Comprehensive Pharmacogenetic Analysis of Irinotecan Neutropenia and Pharmacokinetics

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The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

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ABSTRACT

Purpose

We aim to identify genetic variation, in addition to the *UGT1A1**28 polymorphism, that can explain the variability in irinotecan (CPT-11) pharmacokinetics and neutropenia in cancer patients.

Patients and Methods

Pharmacokinetic, genetic, and clinical data were obtained from 85 advanced cancer patients treated with single-agent CPT-11 every 3 weeks at doses of 300 mg/m² (n = 20) and 350 mg/m² (n = 65). Forty-two common variants were genotyped in 12 candidate genes of the CPT-11 pathway using several methodologies. Univariate and multivariate models of absolute neutrophil count (ANC) nadir and pharmacokinetic parameters were evaluated.

Results

Almost 50% of the variation in ANC nadir is explained by *UGT1A1**93, *ABCC1* *IVS11* -48C>T, *SLCO1B1**1b, ANC baseline levels, sex, and race (*P* < .0001). More than 40% of the variation in CPT-11 area under the curve (AUC) is explained by *ABCC2* -24C>T, *SLCO1B1**5, *HN1A* 79A>C, age, and CPT-11 dose (*P* < .0001). Almost 30% of the variability in SN-38 (the active metabolite of CPT-11) AUC is explained by *ABCC1* 1684T>C, *ABCB1* *IVS9* -44A>G, and *UGT1A1**93 (*P* = .004). Other models explained 17%, 23%, and 27% of the variation in APC (a metabolite of CPT-11), SN-38 glucuronide (SN-38G), and SN-38G/SN-38 AUCs, respectively. When tested in univariate models, pretreatment total bilirubin was able to modify the existing associations between genotypes and phenotypes.

Conclusion

On the basis of this exploratory analysis, common polymorphisms in genes encoding for ABC and SLC transporters may have a significant impact on the pharmacokinetics and pharmacodynamics of CPT-11. Confirmatory studies are required.

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INTRODUCTION

Irinotecan (CPT-11) is a topoisomerase I inhibitor approved worldwide for the treatment of metastatic colorectal cancer. A genetic polymorphism in *UGT1A1* increases the risk of CPT-11 toxicity, particularly when administered as a single agent.^{1,2} The pathophysiology of this susceptibility resides in the decreased glucuronidation of SN-38, the active metabolite of CPT-11.³

Several groups, including ours, have established that white patients who are homozygous for the *UGT1A1**28 allele are at the highest risk of developing severe toxicity of CPT-11, whereas heterozygous patients seem at intermediate risk (for a review, see Kim and Innocenti¹). The *UGT1A1**28 allele seems to confer reduced gene expression compared with the reference *UGT1A1**1 allele,^{4,5} leading to increased exposure of patients to

the cytotoxic metabolite SN-38.⁶ The accumulated evidence prompted the US Food and Drug Administration and the pharmaceutical sponsor to revise the CPT-11 label in June 2005. The label now includes homozygosity for the *UGT1A1**28 genotype as one of the risk factors for severe neutropenia.^{7,8} A US Food and Drug Administration–approved *UGT1A1**28 genotyping method is also commercially available.⁹

Over the last two decades, there have been many studies of the pharmacology of CPT-11 identifying a series of genes to be investigated for their possible contribution to the variability in CPT-11 pharmacokinetics and adverse effects, as shown in the pathway of CPT-11 disposition in the liver (Fig 1). In addition to the conversion to SN-38 by carboxylesterases, CPT-11 undergoes oxidation to the metabolites APC and NPC by the CYP3A4/5 enzymes. Moreover, *UGT1A7* and *UGT1A9* are also

Fig 1. Pathway of irinotecan (CPT-11) metabolism and transport in the hepatocyte. This pathway was obtained from the PharmGKB Web site (<http://www.pharmgkb.org/do/serve?objId=PA2001&objCls=Pathway>). Used with permission, PharmGKB copyright 2004.

involved in the inactivation of SN-38. Whereas UGT1A9 is highly expressed in human liver, UGT1A7 is expressed in extrapatic tissues and is potentially relevant to the enterohepatic circulation of SN-38.^{10,11} Membrane transporters are responsible for the uptake of SN-38 from plasma into the hepatocytes (ie, SLCO1B1) and the elimination of CPT-11 and its metabolites into the bile (ie, ABCB1, ABCC2, and ABCG2). The ABCC1 transporter is responsible for the efflux of SN-38 from the hepatocyte into the interstitial space (Fig 1).

