1521-0103/345/1/95–101$25.00
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

http://dx.doi.org/10.1124/jpet.112.202242
J Pharmacol Exp Ther 345:95–101, April 2013

Refining the *UGT1A* Haplotype Associated with Irinotecan-Induced Hematological Toxicity in Metastatic Colorectal Cancer Patients Treated with 5-Fluorouracil/Irinotecan-Based Regimens[S]

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Received December 5, 2012; accepted January 31, 2013

ABSTRACT

Despite the importance of UDP-glucuronosyltransferase (*UGT*) *1A1*28* in irinotecan pharmacogenetics, our capability to predict drug-induced severe toxicity remains limited. We aimed at identifying novel genetic markers that would improve prediction of irinotecan toxicity and response in advanced colorectal cancer patients treated with folic acid (leucovorin), fluorouracil (5-FU), and irinotecan (camptosar)-based regimens. The relationships between *UGT1A* candidate markers across the gene (n = 21) and toxicity were prospectively evaluated in 167 patients. We included variants in the 3′untranscribed region (3′UTR) of the *UGT1A* locus, not studied in this context yet. These genetic markers were further investigated in 250 Italian FOLFIRI-treated patients. Several functional *UGT1A* variants, including *UGT1A1*28*, significantly influenced risk of severe hematologic toxicity. As previously reported in the Italian cohort, a 5-marker risk haplotype [haplotype II (HII); UGTs 1A9/1A7/1A1] was associated with severe neutropenia in our cohort [odds ratio (OR) = 2.43; P = 0.004]. The inclusion of a 3′UTR single-nucleotide polymorphism (SNP) permitted refinement of the previously defined HI, in which HIa was associated with the absence of severe neutropenia in combined cohorts (OR = 0.55; P = 0.038). Among all tested *UGT1A* variations and upon multivariate analyses, no *UGT1A1* SNPs remained significant, whereas three SNPs located in the central region of *UGT1A* were linked to neutropenia grade 3–4. Haplotype analyses of these markers with the 3′UTR SNP allowed the identification of a protective HI (OR = 0.50; P = 0.048) and two risk haplotypes, HII and HIII, characterized by 2 and 3 unfavorable alleles, respectively, revealing a dosage effect (ORs of 2.15 and 5.28; $P \leq 0.030$). Our results suggest that specific SNPs in *UGT1A,* other than *UGT1A1*28,* may influence irinotecan toxicity and should be considered to refine pharmacogenetic testing.

Introduction

Irinotecan (Camptosar, CPT-11), a topoisomerase I inhibitor, is a standard cytotoxic agent used for the treatment of advanced metastatic colorectal cancer. Despite its clinical efficacy, irinotecan has two major dose-limiting toxicities—myelosuppression and diarrhea—that occur with unpredictable severity (Saltz et al., 2000; Rothenberg et al., 2001). Irinotecan has a narrow therapeutic range, and adverse effects may limit the dose that can be safely administered, and subsequently compromise tumor response and clinical outcome. A greater knowledge of human genetic variations pertaining to these variable outcomes following irinotecan treatment may allow an individualized approach to therapy.

The interindividual variability of irinotecan dose/toxicity and tumor response has been attributed mainly to inherited genetic variations in the *UGT1A1* gene, which encodes UDP-glucuronosyltransferase (UGT) 1A1, a key enzyme in irinotecan metabolism. The human *UGT1A* locus is defined by 13 first exons, which are alternatively spliced to four common exons, leading to mRNA isoforms, nine of which conduct to functionally

This work was supported by the Canadian Institutes of Health Research [CIHR MOP-42392] (to C.G.); and Canada Research Chair Program (to C.G.). E.L. is a recipient of a CIHR clinician-scientist salary award. A.-S.B. was a recipient of a CIHR Frederick Banting and Charles Best studentship award. C.G. is the Canada Research Chair in Pharmacogenomics.
dx.doi.org/10.1124/jpet.112.202242.
[S] This article has supplemental material available at jpet.aspetjournals.org.

