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Refining the *UGT1A* haplotype associated with irinotecan-induced hematological toxicity in metastatic colorectal cancer patients treated with 5-fluorouracil/irinotecan (FOLFIRI)-based regimens.

Éric Lévesque, Anne-Sophie Bélanger, Mario Harvey, Félix Couture, Derek Jonker, Federico Innocenti, Erica Cecchin, Giuseppe Toffoli and Chantal Guillemette

Pharmacogenomics Laboratory, Centre Hospitalier de l'Université Laval (CHU de Québec) Research Center and Faculty of Pharmacy, Laval University, G1V 4G2, Quebec, Canada (E.L., A-S.B., M.H., F.C., C.G.), Hematology-Oncology Department, CHU de Québec Research Center, Hôtel-Dieu de Quebec, Laval University, G1R 2J6, Quebec, Canada (E.L., F.C.), Hematology-Oncology Department, Ottawa Hospital, Ottawa University, K1Y 4E9, Ontario (D.J.), Division of Pharmacotherapy and Experimental Therapeutics, UNC Eshelman School of Pharmacy, University of North Carolina at Chapel Hill, USA (F.I.), Division of Experimental and Clinical Pharmacology, Department of Molecular Biology and Translational Research, National Cancer Institute and Center for Molecular Biomedicine, Aviano (PN), Italy (E.C., G.T.).

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Correspondence: Chantal Guillemette, Ph.D.

Canada Research Chair in Pharmacogenomics

Pharmacogenomics Laboratory

CHU de Québec Research Center, T3-48, 2705 Boul. Laurier, Québec, Canada, G1V 4G2

Tel. (418) 654-2296; Fax (418) 654-2761

E-mail: Chantal.Guillemette@crchul.ulaval.ca

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Abbreviations: UGT, UDP-glucuronosyltransferase; GI, gastrointestinal; PCR, polymerase chain reaction; UTR, untranscribed region; SNP, single nucleotide polymorphism; ECOG, Eastern Cooperative Oncology Group; RECIST, Response Evaluation Criteria in Solid Tumors; NCI-CTCAE, National Cancer Institute-Common Terminology Criteria for Adverse Events; LD, Linkage Disequilibrium; OR, Odd Ratio.

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ABSTRACT

Background: Despite the importance of *UGT1A1**28 in irinotecan pharmacogenetics, our capability to predict drug-induced severe toxicity remains limited. **Objective:** We aimed at identifying novel genetic markers that would improve prediction of irinotecan toxicity and response in advanced colorectal cancer patients treated with FOLFIRI-based regimens. **Approach**: 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'UTR region of UGT1A locus, not studied in this context yet. These genetic markers were further investigated in 250 Italian FOLFIRI-treated patients. **Results:** Several functional *UGT1A* variants including *UGT1A1*28* significantly influenced risk of severe hematological toxicity. As previously reported in the Italian cohort, a 5-markers risk haplotype (HII; UGTs 1A9/1A7/1A1) was associated with severe neutropenia in our cohort (OR=2.43; *p*=0.004). The inclusion of a 3'UTR 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≤0.030). **Conclusions:** 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 tumour 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 tumour response has been attributed mainly to inherited genetic variations in *UGT1A1* gene, which encodes UDP-glucuronosyl transferase (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, of which nine conduct to functionally 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 *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 to 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 non-synonymous 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 suggested 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) 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*s metabolic detoxification pathways.

Here, a cohort of 167 Canadian patients treated with FOLFIRI-based regimens for metastatic colorectal cancer was prospectively studied for hematological 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 previously associated with severe neutropenia by Cecchin et al. 2009 (Cecchin et al., 2009) in an Italian cohort of 250 patients also treated

with FOLFIRI. We replicated that a haplotype II (HII; 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'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 the absence of neutropenia. We report 4-marker haplotypes (SNPs in UGTs 1A9/1A7/1A6/3'UTR) that may help to refine prediction of hematological toxicities, and ultimately improving dosing strategies.