Despite the fact that the *UGT1A1**28 polymorphism represents one of the few validated and widely accepted pharmacogenetic markers to predict drug toxicity in oncology, the performance of this test is far from optimal.^{7,8} Drug toxicity and disposition in patients are complex phenotypes. Their variability is a result of environmental and polygenic factors. It is likely that, in addition to *UGT1A1**28, other genetic variants contribute to the toxicity phenotype. For example, it has been suggested that variation in genes encoding for SLCO1B1, ABC transporters, and other UGT1A enzymes is associated with the pharmacokinetics and pharmacodynamics of CPT-11.¹²⁻¹⁷ In the present study, we provide the first assessment of the association between genetic variants in the vast majority of the known genes in the CPT-11 pathway and the pharmacokinetic parameters of CPT-11, SN-38, SN-38 glucuronide (SN-38G), and APC in patients previously treated with single-agent CPT-11.^{6,18} We also investigated the polygenic basis of neutropenia, a common adverse effect of CPT-11. To our knowledge, a comprehensive investigation like this one has never been conducted, and this study proposes new gene variants as candidates with predictive significance.

PATIENTS AND METHODS

Patients

Human investigations were performed after review and approval by the Biological Sciences Division/University of Chicago Hospitals Institutional Review Board and in accordance with Federalwide Assurance for the protection of human subjects. Patient characteristics are listed in Appendix Table A1 (online only).

Genotyping

All genotyped variants are listed in Tables 2 and 3. The genotyping methods are described in the Appendix (online only).

Statistics

We analyzed associations of gene variants with phenotypes using linear regression. The phenotypes of interest included neutropenia (expressed as absolute neutrophil count [ANC] nadir) and the area under the concentration-time curves (AUC) of CPT-11, SN-38, SN-38G, APC, and the SN-38G/SN-38 AUC ratio. These data were log₁₀ transformed. Although we report the frequency data of genotypes in all races, we performed the regression analyses in patients of white and African American race only because there were too few patients in the other racial categories (8% of the total population of patients).

For the univariate analysis, we first carried out simple regressions on each variant separately. We kept for further analysis those variants with a significance level less than .15. Forward selection of this subset of phenotypes led to the final multivariable regression models, in which we required a $P < .05$ (two sided) to call an association nominally significant. We adjusted all analyses (univariate and multivariate) for sex, race (white or African American), age (as a continuous variable), and CPT-11 dose (either 300 or 350 mg/m²) to avoid potential problems with population stratification. Body-surface area (BSA) was not included to calculate actual dose (based on dose · BSA) because it generally did not perform better than using dose as either 300 or 350 mg/m² in

each model (data not shown). In addition, the logANC nadir values were adjusted for baseline logANC.

In the univariate analyses, we considered that pretreatment bilirubin levels might reflect underlying genetic variation that is relevant to glucuronidation. If variation in pretreatment bilirubin corresponds to genetic variability, pretreatment bilirubin may serve as a surrogate biomarker for the genotypes of one or several single nucleotide polymorphisms (SNPs). We examined this possibility by fitting the univariate models with and without pretreatment bilirubin and comparing the estimated SNP effects in both cases. For example, suppose that SNP1 is already in the model and its association with the phenotype arises because it is in linkage disequilibrium (LD) with another SNP (SNP2), which is not in the model. If both SNPs are in a gene that alters total bilirubin, then total bilirubin may explain a greater percentage of the phenotype variation in our study population. If it does explain a greater percentage of the phenotype's variation, then the regression estimate of SNP1 will get smaller (ie, go to zero) if bilirubin is in the model. We describe situations where this phenomenon occurred. Additional statistical evaluations are described in the Appendix.

RESULTS

The genotype frequencies in each race are listed in Tables 1 and 2.

Univariate Analysis

Table 3 summarizes the univariate regression analysis for each parameter. *UGT1A1**28 and *93 were associated with decreased ANC nadir and SN-38G/SN-38 AUC, as well as increased SN-38 AUC. *UGT1A1**60 was also associated with decreased SN-38G/SN-38 AUC, as well as decreased ANC nadirs. *UGT1A7* 387G>T was associated with increased APC AUC (similar to *UGT1A9**1b), and *UGT1A7**4 was associated with decreased SN-38G/SN-38 AUC.

Concerning the CPT-11 transporters, *ABCC1* 1684T>C and *IVS18-30C*>G were associated with variation in APC AUC and SN-38G/SN-38 AUC, respectively, and *ABCC1* 1684T>C was associated with increased SN-38 AUC. *ABCC1* IVS11 -48C>T was associated with decreased ANC nadir. *SLCO1B1**5 was associated with increased CPT-11 AUC, and *SLCO1B1**1b was associated with increased ANC nadir. Several *ABCC2* variants were associated with variability in CPT-11, SN-38, SN-38G, and APC AUC, without any notable effect on ANC nadir. Two *ABCB1* variants were associated with variability in SN-38 AUC (Table 3).