ABBREVIATIONS: FOLFIRI, folic acid (leucovorin), fluorouracil (5-FU), and irinotecan (camptosar) regimen; GI, gastrointestinal; H, haplotype; LD, linkage disequilibrium; MAF, minor allele frequency; OR, odds ratio; SN-38, 7-ethyl-10-hydroxycamptothecin; SNP, single-nucleotide polymorphism; UGT, UDP-glucuronosyltransferase; UTR, untranscribed region.

active enzymes. Indeed, following intravenous administration, irinotecan is converted in vivo to the highly potent active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38) by carboxylesterase-mediated hydrolysis (Kawato et al., 1991; Kojima et al., 1993). SN-38 is conjugated with glucuronic acid by hepatic and extrahepatic UGTs to form inactive SN-38-glucuronide. Several studies have identified specific inherited differences in irinotecan glucuronidation capacity that influence toxicity (Ando et al., 2000; Iyer et al., 2002; Innocenti et al., 2004; Marcuello et al., 2004; Mathijssen et al., 2004; Carlini et al., 2005; de Jong et al., 2006; McLeod et al., 2006; Toffoli et al., 2006). An increased number of dinucleotide repeats in the atypical TATA-box region of the *UGT1A1* promoter (*UGT1A1*28* allele) leads to a decreased rate of transcription initiation/expression of *UGT1A1* (Beutler et al., 1998). Several studies suggest that patients homozygous for *UGT1A1*28* are more likely to develop dose-dependent severe neutropenia compared with individuals with the reference genotype (*1/*1) (Iyer et al., 2002; Innocenti et al., 2004; Marcuello et al., 2004; Carlini et al., 2005; Soepenberg et al., 2005; Toffoli et al., 2006; Hoskins et al., 2007). Other genetic variations also linked to toxicity, such as the nonsynonymous coding variant G71R (*UGT1A1*6* allele), are particularly prevalent in Asians (frequency of 0.13–0.25), and lead to variable enzyme activity (Jada et al., 2007). Additionally, there are few data regarding the relationship with diarrhea, the other major adverse effect (Carlini et al., 2005; Toffoli et al., 2006). Thus, the clinical value of *UGT1A1* polymorphisms as predictors of irinotecan-associated toxicity has limitations, supporting the need for additional studies before implementation of individualized irinotecan dosing.

Along with UGT1A1 enzyme, several studies have revealed the importance of UGT1A9 in the hepatic conjugation of SN-38, whereas UGT1A7 is predominantly involved in its extrahepatic metabolism (Hanioka et al., 2001; Gagne et al., 2002). UGT1A6 has catalytic activity toward SN-38 in vitro (Gagne et al., 2002), but the effect on irinotecan metabolism is relatively undefined in vivo. Recent observations suggest that a combined signature of the haplotypes of *UGT1A1, UGT1A6, UGT1A7*, and *UGT1A9* might provide more precise information about irinotecan pharmacokinetics, pharmacodynamics, and time to progression defined as the interval between the first drug [FOLFIRI; folic acid (leucovorin; Pfizer, Saint-Laurent, QC, Canada), fluorouracil (5-FU; Hospira, Montreal, QC, Canada), and irinotecan (camptosar; Pfizer)] administration and the date of first disease progression (documented by computed tomography scans of measurable lesions) or last follow-up (Cecchin et al., 2009). Therefore, clinical outcome is likely the result of complex interplay, at least in part, between key genomic variations in UGT metabolic detoxification pathways.

Here, a cohort of 167 Canadian patients treated with FOLFIRI-based regimens for metastatic colorectal cancer was prospectively studied for hematologic and gastrointestinal (GI) toxicities in relation to germline polymorphisms in the major *UGT1A* gene. A first series of analyses focused on specific *UGT1A* variants, including the *UGT1A1*28*, and their haplotypes that were previously associated with severe neutropenia by Cecchin et al. (2009) in an Italian cohort of 250 patients also treated with FOLFIRI. We replicated the idea that a haplotype II [HII; single-nucleotide polymorphisms (SNPs) in *UGT1As 1A9/1A7/1A1*) is associated with increased risk of neutropenia, as reported in the Italian cohort (Cecchin et al., 2009). We also tested the inclusion of a 3′untranscribed region (3′UTR) variant common to all *UGT1As*, and defined a novel haplotype associated with the absence of neutropenia (HIa) in the combined analysis of Canadian and Italian patients. In a second series of investigations, we tested a broader range of variations across the *UGT1A* gene ($n = 21$) genotyped in Canadian patients, with the aim to identify a better combination of *UGT1A* markers (haplotypes) associated with the presence and absence of neutropenia. We report 4-marker haplotypes (SNPs in UGTs 1A9/1A7/1A6/3′UTR) that may help to refine prediction of hematologic toxicities, and ultimately improve dosing strategies.

