39	0	0	1.00	0.00	
<i>CYP3A4</i> 23172 T>C	<i>CYP3A4</i> *3	M445T	62	2	0	0.98 (0.91–1)	0.02 (0–0.09)	
<i>CYP3A5</i> 22893 G>A	<i>CYP3A5</i> *3C	Splice variant	56	8	0	0.94 (0.85–0.98)	0.06 (0.02–0.15)	
<i>CYP3A5</i> 30597 G>A	<i>CYP3A5</i> *6	Splice variant	63	0	0	1.00	0.00	
<i>UGT1A1</i> (TA) _n ^f	<i>UGT1A1</i> *28	Promoter	34	22	2	0.78 (0.66–0.87)	0.22 (0.13–0.34)	
<i>UGT1A1</i> 1456 T>G	<i>UGT1A1</i> *7	Y486D	62	0	0	1.00	0.00	
<i>XRCC1</i> 26304 C>T	n/a	R194W	35	8	0	0.91 (0.79–0.96)	0.09 (0.04–0.21)	
<i>XRCC1</i> 27466 G>A	n/a	R280H	60	2	0	0.98 (0.91–1)	0.02 (0–0.09)	
<i>XRCC1</i> 28152 G>A	n/a	R399Q	25	27	5	0.68 (0.55–0.79)	0.32 (0.21–0.45)	

^a Number represents position in nucleotide sequence.

^b Number represents amino acid codon.

^c Number represents number of patients.

^d Hardy-Weinberg notation was used for allele frequencies (p, q, and r).

^e n/a, not available; CI, confidence intervals; Wt, Wild type patient; Het, Heterozygous variant type patient; Var, Homozygous variant type patient.

^f p = 6 dinucleotide repeats, q = 7 dinucleotide repeats.

Table 5 Summary of genotype-phenotype associations

Polymorphism	Phenotypic consequence ^a	P ^b
<i>ABCB1</i> 1236C>T	Irinotecan total AUC increased	0.038 (46)
	SN-38 total AUC increased	0.031 (46)
	SN-38 lactone CL reduced	0.015 (46)
<i>ABCB1</i> 3435C>T	Irinotecan lactone AUC	0.100 (59)
	Irinotecan lactone CL	0.083 (53)
<i>ABCC1</i> 14008G>A	SN-38 lactone CL	0.127 (64)
<i>ABCC1</i> 34215C>G	APC AUC	0.345 (60)
<i>CYP3A4</i> *1B	SN-38 lactone CL	0.213 (65)
<i>CYP3A4</i> *3	Irinotecan lactone CL	0.059 (64)
<i>CYP3A5</i> *3C	Irinotecan carboxylate CL	0.174 (64)
<i>UGT1A1</i> *28	SN-38G/SN-38 AUC ratio	0.221 (53)

^a Represents the best association (lowest P) between a given polymorphism (wild-type versus heterozygous variant versus homozygous variant type patients) and a change in a pharmacokinetic parameter, with number of patients in parenthesis.

^b Kruskal-Wallis test following logarithmic transformation for data with a skewed distribution; except for *ABCB1* 1236 C>T, no statistically significant associations were found.

Recent investigations indicated that the metabolism of irinotecan is substantially influenced by a nucleotide polymorphism in the TATA-box sequences of *UGT1A1*. This gene encodes the enzyme UGT1A1 that is responsible for the glucuronidation of several compounds, including SN-38 (7). An extra (7th) TA-repeat [A(TA)₇TAA] in one allele results in ~70% reduction in transcriptional activity compared with wild-type *UGT1A1* [A(TA)₆TAA]. Inheritance of the promoter containing

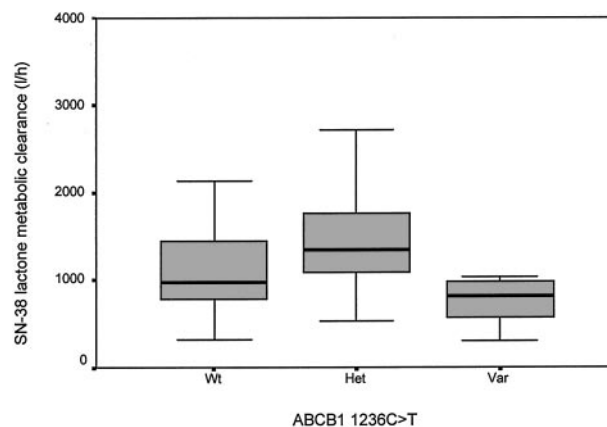


Fig. 2 *ABCB1* 1236C>T variants in relation to the metabolic CL of SN-38 lactone. Wt, wild-type patient; Het, heterozygous variant type patient; Var, homozygous variant type patient.