Effect of Pretreatment Total Bilirubin in the Univariate Analysis

When pretreatment bilirubin was included in the ANC nadir model, the estimates of the *UGT1A1* *28/*28 and *93/*93 genotypes retained significance but were reduced by approximately 30% compared with the unadjusted values. For CPT-11 AUC, bilirubin was a significant covariate ($P = .02$ to $.05$) for the four independent variables but had a minimal effect on the estimate ($< 8\%$).

For SN-38 AUC, pretreatment bilirubin was a significant covariate ($P < .0001$) for each independent variable, the most notable change being on *UGT1A1* *93/*93 and *28/*28, where the regression estimates became smaller (-54% and -49% reduction, respectively) in the presence of bilirubin. The smaller effect was also evident in the nonsignificant P value for these polymorphisms with bilirubin in the model, even though the SEs did not change; for *UGT1A1* *93/*93, the change in P value went from .002 (unadjusted) to .14 (adjusted), and for *UGT1A1* *28/*28, the P value increased from .002 to .09 after adding bilirubin to the model.

Table 1. Genotype Frequencies of Drug Metabolizing Genes and the *HNF1A* Gene

Gene and Genotype	rs No.	% of Patients			HWE Exact <i>P</i> (white)
		African American (n = 11)	White (n = 67)	Other (n = 7)	
<i>CES2</i> –363C>G	11075646				1.000
C/C		27.3	82.1	100	
C/G		54.5	16.4	0	
G/G		18.2	1.5	0	
<i>CES2</i> IVS1+ 1361A>G	4783745				.474
A/A		18.2	70.1	71.4	
A/G		54.5	28.4	28.6	
G/G		27.3	1.5	—	
<i>CES2</i> 108C>G*	11568313				—
C/C		100	100	85.7	
C/G		0	0	14.3	
GG		0	0	0	
<i>CYP3A4</i> *1B –392A>G	2740574				.017
A/A		54.5	83.6	100	
A/G		45.5	11.9	0	
G/G		0	4.5	0	
<i>CYP3A5</i> *3 IVS3+ 6986A>G	776746				.010
A/A		9	4.5	0	
G/A		54.6	10.4	42.9	
G/G		36.4	85.1	57.1	
<i>HNF1A</i> 79A>C (I27L)	1169288				.075
A/A		27.3	55.2	14.3	
A/C		72.7	31.3	71.4	
C/C		0	13.5	14.3	
<i>UGT1A1</i> *6 211G>A (G71R)	4148323				—
G/G		100	100	100	
G/A		0	0	0	
A/A		0	0	0	
<i>UGT1A1</i> *27 686C>A (P229Q)	35350960				1.000
C/C		100	98.5	100	
C/A		0	1.5	0	
A/A		0	0	0	
<i>UGT1A1</i> *28 –53(TA) ₆ > ₇ TAA	8175347				.284
*1/*1		45.5	46.3	57.1	
*1/*28		45.5	38.8	42.9	
*28/*28		9.0	14.9	0	
<i>UGT1A1</i> *60 –3279T>G	4124874				.625
T/T		18.2	31.3	28.6	
G/T		54.5	46.3	71.4	
G/G		27.3	22.4	0	
<i>UGT1A1</i> *93 –3156G>A	1092302				.265
G/G		72.7	49.3	57.1	
G/A		27.3	37.3	42.9	
A/A		0	13.4	0	
<i>UGT1A9</i> *1b –118(dT) ₉ > ₁₀	35426722				1.000
9/9		18.2	37.3	14.2	
9/10		63.6	47.8	42.9	
10/10		18.2	14.9	42.9	
<i>UGT1A9</i> –275T>A	6714486				1.000
T/T		81.8	85.1	100	
T/A		18.2	14.9	0	
AA		0	0	0	
<i>UGT1A9</i> –2152C>T	17868320				1.000
C/C		100	86.6	100	
C/T		0	13.4	0	
TT		0	0	0	

(continued on following page)

Table 1. Genotype Frequencies of Drug Metabolizing Genes and the *HNF1A* Gene (continued)

Gene and Genotype	rs No.	% of Patients			HWE Exact <i>P</i> (white)
		African American (n = 11)	White (n = 67)	Other (n = 7)	
<i>UGT1A7</i> 387T>G (N129K)	17868323				.803
T/T		27.3	13.4	42.9	
T/G		72.7	49.3	42.9	
G/G		0	37.3	14.2	
<i>UGT1A7</i> 391C>A (R131K)	17863778				.803
C/C		27.3	13.4	42.9	
C/A		72.7	49.3	42.9	
A/A		0	37.3	14.2	
<i>UGT1A7</i> 392G>A (R131K)	17868324				.804
G/G		27.3	13.4	42.9	
G/A		72.7	49.3	42.9	
A/A		0	37.3	14.2	
<i>UGT1A7</i> *4 622T>C (W208R)	11692021				.204
T/T		72.7	31.3	71.4	
T/C		27.3	56.7	14.3	
C/C		0	11.9	14.3	