Materials and Methods

Study Design and Patients

This multi-institution prospective study involved patient recruitment from 2003 to 2012 at three medical centers in eastern Canada: Hotel-Dieu de Québec in Québec City, QC; Hotel-Dieu de Lévis in Lévis, QC; and The Ottawa Hospital in Ottawa, ON. The ethics committee of each participating institution approved the study protocol, and all patients signed a written informed consent before entering the study. Eligibility criteria included patients (18–90 years old) initiating their first irinotecan-based chemotherapy with a histologically confirmed metastatic colorectal cancer, a life expectancy of at least 3 months, and a good performance status (Eastern Cooperative Oncology Group ≤ 2). Table 1 summarizes patient characteristics, such as age, gender, tumor site, treatment, and toxicity. The primary objective was to assess the relationship between SNPs in candidate genes and irinotecan-induced toxicity. The second cohort is composed of 250 metastatic cases and was previously described elsewhere (Toffoli et al., 2006; Cecchin et al., 2009).

Treatments

Patients were treated with one of the following FOLFIRI-based chemotherapies. Patients treated with the modified FOLFIRI regimen received irinotecan (180 mg/m^2 i.v.) for 2 hours on day 1 plus a bolus of 5-fluorouracil (400 mg/m^2) followed by continuous infusion of 5-fluorouracil (2400 mg/m^2) plus leucovorin (200 mg/m^2) over 46 hours. Patients received this treatment cycle every two weeks. Sixty-nine patients also received the monoclonal antibody bevacizumab (Avastin; Genentech, San Francisco, CA) in coadministration with their regimen, and 6 patients received either an experimental drug or placebo.

TABLE 1
Demographic and clinical characteristics

Characteristics	*N*	%
Total number	167	
Sex (male/female)	110/57	
Age median (years)	61.5	
Primary tumor site		
Colon	122	73.1
Rectum	42	25.1
Unknown	3	1.8
Regimen		
FOLFIRI	167	41.3
Cotreatment	69	3.6
Avastin/bevacizumab	6	
Other drugs		
Toxicity		
Diarrhea (grade 3-4)	24	14.4
Neutropenia (grade 3-4)	28	16.8

Efficacy Assessment. Computed tomography scans of measurable lesions were recorded prior to irinotecan chemotherapy and every four to eight doses after the start of treatment. Objective response and duration of response were assessed by Response Evaluation Criteria in Solid Tumors. Patients were considered evaluable for response if they had at least four doses of chemotherapy.

Toxicity Assessment. Toxicity was evaluated prospectively and according to National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 criteria. The toxicity endpoints consisted of both GI and hematologic toxicities, and were analyzed separately. For GI toxicities, all patients completed a daily report of GI toxicities during the first 14 days of each cycle to record the incidence and severity of nausea, vomiting, and diarrhea. For hematologic toxicities, laboratory parameters were collected before each cycle of chemotherapy and/or when the treatment was delayed. The most severe toxicity reported was used for data analysis. GI toxicity was evaluable for all patients except for one who died before toxicity assessment, and another who did not fill out the GI toxicity diary, while hematologic toxicity was evaluable for 166 of 167 patients. For the Italian cohort, details on eligibility, modalities of treatment, data collection, and definitions have been published previously (Toffoli et al., 2006; Cecchin et al., 2009).