PATIENTS AND METHODS

Study design and patients

This multi-institution prospective study involved patient recruitment from 2003 to 2012 at three medical centres in eastern Canada: Hotel-Dieu de Québec in Québec City, Quebec; Hotel-Dieu de Lévis in Lévis, Québec; and The Ottawa Hospital in Ottawa, Ontario. 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 (ECOG $\leq$ 2). The Table 1 summarizes patient characteristics, such as age, gender, tumour 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) intravenously for 2 h on day 1 plus a bolus of 5-fluorouracil (400 mg/m^2) followed by continuous infusion of 5-fluorouracil (2,400 mg/m^2) plus leucovorin (200 mg/m^2) over 46 h. Patients received this treatment cycle every two weeks. Sixty-nine patients also received the monoclonal antibody bevacizumab (Avastin®) in co-administration with their regimen, and 6 patients received either an experimental drug or placebo.

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 RECIST criteria. Patients were considered evaluable for response if they had at least four doses of chemotherapy.

Toxicity assessment. Toxicity was evaluated prospectively and according to NCI-CTCAE v3.0 criteria. The toxicity end points consisted of both GI and hematological 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 hematological 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 one that did not fill out the GI toxicity diary, while hematological toxicity was evaluable for 166 out 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 section (**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 enrolment, 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 (PCR) products using an ABI PRISM 3730XL DNA Analyzer (Applied Biosystems, Foster City, CA). All PCR reactions were carried out in a final volume of 50 µl, containing 30 ng of genomic DNA, 3 mM $MgCl_2$, 200nM of each dNTP, 300 nM of each primer, 5% acetamide and 1 U Taq polymerase. Each reaction was incubated at 94°C for 30 s followed by 35 cycles at 94°C for 30 s, 55-

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58°C for 30 s, and 72°C for 30 min, with a final step at 72°C for 5 min. All sequences were analyzed with the Staden package (Open Source Technology Group, http://staden.sourceforge.net/) and compared to the reference sequence to assess genetic variations. Samples given ambiguous sequencing chromatogram were systematically re-amplified and re-sequenced. 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 v2.1.1 program. 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 for $p \leq 0.05$ as calculated by JMP4.0.2 software 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.

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RESULTS

Patient characteristics for the Canadian cohort are summarized in **Table 1**. Rates of grade 3-4 hematological 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 hematological and GI related toxicities. The observed allele frequency for selected SNP 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.* (Cecchin et al., 2009), to allow comparison between studies thereby and avoid nomenclature confusion. Four haplotypes were inferred by 5 markers and occur at a frequency ≥5% in the Canadian cohort. We observe that haplotype HII characterized by the co-occurrence of SNPs susceptibility alleles including the *UGT1A1*28,* is associated with higher risk of severe neutropenia (OR=2.43; p=0.004), as reported by Cecchin *et al.* for the Italian cohort. (Cecchin et al., 2009) (**Fig.1**). The inclusion of an additional UGT1A-associated SNP located in the 3'UTR region described two HI-related haplotype alleles, called HIa and HIb. . While the HIb is evenly distributed between patients having experienced neutropenia or not, the HIa allele is largely associated to severe neutropenia in both Canadian and Italian cohorts (n=417; OR=0.55; p=0.038).

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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 with GI toxicities (data not shown). 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), 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). Odds ratios (ORs) and *p*-values for association with hematological 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 *UGT1A6* c.181A allele (OR=2.32 95% CI [1.03-3.30], p=0.045) and *UGT1A7* c.208C allele (OR=2.00 95% CI [1.12-3.58], p=0.025) were significant predictors of severe neutropenia.

Upon multivariate analyses, no SNPs located in *UGT1A1* first exon or its promoter region including the *UGT1A1*28* (seven TA repeats) and the *UGT1A1* c.-3156A alleles remained significant in the Canadian patients. However, three markers situated in the central region of *UGT1A* were associated with a two-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 UGT1A7 first exon (p.W208R; MAF of 0.412) and in 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) unfavourable alleles respectively, revealing a dosage effect (**Figure 2**). This combination has not been tested in the Italian population due to missing genotype for position -688 of UGT1A9.