NOTE. Alleles for which a * nomenclature has not yet been assigned have been reported according to their genomic position relative to the ATG start site. The reference sequences used for genotyping are the following: *CES2*, AC009084.6; *HNF1A*, NM_000545.3; and *UGT1A1*, *UGT1A7*, and *UGT1A9*, AF297093.1. The amino acid change has been indicated within parentheses.

Abbreviations: rs, dbSNP reference number; HWE, Hardy-Weinberg equilibrium; UTR, untranslated region.

*3'UTR.

For SN-38G AUC, bilirubin was a marginally significant covariate ($P = .04$), with no notable effect on the estimate (2.9%). For APC AUC, including bilirubin did not significantly change the estimated effect of each polymorphism ($\leq 2.5\%$). It was never a significant covariate ($P \geq .5$).

For SN-38G/SN-38 AUC ratio, bilirubin was a significant covariate in the models with *ABCC1* (1684T>C and *IVS18-30G*>C) and *UGT1A7**4. Including bilirubin in the model led to the largest change for the effect of *UGT1A1**60 (−17%). It had the smallest change on the effect of *ABCC1* 1684 T>C (3%).

Multivariate Analysis

The model for ANC nadir explains approximately 50% of the variation in neutropenia. ANC nadirs are negatively affected by the *UGT1A1**93 genotype according to an additive model in which the trend is for lower nadirs as one goes from *1/*1 to *93/*93 genotypes. The C allele of *ABCC1* *IVS11* −48C>T appears dominant, with the TT genotype having lower nadirs. A smaller effect (compared with *UGT1A1* and *ABCC1*) is observed for the *SLCO1B1**1b variant (Fig 2). Significant adjusting covariates in this model are sex (females associated with significantly reduced ANC nadir, $P = .009$; 4.9% of the total percent variation), dose ($P = .015$, 2.5%), and baseline logANC ($P = .005$, 7%).

The CPT-11 AUC model explains approximately 40% of the variation in CPT-11 exposure. *ABCC2* −24C>T and *SLCO1B1**5 increase the exposure of patients to CPT-11 according to a dominant model; in addition, *HNF1A* 79A>C has an additive effect. Significant adjusting covariates in this model are age ($P = .002$) and CPT-11 dose ($P < .0001$).

The SN-38 AUC model explains close to 30% of the variation in SN-38 exposure. *ABCC1* 1684T>C has a dominant effect, increasing the exposure of patients to SN-38, whereas *ABCB1* *IVS9* −44A>G has a similar dominant effect but decreases the exposure of patients

to SN-38. *UGT1A1**93 is associated with increased SN-38 AUC, according to an additive model. No significant adjusting covariates were found.

The SN-38G AUC model explains just 17% of the variation in SN-38G exposure. *ABCC2* 3972C>T is associated with increased exposure to SN-38G. The only significant adjusting covariate in this model is sex (females associated with higher SN-38G AUC, $P = .05$).

The APC AUC model explains 23% of the total variation in APC exposure. The only significant genotype in this model is *ABCC2* 3972C>T, which increases APC exposure. The only significant adjusting covariate in this model is CPT-11 dose ($P = .012$).

The SN-38G/SN-38 AUC model explains 27% of the total variation in this parameter. *UGT1A1**28 (as an additive model) and *ABCC1* 1684T>C (as a dominant model) are associated with reduced ratios. No covariates are significant in this model.

DISCUSSION

This study provides the most comprehensive survey of genetic variation of known drug-metabolizing enzyme and transporter genes of CPT-11 and has identified potential new genetic determinants of its neutropenic effects. The availability of detailed pharmacokinetics has been pivotal to interpreting the possible pharmacologic basis of the observed associations and selecting gene variants of potential interest, as well as to excluding others from further evaluation.