Genotyping

Polymorphisms included in this study and their amplification strategies including primer sequences are described in the supplementary materials (Supplemental Tables 1 and 2). Variations linked at $r^2 \geq 0.95$ with another variant included or determined to be relatively rare [minor allele frequency (MAF) of <0.5%] were omitted in further analyses. At the time of patient enrollment, genomic DNA was obtained from a blood sample using a genomic DNA extraction kit (QIAamp DNA Blood Mini kit; Qiagen, Mississauga, ON, Canada). We identified polymorphisms by sequencing polymerase chain reaction products using an ABI PRISM 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA). All polymerase chain reactions were carried out in a final volume of 50 μl, containing 30 ng of genomic DNA, 3 mM $MgCl_2$, 200 nM of each deoxynucleotide triphosphate, 300 nM of each primer, 5% acetamide, and 1 U Taq polymerase. Each reaction was incubated at 94°C for 30 seconds followed by 35 cycles at 94°C for 30 seconds, 55–58°C for 30 seconds, and 72°C for 30 minutes, with a final step at 72°C for 5 minutes. All sequences were analyzed with the Staden package (Open Source Technology Group, Fairfax, VA; http://staden.sourceforge.net/) and compared with the reference sequence to assess genetic variations. Samples given an ambiguous sequencing chromatogram were systematically reamplified and resequenced. Genotyping was performed independently without knowledge of the clinical evaluations.

Data Analyses and Statistics

Deviations from the Hardy-Weinberg equilibrium of allele and genotype frequencies for the various genetic variations were assessed by Fisher's exact test. Haplotypes were inferred using the Phase version 2.1.1 program (Stephens et al., 2001; Stephens and Donnelly, 2003). Pairwise linkage disequilibrium (LD) was determined with HAPLOVIEW 3.32 (www.broad.mit.edu/mpg/haploview). Pairwise LD between polymorphisms was estimated by a log-linear model, and the extent of disequilibrium was expressed in terms of D', which is the ratio of the unstandardized coefficient to its maximal/minimal value. The possibility of genetic association was examined by testing the null hypothesis using two-tailed Fisher's exact test, and was considered statistically significant at $P \leq 0.05$ as calculated by JMP4.0.2 software (JMP Statistical Discovery, Cary, NC) or the SAS statistics package (SAS Institute, Cary, NC). When a particular variant was infrequent in the studied cohort ($n \leq 3$), homozygous and heterozygous genotypes were combined for statistical analyses. Genetic variants deemed positive ($P < 0.05$) or with a trend ($P < 0.10$) by univariate analysis were included in a stepwise logistic regression analysis. No adjustments were made for multiple comparisons because of the exploratory nature of this study.

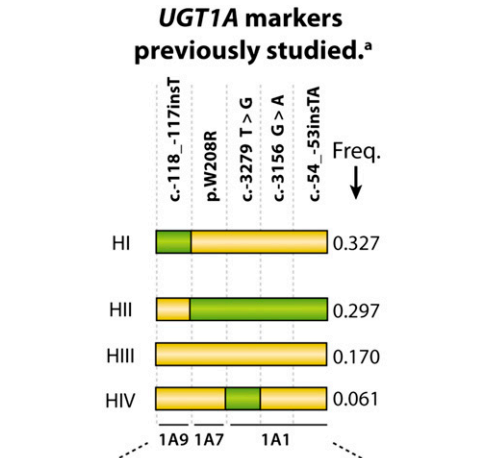
Results

Patient characteristics for the Canadian cohort are summarized in Table 1. Rates of grade 3–4 hematologic and GI toxicities prospectively evaluated were in keeping with previous reports (Schulz et al., 2009) (Supplemental Table 3). We studied 21 SNPs of the *UGT1A* gene genotyped in the cohort of 167 Canadian patients in relation to hematologic and GI-related toxicities. The observed allele frequency for selected SNPs was in agreement with previous analyses, and all of the SNP markers under study are in Hardy-Weinberg equilibrium, except for rs10929302 ($P = 0.01$) (Supplemental Table 2) (Maitland et al., 2006; Thomas et al., 2006; Menard et al., 2009). Pairwise LD analysis was performed with variations having a MAF > 0.05. As expected, the high LD observed for *UGT1A* variants agreed with data from a recent published analysis of a population from the same geographic region (Menard et al., 2009).