DISCUSSION

Irinotecan combination chemotherapy causes severe and unpredictable hematological and GI toxicities in a substantial percentage of patients (Negoro et al., 1991; Rothenberg et al., 1993; Rougier et al., 1998; Saltz et al., 2000; Rothenberg et al., 2001; 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 complications, including severe diarrhea as well as to predict clinical response would be helpful to patients and medical oncologists. Currently, pharmacogenetic data suggests that *UGT1A1*28/*28* genotype confers the highest risk of severe neutropenia due to increase 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; Kweekel et al., 2008; Ruzzo et al., 2008) (Hoskins et al., 2007; 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 and collaborators, and include the *UGT1A1*28* allele (**Figure 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 to experience severe neutropenia (by 2-fold) and therefore could potentially tolerate irinotecan with less hematological 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* non-carriers would be protected from severe neutropenia, the assessment of haplotype HIa seems to better identify those who have low risk of irinotecan induced neutropenia, presumably owing to the high glucuronidation activity of this 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 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, defines 4 common haplotypes, of which one is protective 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 highest 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, while both UGT1A6 and UGT1A9 are both 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, 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.

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 that 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.

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Authors contribution:

Participated in research design: Guillemette, Lévesque.

Conducted experiments: Harvey, Bélanger, Cecchin.

Performed data analysis: Lévesque, Harvey, Bélanger, Couture, Jonker, Innocenti, Cecchin, Toffoli, Guillemette

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

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Footnotes

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FIGURE LEGENDS

Figure 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*. JCO 2009. Freq.: frequency; H: haplotype; C: carrier; NC: non-carrier; OR: odds ratio; CI 95%: 95% confidence interval.

Figure 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. Freq.: frequency; H: haplotype; C: carrier; NC: non-carrier; OR: odds ratio; CI 95%: 95% confidence interval.

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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	
Co-treatment:		
Avastin/bevacizumab	69	41.3
Other drugs	6	3.6
Toxicity		
Diarrhea (grade 3-4)	24	14.4
Neutropenia (grade 3-4)	28	16.8

Table 2. Polymorphisms in the *UGT1A* gene positively associated with severe neutropenia under allelic or genotypic analyses.

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

Odd ratios have been calculated under the following models as specified: Dominant (a), recessive (b). OR: odd ratios; CI95%: confidence intervals; P: p-value. *N : number of individuals with available genotyping data and toxicity information.

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Table 3. Stepwise logistic regression model for severe neutropenia.

Severe neutropenia	Estimate	SE	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

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. SE: standard errors; OR: odd ratios; CI95%: confidence intervals; P: p value.

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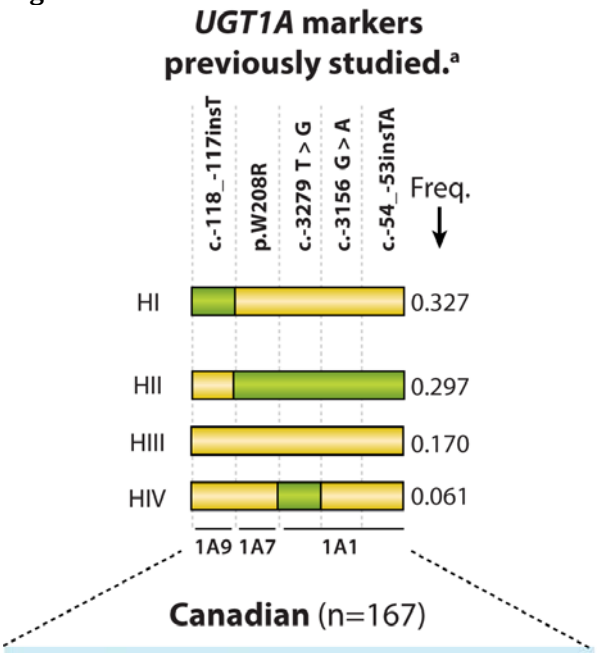
Figure 1



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	-

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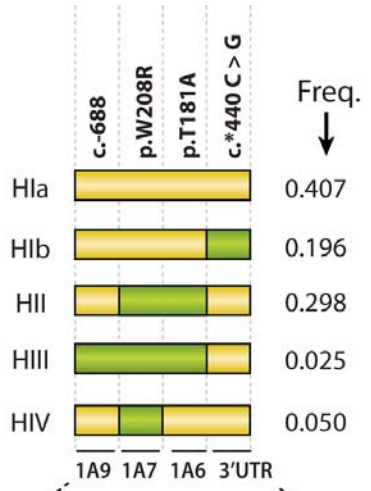
Figure 2



Allele	Neutropenia Freq (%) 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)