Our multivariate genetic model of neutropenia can explain almost half of the observed variation. Three SNPs explain 28% of ANC nadir variation, which is a significant contribution to the overall model, considering that five other nongenetic covariates contribute to the remaining 19% of the variation (Table 4). Among all the variables in the model, *UGT1A1**93 seems to have the strongest effect. We first

Table 2. Genotype Frequencies of Transporter Genes

Gene and Genotype	rs No.	% of Patients			HWE Exact <i>P</i> (white)
		African American (n = 11)	White (n = 67)	Other (n = 7)	
<i>ABCC1</i> IVS9 +8A>G	35588				1.000
A/A		27.3	46.3	57.1	
A/G		45.4	43.3	42.9	
G/G		27.3	10.4	0	
<i>ABCC1</i> IVS11 -48C>T	3765129				.092
C/C		81.8	71.6	71.4	
C/T		18.2	22.4	28.6	
T/T		0	6.0	0	
<i>ABCC1</i> 1684T>C	35605				.738
T/T		9.1	6.0	14.3	
T/C		27.3	34.3	14.3	
C/C		63.6	59.7	71.4	
<i>ABCC1</i> IVS18 -30C>G	2074087				.464
C/C		0	6.0	14.3	
C/G		36.4	29.8	14.3	
G/G		63.6	64.2	71.4	
<i>ABCC1</i> 4002G>A	2230671				.392
G/G		63.6	47.8	71.4	
G/A		36.4	46.3	14.3	
A/A		0	6.0	14.3	
<i>ABCC1</i> IVS30 +18A>G	212088				.392
A/A		18.2	4.5	0	
A/G		18.2	25.4	14.3	
G/G		63.6	70.1	85.7	
<i>ABCC2</i> -1549A>G	1885301				.602
A/A		27.3	16.4	14.3	
A/G		27.3	43.3	28.6	
G/G		45.4	40.3	57.1	
<i>ABCC2</i> -1019A>G	2804402				.792
A/A		54.6	40.3	57.1	
A/G		45.4	44.8	28.6	
G/G		0	14.9	14.3	
<i>ABCC2</i> -24C>T	717620				.678
C/C		100	65.7	85.7	
C/T		0	32.8	14.3	
T/T		0	1.5	0	
<i>ABCC2</i> 1249G>A (V417I)	2273697				.043
G/G		54.5	62.7	85.7	
G/A		45.5	26.9	14.3	
A/A		0	10.4	0	
<i>ABCC2</i> IVS26 -34T>C	17216177				1.000
T/T		72.7	94.0	85.7	
T/C		27.3	6.0	14.3	
C/C		0	0	0	
<i>ABCC2</i> 3972C>T	3740066				.790
C/C		45.5	44.8	85.7	
C/T		45.5	43.3	0	
T/T		9.0	11.9	14.3	
<i>ABCB1</i> -129T>C	3213619				.006
T/T		100	92.5	100	
T/C		0	4.5	0	
C/C		0	3.0	0	
<i>ABCB1</i> IVS4 -25G>T	2235015				.700
G/G		72.7	65.7	100	
G/T		27.3	29.8	0	
T/T		0	4.5	0	

(continued on following page)

Table 2. Genotype Frequencies of Transporter Genes (continued)

Gene and Genotype	rs No.	% of Patients			HWE Exact <i>P</i> (white)
		African American (n = 11)	White (n = 67)	Other (n = 7)	
<i>ABCB1</i> IVS9 -44A>G	10276036				.012
A/A		45.4	37.3	0	
A/G		36.4	34.3	57.1	
G/G		18.2	28.4	42.9	
<i>ABCB1</i> 1236C>T	1128503				.012
C/C		45.5	38.8	0	
C/T		54.5	34.3	57.1	
T/T		0	26.9	42.9	
<i>ABCB1</i> IVS13 +24C>T	2235033				.027
C/C		27.3	29.9	0	
C/T		45.4	35.8	57.1	
T/T		27.3	34.3	42.9	
<i>ABCB1</i> IVS14 +38A>G	2235013				.027
A/A		18.2	29.9	0	
A/G		54.5	35.8	57.1	
G/G		27.3	34.3	42.9	
<i>ABCB1</i> 2677G>A/T (A893T/S)	2032582				.046
G/G		40.0	36.9	40.0	
G/T		60.0	36.9	60.0	
T/T		0	26.2	0	
<i>ABCB1</i> 3435C>T	1045642				.212
C/C		36.4	28.4	57.1	
C/T		54.5	41.8	42.9	
T/T		9.1	29.8	0	
<i>ABCG2</i> 34G>A (V12M)	2231137				.113
G/G		100	92.5	28.6	
G/A		0	6.0	57.1	
A/A		0	1.5	14.3	
<i>ABCG2</i> 421C>A (Q141K)	2231142				1.000
C/A		9.1	14.9	42.9	
C/C		90.9	85.1	57.1	
A/A		0	0	0	
<i>SLCO1B1</i> *1b 388A>G (N130D)	2306283				.624
A/A		18.2	31.3	0	
A/G		63.6	46.3	71.4	
G/G		18.2	22.4	28.6	
<i>SLCO1B1</i> *5 521T>C (V174A)	4149056				1.000
T/T		81.8	65.7	57.1	
T/C		18.2	31.3	42.9	
C/C		0	3.0	0	