We initially tested previously reported haplotypes of the *UGT1A* locus named according to Cecchin et al. (2009) to thereby allow comparison between studies and avoid nomenclature confusion. Four haplotypes were inferred by 5 markers and occurred at a frequency of ≥5% in the Canadian cohort. We observed that haplotype HII, characterized by the co-occurrence of SNP susceptibility alleles including *UGT1A1*28*, is associated with a higher risk of severe neutropenia [odds ratio (OR) = 2.43; $P = 0.004$], as reported by Cecchin et al. (2009) for the Italian cohort (Fig. 1). The inclusion of an additional *UGT1A*-associated SNP located in the 3′UTR region resulted in two HI-related haplotype alleles, called HIa and HIb. Whereas the HIb is evenly distributed between patients who have or have not experienced neutropenia, the HIa allele is largely associated with severe neutropenia in both the Canadian and Italian cohorts ($n = 417$; OR = 0.55; $P = 0.038$).

In a second series of analyses, we included 21 variations across the *UGT1A* gene genotyped in the Canadian cohort. In univariate analyses of the Canadian cohort, *UGT1A* variants were linked to severe neutropenia but not GI toxicities (unpublished data). Severe neutropenia was associated with numerous variants with a MAF > 5% at the *UGT1A* locus ($P < 0.05$), including functional coding variants of *UGT1A6* and *UGT1A7*; three promoter polymorphisms of *UGT1A9* [c.-1212 (G/A), c.-688 (A/C), and c.-440 (C/T)], the common promoter *UGT1A1*28* (c.-54_-53 $TA_{6/7}$) allele; and promoter variant c.-3156 (G/A), most of which are known to impair gene expression or function (Bosma et al., 1995; Beutler et al., 1998). ORs and *P* values for association with hematologic toxicities are indicated in Table 2. For instance, the *UGT1A1**28 allele was associated with a 1.84-fold increased risk of developing severe neutropenia ($P = 0.045$). Both the *UGT1A6* c.181A allele (OR = 2.32; 95% confidence interval 1.03–3.30; $P = 0.045$) and the *UGT1A7* c.208C allele (OR = 2.00 95% confidence interval 1.12–3.58; $P = 0.025$) were significant predictors of severe neutropenia.

Upon multivariate analyses, no SNPs located in the *UGT1A1* first exon or its promoter region, including the *UGT1A1*28* (seven TA repeats) and the *UGT1A1* c.-3156A



Canadian (n=167)

Allele	Neutropenia Freq (%) 3-4/0-2	*P*	*OR (CI95%)*
I	0.27/0.34	0.350	0.71 (0.38-1.35)
II	**0.46/0.26**	**0.004**	**2.43 (1.35-4.39)**
III	0.18/0.17	0.846	1.08 (0.51-2.29)
IV	0/0.07	0.032	-

Fig. 1. Schematic representation of *UGT1A* and the haplotype analyses associated with severe neutropenia based on previously investigated markers. Variations are represented by squares: a yellow square represents a reference nucleotide, whereas a green square represents a variant (relative to the AF297093 sequence). Only haplotypes with an MAF > 5% in the present study are shown. [a]Based on markers studied by Cecchin et al. (2009). CI95%, 95% confidence interval; Freq., frequency.

alleles, remained significant in the Canadian patients. However, three markers situated in the central region of *UGT1A* were associated with a 2-fold increased risk of neutropenia grade 3–4 (Table 3), and are located in the *UGT1A9* promoter at position −688 (MAF of 0.025), in the *UGT1A7* first exon (p.W208R; MAF of 0.412), and in the *UGT1A6* first exon (p.T181A; MAF of 0.352). Thus, in a second series of haplotype analysis, we tested these three SNPs with the 3′UTR SNP and revealed a protective HI (OR = 0.50; $P = 0.048$) and two risk haplotypes, HII and HIII, characterized by the presence of 2 (OR = 2.18; $P = 0.014$) and 3 (OR = 5.28; $P = 0.030$) unfavorable alleles, respectively, revealing a dosage effect (Fig. 2). This combination has not been tested in the Italian population due to a missing genotype for position −688 of *UGT1A9*.