[A(TA)₇TAA] is one of the most common genotypes leading to Gilbert's syndrome (29), which is characterized by mild nonhemolytic, unconjugated bilirubinemia. Genetic abnormalities in *UGT1A1* are also associated with the Crigler-Najjar hepatic syndromes with absent (type I) or reduced (type II) UGT1A1 activity (29). As such patients cannot adequately metabolize SN-38, they might be at increased risk for severe drug-related toxicities. Indeed, it has been suggested that screening for

Table 6 Effect of *UGT1A1**28 on the extent of SN-38 glucuronidation

Genotype	Reference	n	AUC _{0-t} (0–24 h) ratio ^a SN-38G/SN-38 Mean ± SD (median)	AUC _{0-inf} (0–100 h) ratio ^b SN-38G/SN-38 Mean ± SD (median)
Wt ^c	This study	32	7.6 ± 4.1 (6.6)	6.9 ± 3.5 (6.4)
	Iyer <i>et al.</i> (16)	9	9.3 ± 11	n/a
Het	This study	19	7.1 ± 3.6 (6.6)	6.7 ± 3.2 (6.1)
	Iyer <i>et al.</i> (16)	7	4.0 ± 1.7	n/a
Var	This study	2	2.2, 5.2 (3.7)	2.5, 4.6 (3.6)
	Iyer <i>et al.</i> (16)	4	2.4 ± 1.1	n/a

^a SN-38G/SN-38 AUC ratio based on nonparametric analysis of samples taken up to 24 h after irinotecan administration [trend analysis (Wt>Het>Var), *P* = 0.272].

^b SN-38G/SN-38 AUC ratio based on NONMEM model [trend analysis (Wt>Het>Var), *P* = 0.300]; Iyer *et al.* (16) [trend analysis (Wt>Het>Var), *P* = 0.001].

^c Wt, Wild type patient; Het, Heterozygous variant type patient; Var, Homozygous variant type patient; n/a, not available.

*UGT1A1**28 before treatment might identify patients with lower SN-38 glucuronidation rates and greater susceptibility to irinotecan-induced hematological and nonhematological toxicities (30). Unfortunately, these results could not be confirmed in our study, although the study settings were comparable. One reason for this discrepancy may be found in the allele frequency of the variant allele in our population that was clearly lower than mentioned in literature for a normal Caucasian population (25). However, although differences in our study among genotypes were not statistically significant, an overall trend in reduced SN-38 glucuronidation rate (*i.e.*, REG) in homozygous variants of *UGT1A1**28 could be observed (Table 6), particularly in the median values, both with pharmacokinetic data based on non-compartmental analysis or those based on the NONMEM population model. Furthermore, it is particularly noteworthy that one of the two patients with the variant *UGT1A1* allele in our study with data for REG was the only individual that experienced grade 4 diarrhea in the entire cohort. Clearly, additional investigation is required to unambiguously define the association between genetic variation in *UGT1A1* and irinotecan pharmacokinetics and pharmacodynamics.

Novel SNPs in *ABCC1*, *ABCG2*, *CES1*, and *CES2* were identified and evaluated in this patient set. Because of the rapid expansion of SNP discovery and the present lack of overlap among the various SNP databases, it is possible that additional functional polymorphisms in these genes are still to be described (17). For example, recent data suggest that molecular determinants of SN-38 glucuronidation and irinotecan response might include common allelic variants of the hepatic UGT1A9 isozyme (31). This may eventually provide additional refinement of the predictive strategies for irinotecan.

One might seriously question if our current findings already provide meaningful tools for medical decision making in clinical practice. It is probably too simplistic to think that the complex metabolism of irinotecan can be predicted by screening for one or even a few genetic variants. Because every individual represents a combination of transporter and drug metabolizer phenotypes and given the many enzymes involved in irinotecan metabolism, it is apparent that some individuals are destined to have unusual reactions to this agent because of the coincidental occurrence of multiple genetic defects. Furthermore, it has been shown before that various physiological and environmental factors are also involved in the way patients react to the irinotecan

therapy (2). Besides the condition of the patient and measures of hepatic dysfunction, the role of comedication and dietary supplements should not be underestimated. For example, sedatives, antiepileptic drugs, and some corticosteroids, which are very commonly used among patients on chemotherapy treatment, can induce UGT1A1 and CYP3A4 activity (32, 33). In addition, coadministration of a wide variety of agents can result in competitive inhibition of CYP3A4 activity (34), whereas recommended levels of the natural product St. John's wort significantly induce the activity of this enzyme, resulting in altered SN-38 concentrations in plasma (35). Therefore, the next step in predicting the pharmacokinetic and pharmacodynamic outcome of therapy would be by focusing on phenotyping strategies because these combine physiological, environmental, and genetic factors. Hopefully, these procedures will eventually lead toward individualized dosing of this drug. Trials implementing a strategy to phenotype total CYP3A expression by using the erythromycin breath test and/or midazolam CL as surrogate markers of enzyme activity before treatment with irinotecan are currently ongoing.

In conclusion, individuals homozygous for the T allele of *ABCB1* 1236 C>T appear to have altered irinotecan plasma concentrations in comparison with heterozygous and wild-type patients. Although this polymorphism does not completely explain the differences in irinotecan pharmacokinetics among patients, this observation may be of relevance to achieve individualized treatment strategies with this agent. Future studies will focus on the inclusion of genotype data as a covariate in a population model and will assess relationships between irinotecan disposition and the rare variant genotypes, especially in other ethnic groups.

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