NOTE. Alleles for which a * nomenclature has not yet been assigned have been reported according to their genomic position related to the ATG start site. The reference sequences used for genotyping are the following: *ABCG2*, NM_004827; *SLCO1B1*, NM_006446; *ABCB1*, NM_000927; *ABCC1*, NM_004987; and *ABCC2*, NM_000339. The amino acid change has been indicated within parentheses. Deviation from Hardy-Weinberg equilibrium ($P < .01$) was observed for *ABCB1* -129T>C and is most likely a result of the small sample size and the relatively low minor allele frequency.

Abbreviations: rs, dbSNP reference number; HWE, Hardy-Weinberg equilibrium.

discovered this variant during a resequencing study of the region 5' to the *UGT1A* exon 1¹⁹ and hypothesized that *UGT1A1**93 was a better predictor of neutropenia than *UGT1A1**28.⁶ This variant has unknown function at the molecular level and, in our study, is associated with increased exposure of patients to SN-38. Despite its high LD with the *UGT1A1**28 allele, our data are consistent with the recent results of a study identifying *UGT1A1**93 as the only predictor of severe hematologic toxicity in colorectal cancer patients receiving fluorouracil, leucovorin, and CPT-11.²⁰

In addition to *UGT1A1*, our data suggest that *ABCC* transporter genes play a major role in the pharmacology of CPT-11. Different variants of *ABCC1* (*IVS11* -48C>T and 1684T>C) are associated

with ANC nadir, SN-38 AUC, and SN-38G/SN-38 AUC (Table 4). Two *ABCC1* intronic variants that were not genotyped in the present study were previously shown to have no effect on the pharmacokinetics of CPT-11.¹⁶ The clinical relevance of *ABCC1* variation is relatively unexplored compared with that of *ABCB1*, and functional studies would be important to validate our preliminary findings. In an analysis of resequencing data from 24 SLC and ABC membrane transporters, *ABCC1* was found to have one of the lowest values for heterozygosity at nonsynonymous sites, indicating very little genetic variation that results in changes in the MRP1 amino acid sequence.²¹ Several rare variants of *ABCC1* do affect transport function, but their low allele frequency precludes a major role in human drug

Table 3. Univariate Analysis

Phenotype and Variant	Genotype	Estimate	SE	P
Log ANC nadir, log cells/ μ L				
<i>ABCC1</i> IVS11 -48C>T	TT, CC, CT	-0.505	0.158	.002
<i>UGT1A1</i> *28	*1*1 = 1, *1*28 = 2, *28*28 = 3	-0.190	0.051	< .001
<i>UGT1A1</i> *93	*93*93, *1*1 , *1*93	-0.461	0.115	< .001
<i>UGT1A1</i> *60	*60*60, *1*1 , *1*60	-0.227	0.092	.016
<i>SLCO1B1</i> *1b	*1a*1a, *1a*1b , *1b*1b	-0.179	0.083	.034
Log CPT-11 AUC, log h $\cdot$ ng/mL				
<i>ABCC2</i> -24C>T	CC, CT, TT	-0.089	0.034	.010
<i>ABCC2</i> -1549A>G	AA, AG, GG	0.082	0.040	.043
<i>HNF1A</i> 79A>C	CC = 1, AC = 2, AA = 3	0.048	0.022	.034
<i>SLCO1B1</i> *5	*1a*1a, *1a*5 , *5*5	-0.076	0.033	.023
Log SN-38 AUC, log h $\cdot$ ng/mL				
<i>ABCC1</i> 1684T>C	TC, CC, TT	0.269	0.123	.032
<i>UGT1A1</i> *28	*1*1 = 1, *1*28 = 2, *28*28 = 3	0.133	0.042	.002
<i>UGT1A1</i> *93	*1*1 = 1, *1*93 = 2, *93*93 = 3	0.142	0.044	.002
<i>ABCB1</i> IVS9 -44A>G	AA, AG, GG	0.141	0.064	.030
<i>ABCC2</i> 3972C>T	TT, CC, CT	0.246	0.095	.011
<i>ABCB1</i> G2677A/T	TT, TG, TA, GG, GA	-0.134	0.064	.041
Log SN-38G AUC, log h $\cdot$ ng/mL				
<i>ABCC2</i> 3972C>T	TT, TC, CC	0.399	0.111	< .001
Log APC AUC, log h $\cdot$ ng/mL				
<i>ABCC2</i> -1549A>G	AA, AG, GG	0.250	0.097	.012
<i>ABCC2</i> -1019A>G	A/A = 1, A/G = 2, G/G = 3	0.147	0.055	.009
<i>ABCC2</i> -24C>T	CC, CT, TT	-0.191	0.084	.026
<i>ABCC1</i> 1684T>C	TT, CC, TC	0.345	0.136	.013
Log SN-38G/SN-38 AUC ratio, log of the AUC ratio				
<i>ABCC1</i> IVS18 -30G>C	CC, CG, GG	0.322	0.154	.040
<i>UGT1A1</i> *28	*1*1 = 1, *1*28 = 2, *28*28 = 3	-0.192	0.045	< .0001
<i>UGT1A1</i> *60	*1*1 = 1, *1*60 = 2, *60*60 = 3	-0.139	0.046	.004
<i>UGT1A1</i> *93	*1*1 = 1, *1*93 = 2, *93*93 = 3	-0.168	0.049	.001
<i>UGT1A7</i> *4	*4/*4, *1/*1 , *1/*4	-0.292	0.112	.011