Discussion

Irinotecan combination chemotherapy causes severe and unpredictable hematologic and GI toxicities in a substantial percentage of patients (Negoro et al., 1991; Rothenberg et al., 1993, 2001; Rougier et al., 1998; Saltz et al., 2000; Vanhoefer et al., 2001; Fuchs et al., 2003). Despite several published studies on genetic markers that help predict irinotecan-associated severe neutropenia [reviewed in Hoskins et al. (2007)], much work is still required to optimize individualized treatment. Hence, better molecular markers to identify patients at risk for complications, including severe diarrhea, as well as to predict clinical response would be helpful to patients and medical oncologists. Currently, pharmacogenetic data suggest that the *UGT1A1*28/*28* genotype confers the highest risk of severe neutropenia due to increased exposure to SN-38 (Ando et al., 2000; Iyer et al., 2002; Innocenti et al., 2004; Marcuello et al., 2004; Mathijssen et al., 2004; Rouits et al., 2004; de Jong et al., 2006; Massacesi et al., 2006; McLeod et al., 2006; Pillot et al., 2006; Toffoli et al., 2006; Cote et al., 2007; Hoskins et al., 2007; Kweekel et al., 2008; Ruzzo et al., 2008; Glimelius et al., 2011). Our current study confirms that this genotype is associated with an increased risk of severe neutropenia but in univariate analyses only, whereas a more comprehensive analysis of variations at the *UGT1A* locus suggests that other markers in the central region of the gene and in the 3′UTR region might better predict this toxicity.

As previously reported, polymorphisms at the *UGT1A* locus exhibit strong LD (Kohle et al., 2003; Peters et al., 2003; Menard et al., 2009). There has been sporadic conflicting information on the role of functional variants in the *UGT1A1* promoter and coding regions and other *UGT1A* genes involved in irinotecan metabolism (Schulz et al., 2009). However, considering that *UGT1A1*28* is a well-accepted predictor of severe neutropenia, and that strong LD is observed between several functional genetic variations at the *UGT1A* locus in diverse populations, it is thus not surprising to find an association between severe neutropenia and other common deleterious variations in *UGT1A* genes encoding SN-38–metabolizing enzymes (Table 2) (Iyer et al., 1998; Ciotti et al., 1999; Hanioka et al., 2001; Gagne et al., 2002).

Several *UGT1A* variations were individually associated with severe neutropenia, and their presence is inferred in the haplotype HII defined by a 5-marker haplotype across *UGT1A* first exons previously reported by Cecchin et al. (2009), and include the *UGT1A1*28* allele (Fig. 2). We further described a protective *UGT1A* haplotype allele (HIa) defined by the reference sequence for these 5 markers, but also a variation in the 3′UTR region of the *UGT1A* gene common to all UGT1A-derived enzymes. Individuals with this haplotype have less chance of experiencing severe neutropenia (by 2-fold), and therefore could potentially tolerate irinotecan with less hematologic toxicity. Indeed, it has also been hypothesized that higher irinotecan doses can be safely administered to patients homozygous for the reference genotype UGT1A1*1/*1 owing to their relatively good tolerance of this drug (Schulz et al., 2009). Only the *UGT1A* HIa haplotype was associated with a reduced incidence of neutropenia, indicating that the simple exclusion of patients with the *UGT1A1*28/*28* genotype may be insufficient to predict good tolerance to irinotecan with respect to severe neutropenia. Instead of identifying a risk haplotype and inferring that *UGT1A1*28* noncarriers would be protected from severe neutropenia, the assessment of haplotype HIa seems to better identify those who have a low risk of irinotecan-induced neutropenia, presumably owing to the high glucuronidation activity of this

TABLE 2
Polymorphisms in the *UGT1A* gene positively associated with severe neutropenia under allelic or genotypic analyses
Odds ratios (OR) have been calculated under the following models as specified: Dominant (a), recessive (b).

Gene	Variation	Alleles	Neutropenia 0–2	Neutropenia 3–4	OR (95% CI)	*P*	Genotypes	Neutropenia 0–2	Neutropenia 3–4	OR (95% CI)	*P*	*N*[a]
UGT1A9	c.-1212	G	189	29	2.12 (1.17–3.84)		GG	71	8	3.24[b] (1.32–7.98)	0.010	160
		A	77	25		0.016	GA	47	13			
							AA	15	6			
	c.-688	A	264	50	5.28 (1.28–21.81)		AA	130	23	5.65[b] (1.32–24.22)	0.028	161
		C	4	4		0.030	AC	4	4			
							CC	0	0			
	c.-440	C	182	29	1.97 (1.10–3.53)		CC	65	8	2.36[b] (0.97–5.72)	0.062	162
		T	86	27		0.030	CT	52	13			
							TT	17	7			
UGT1A7	p.W208R	T	168	25	2.00 (1.12–3.58)		TT	53	6	2.59[c] (1.03–6.51)	0.057	164
		C	104	31		0.025	TC	62	13			
							CC	21	9			
UGT1A6	p.S7A	T	160	24	2.00 (1.12–3.57)		TT	53	6	2.59[c] (1.03–6.51)	0.057	164
		G	110	33		0.019	TG	62	13			
							GG	21	9			
	p.T181A	A	186	27	2.32 (1.30–4.16)		AA	66	8	3.55[c] (1.38–9.18)	0.017	164
		G	86	29		0.005	AG	54	11			
							GG	16	9			
	p.R184S	A	180	26	2.26 (1.26–4.04)		AA	62	8	3.64[c] (1.45–9.13)	0.010	164
		C	92	30		0.006	AC	56	10			
							CC	18	10			
UGT1A1	c.-3156	G	193	29	2.28 (1.27–4.09)		GG	73	9	3.00[c] (1.14–7.93)	0.036	164
		A	79	27		0.007	GA	47	11			
							AA	16	8			
	c.-54_-53	6	185	30	1.84 (1.03–3.30)		66	66	9	2.33[c] (0.86–6.31)	0.137	164
		7	87	26		0.045	67	53	12			
							77	17	7			

95% CI, 95% confidence interval.
[a] N: number of individuals with available genotyping data and toxicity information.
[b] Dominant model.
[c] Recessive model.

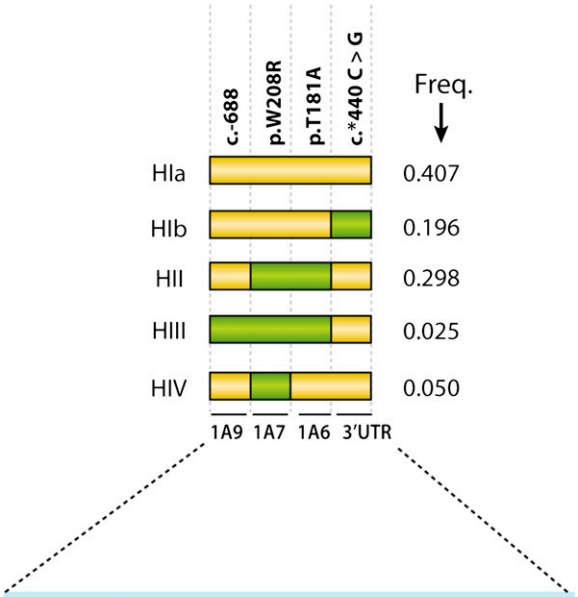
allele. Indeed, this haplotype, as well as the haplotype HIb but with variation in the 3′UTR, contains *UGT1A1*1*, the reference *UGT1A6/1A7*, and *UGT1A9,* all of which are associated with high UGT expression and glucuronidation activity. These functional alleles may act synergistically to enhance SN-38 conjugation in the liver and extrahepatic tissues. It is thus tempting to speculate that genetic variations at the 3′ end affect gene expression. More studies are definitely needed to confirm these findings and elucidate the exact molecular mechanisms underlying our observation, and to assess the functionality of 3′UTR variations in *UGT1A*.