NOTE. Significant variants for each phenotype in univariate analysis were adjusted for age, sex, race, and CPT-11 dose. In addition, the logANC nadir values were adjusted for baseline logANC. Regression coefficients are shown for significant ($P \leq .05$) correlations. Results are for African Americans and whites only. APC concentrations were measured using an assay previously described for CPT-11 and metabolites.¹⁸ APC concentrations were estimated using the CPT-11 standard curve (range, 15 to 6,031 ng/mL) and applying a correction factor based on the fluorescence of both compounds. References values are indicated in bold font. Abbreviations: ANC, absolute neutrophil count; CPT-11, irinotecan; AUC, area under the concentration-time curve.

disposition.²²⁻²⁵ Similarly, there is no evidence that *ABCC1* polymorphisms are associated with mRNA levels in lymphocytes or duodenal enterocytes.^{26,27} The lack of association of the synonymous 1684T>C variant with lymphocyte *ABCC1* mRNA levels provides no support for our observed association of this polymorphism with SN-38 AUC and the SN-38G/SN-38 AUC ratio. The positive association between *ABCC1* 1684T>C and SN-38 AUC is consistent with increased trans-

port of SN-38 from the hepatocyte into the plasma. The functional basis for this association requires further investigation.

Recent data point toward *ABCC2* variation as a determinant of CPT-11 diarrhea.^{12,15} The 3972C>T variant (which we typed in this study) and a haplotype of the six variants typed in this study were associated with severe diarrhea in both Korean and European patients.^{12,15} In the present study, we could not ascertain the effect of

Fig 2. Association of *UGT1A1**93, *SLCO1B1**1b, and *ABCC1* IVS11 -48C>T with absolute neutrophil count (ANC) nadir normalized to the baseline (pretreatment) ANCs. Data are expressed as medians and 25th and 75th percentiles.

Table 4. Multivariate Analysis

Phenotype and Variant	R ²	ΔR ²	P	Genotype	Estimate	SE	P
Log ANC nadir, log cells/ μ L	0.471	0.283	< .0001	TT, CC, CT	-0.401	0.148	.009
<i>ABCC1</i> IVS11 -48C>T				*93*93, *93*1, *1*1	-0.373	0.111	.001
<i>UGT1A1</i> *93				*1a*1a, *1a*1b, *1b*1b	-0.211	0.071	.004
<i>SLCO1B1</i> *1b							
Log CPT-11 AUC, log h · ng/mL	0.416	0.187	< .0001	CC, CT, TT	-0.102	0.031	.002
<i>ABCC2</i> -24C>T				CC = 1, AC = 2, AA = 3	0.057	0.020	.007
<i>HNF1A</i> 79A>C				*1a*1a, *1a*5, *5*5	-0.075	0.030	.015
<i>SLCO1B1</i> *5							
Log SN-38 AUC, log h · ng/mL	0.292	0.213	.004	TT, TC, CC	-0.256	0.116	.031
<i>ABCC1</i> 1684T>C				AA, AG, GG	0.147	0.059	.016
<i>ABCB1</i> IVS9 -44A>G				*1*1 = 1, *1*93 = 2, *93/*93 = 3	0.119	0.043	.007
<i>UGT1A1</i> *93							
Log SN-38G AUC, log h · ng/mL	0.165	0.078	.02	TT, CC, CT	0.246	0.095	.012
<i>ABCC2</i> 3972C>T							
Log APC AUC, log h · ng/mL	0.229	0.138	.002	CC, CT, TT	-0.399	0.111	.001
<i>ABCC2</i> 3972C>T							
Log (SN-38G/SN-38 AUC) (log of the AUC ratio)	0.266	0.258	.001	TT, CC, TC	0.287	0.124	.023
<i>ABCC1</i> 1684T>C				*1*1 = 3, *1*28 = 2, *28*28 = 1	-0.180	0.044	< .0001
<i>UGT1A1</i> *28							

NOTE. Significant variants for each phenotype in the final genetic model are shown, while adjusting for age, sex, race, and CPT-11 dose. The logANC nadir values were adjusted for baseline logANC. Regression coefficients are shown for significant ($P < .05$) correlations. Results are for African Americans and whites only. References values are indicated in bold font.