Additional analyses reveal that other variants in the central region of the *UGT1A* gene, namely, those located in *UGT1A9* and in exons *UGT1A7* and *UGT1A6*, are significant predictors of severe neutropenia with at least a 2-fold increased risk in multivariate analyses. Some of these SNPs are only partially linked to the *UGT1A1*28* allele (r^2 values between 0.028 and 0.82). Haplotype analyses with these markers and the 3′UTR variation, for a total of 4 markers located in *UGT1A9, UGT1A7, UGT1A6,* and 3′UTR, define four common haplotypes, of which one is protective and is referred to as HI = ATA (OR = 0.50) and two, HII = ACG and HIII = CCG, that are linked to a significantly higher risk of severe neutropenia. We further reveal a dosage effect with a higher risk in patients carrying 2 markers and the highest risk in those with 3 markers, also carrying the reference 3′UTR allele that does not confer protection. This set of *UGT1A* markers seems to improve risk prediction for severe neutropenia. Previous in vitro reports support the contribution of UGT1A9, UGT1A7, and UGT1A6 enzymes in the conjugation of SN-38 (Ciotti et al., 1999). Despite the uncertainty of the extent of the contribution of these other enzymes to SN-38 inactivation, several studies have found an association between the *UGT1A7*3* allele (p.W208R) and irinotecan-induced toxicities (Ando et al., 2002; Carlini et al., 2005; Lankisch et al., 2008). UGT1A7 is one of the extrahepatic enzymes expressed mainly in the upper GI tract, whereas both UGT1A6 and UGT1A9 are expressed in the liver and other tissues (Nakamura et al., 2008). These *UGT1A* variations and the identified set of *UGT1A* markers should be carefully evaluated in future studies of irinotecan toxicity. The limitations of the study are its exploratory nature; the limited sample size, particularly for haplotype analyses; and the population studied that might be relatively genetically homogeneous. Other limitations are related to a focus on specific SNPs within the *UGT1A* gene, the scarcity of functional data for some of the positive markers, and a need for validation and study of additional cohorts.

TABLE 3
Stepwise logistic regression model for severe neutropenia
Variables entered in the first step: *UGT1A9* c.-1212, *UGT1A9* c.-688, *UGT1A9* c.-440, *UGT1A7* p.W208R, *UGT1A6* p.S7A, *UGT1A6* p.T181A, *UGT1A6* p.R184S, *UGT1A1* c.-3156, *UGT1A1* c.-54_53insTA. Age and treatment type were also included in the model.

Severe Neutropenia	Estimate	S.E.	OR (95% CI)	*P*
UGT1A6 p.T181A	0.741	0.317	2.10 (1.13–3.91)	0.020
UGT1A7 p.W208R	0.701	0.311	2.00 (1.12–3.57)	0.024
UGT1A9 c.-688	1.509	0.810	4.52 (0.92–22.15)	0.063

95% CI, 95% confidence interval; OR, odds ratio.



Neutropenia Freq (%) Allele	3-4/0-2	P	OR (CI95%)
Ia	**0.28/0.43**	**0.048**	**0.50 (0.27-0.96)**
Ib	0.17/0.20	0.707	0.79 (0.37-1.72)
II	**0.44/0.27**	**0.014**	**2.18 (1.19-3.97)**
III	**0.07/0.01**	**0.030**	**5.28 (1.28-21.81)**
IV	0.02/0.06	0.488	0.32 (0.04-2.46)

Fig. 2. Schematic representation of *UGT1A* and the haplotype analyses associated with severe neutropenia based on markers across the *UGT1A* locus. Variations are represented by squares: a yellow square represents a reference nucleotide, whereas a green square represents a variant (relative to the AF297093 sequence). Only haplotypes with an MAF > 5% in the present study are shown. C, carrier; CI95%, 95% confidence interval; Freq., frequency; NC, noncarrier.

In conclusion, the ultimate objective of pharmacogenetic studies is to develop tests that can be used to identify patients more likely to respond to a particular therapy and individuals who are more liable to suffer adverse reactions. In our study, we characterize *UGT1A* haplotypes that could potentially lead to more robust predictive tests. Additional studies that include a more comprehensive assessment of variations in *UGT1A*, including variations in the 3′UTR region and those across the locus, are warranted in irinotecan-containing dosage regimens, and may help clarify the role of *UGT1A* in the management of irinotecan toxicity and response.

Acknowledgments

The authors thank all the participants in this study as well as the research nurses from Québec and Ottawa hospitals for their contributions. The authors also thank Anne Dionne from Laval University for help in the design of the toxicity records. The authors also acknowledge the contribution of other laboratory members for work related to the support of genotyping and handling of samples.

Authorship Contributions

Participated in research design: Guillemette, Lévesque.

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Wrote or contributed to the writing of the manuscript: Lévesque, Harvey, Bélanger, Couture, Jonker, Innocenti, Cecchin, Toffoli, Guillemette.

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