Abbreviations: ANC, absolute neutrophil count; CPT-11, irinotecan; AUC, area under the concentration-time curve.

ABCC2 variation on severe diarrhea because of the low incidence of this event on this schedule. However, we observed associations between -24C>T and higher CPT-11 AUC and between 3972C>T and higher SN-38G and APC AUCs. The -24C>T variant is in high LD with 3972C>T,²⁸ has been found to reduce promoter activity in an in vitro assay, and is associated with lower *ABCC2* mRNA levels in human kidney.^{28,29} The observed increases in exposure of patients to CPT-11 and its metabolites might be a result of impaired excretion as a result of a genetically determined reduction of *ABCC2* expression.

The variability in SN-38 AUC was a strong determinant of neutropenia,^{6,18} but only one third of its variability can be accounted for by the covariates we analyzed, although genetic variants predict the majority of such variability (Table 4). In addition to *UGT1A1**93 and *ABCC1* 1684T>C, *ABCB1* IVS9 -44A>G is associated with reduced SN-38 AUC. There is no evidence of function for this intronic variant, but it is possible that it is in LD with a causative SNP. *ABCB1* 1236C>T has been previously associated with increased SN-38 AUC,¹⁶ but this was not replicated in the current study. There is also no indication that variation in the *CES* gene controlling SN-38 formation is a major determinant of SN-38 exposure. Clearly, more research is required to discover genetic and environmental determinants of exposure of patients to SN-38.

In addition to the ABC transporters, our study highlights the importance of *SLCO1B1* for the exposure of patients to parent drug and its neutropenic effects. The *SLCO1B1**5 allele increases the exposure of patients to CPT-11, similar to studies with statins, where the deficient *5 allele confers impaired drug uptake by the liver after dosing.^{30,31} Although increased exposure to statins clearly results in increased toxicity risk of myopathy,³² we did not observe similar increased neutropenia in *5 carriers because CPT-11 AUC is not associated with increased toxicity in our study. However, the *SLCO1B1**5 allele was associated with increased risk of severe neutro-

penia and increased SN-38 AUC (and no effect on CPT-11 AUC) after administration of CPT-11 in Asian patients.¹² Our results also show that the *SLCO1B1**1b allele seems to be protective of neutropenia. This result is difficult to interpret because the *1b allele showed no neutropenic effect in Asians patients.¹² A different *SLCO1B1* haplotype structure has been recently reported in Japanese compared with Europeans,³³ and caution should be used when extrapolating interpretation from studies conducted in different ethnicities. More importantly, our findings on *SLCO1B1*, similar to all the other associations, should be taken with caution. Because of the retrospective and exploratory nature of this analysis, they require independent confirmation.

In this study, we also have clarified the significance of nongenetic factors. Our data suggest that females tend to be more prone to neutropenia, a finding we observed in our previous publication when only *UGT1A1* variants were surveyed⁶ and in another recent preliminary report.³⁴ This effect does not seem to be mediated by differences in BSA because BSA did not affect the pharmacokinetics of CPT-11 in our and other studies.³⁵⁻³⁷ Finally, the effect of pretreatment total bilirubin on the association for each gene variant suggests that total bilirubin not only carries information on the genes we surveyed (ie, *UGT1A1*, *ABCC2*, *SLCO1B1*) but might also be a proxy for other hitherto unknown genes and/or for the overall excretory capability of the liver. Total bilirubin remains to be validated as a marker to predict the risk of severe neutropenia in CPT-11 patients in the absence of *UGT1A1* genetic information, although some studies are suggestive of its association with severe neutropenia.^{6,38-40}

The development of pharmacogenetic algorithms to predict the risk/benefit ratio of patients will become increasingly available to practicing physicians; warfarin algorithms are currently applied in the clinic.⁴¹ In the present study, we provide information on genetic variation that is likely to affect the neutropenic effects of CPT-11. If

confirmed in independent studies, these findings may have direct clinical application because they could help establish methods for assessing the toxicity risk and help physicians individualize therapy for colorectal cancer patients. A composite pharmacogenetic test is likely to be more predictive than the *UGT1A1**28 test alone.

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