THE NOVEL UGT1A9 INTRONIC I399 POLYMORPHISM APPEARS AS A PREDICTOR OF 7-ETHYL-10-HYDROXYCAMPTOTHECIN GLUCURONIDATION LEVELS IN THE LIVER

Hugo Girard, Lyne Villeneuve, Michael H. Court, Louis-Charles Fortier, Patrick Caron, Qin Hao, Lisa L. von Moltke, David J. Greenblatt, and Chantal Guillemette

ABSTRACT:
Polymorphisms in UGT1A9 were associated with reduced toxicity and increased response to irinotecan in cancer patients. UDP-glucuronosyltransferase (UGT) protein expression, glucuronidation activities for 7-ethyl-10-hydroxycamptothecin (SN-38), and probe substrates of the UGT1A9 and UGT1A1 were measured in 48 human livers to clarify the role of UGT1A9 variants on the in vitro glucuronidation of SN-38. Genotypes were assessed for UGT1A9 (−2152C>T, −275T>A, and −118T>T10), three novel UGT1A9 variants (−5366G>T, −4549T>C, and I399C>T), and UGT1A1 (−53TA6, −3156G>A, and −3279T>G). Of all the variants, the UGT1A9 I399C>T was associated with the most dramatic change in SN-38-glucuronide (SN-38G) (2.64-fold; p = 0.0007). Compared with UGT1A9 I399C/G, homozygous I399T/T presented elevated UGT1A1 and UGT1A9 proteins and higher glucuronidation of UGT1A9 and UGT1A1 substrates (p < 0.05). The very low linkage disequilibrium (r² < 0.19) between UGT1A9 I399 and all the other UGT1A1 and UGT1A9 variants suggests a direct effect or linkage to unknown functional variant(s) relevant to SN-38 glucuronidation. The UGT1A9 −118T>T10 was also linked to alteration of SN-38 glucuronidation profiles in the liver (p < 0.05) and was associated with higher UGT1A1 protein (p = 0.03). However, UGT1A9 −118T>T10 appears to have low functional impact as a result of the lack of correlation with UGT1A9 protein levels and a modest 1.4-fold higher reporter gene expression associated with the −118T>T10 allele in HepG2 cells (p = 0.004). In contrast, the UGT1A9 −5366T, −4549C, −2152T, and −275A, associated with higher UGT1A9 protein (2-fold; p < 0.05), have no influence on SN-38G. Despite limitations resulting from sample size, results indicate that UGT1A9 I399 and −118T>T10 may represent additional candidates in combination with UGT1A1 promoter haplotypes for the prediction of SN-38 glucuronidation profile in vivo.

Irinotecan (or CPT-11) is a topoisomerase inhibitor used to treat metastatic colorectal cancer (Douillard et al., 2000; Saltz et al., 2000). Its metabolism is complex and includes oxidation by cytochrome P450 and activation by carboxylesterase to form the active metabolite SN-38 (Kawato et al., 1991). Glucuronidation catalyzed by UDP-glucuronosyltransferases (UGTs) represents the major inactivation pathway for SN-38 (Gupta et al., 1994). Of the 16 functional human UGTs, UGT1A1 and UGT1A9 were identified as the main hepatic enzymes involved in the inactivation of SN-38 to SN-38-glucuronide (SN-38G) (Hanioka et al., 2001; Gagne et al., 2002). UGT1A1 and UGT1A9 are encoded by a single gene by exon sharing of individual exons 1 with common exons 2 to 5, whereas at least six other UGT1A1 proteins originate from this gene (Gong et al., 2001).

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Several studies revealed the importance of UGT1A1 and UGT1A9 in the hepatic metabolism of SN-38 (Iyer et al., 1998; Gagne et al., 2002; Carlini et al., 2005). A number of polymorphisms in UGT1A1 and UGT1A9 affecting expression and protein function have been identified and could potentially modulate the metabolism of SN-38 in vivo (Zheng et al., 2001; Guillemette, 2003; Villeneuve et al., 2003; Girard et al., 2004; Yamanaka et al., 2004), as well as response to irinotecan-based chemotherapy (Ando et al., 2000; Innocenti et al., 2004; Marcuello et al., 2004; Rouits et al., 2004; Carlini et al., 2005). Among these genetic variations, patients with the UGT1A1*28 variant, associated with Gilbert’s syndrome (Bosma et al., 1995), show higher levels of SN-38 and experienced severe diarrhea and neutropenia (Ando et al., 2000; Innocenti et al., 2004; Marcuello et al., 2004; Rouits et al., 2004). Additional polymorphisms in the UGT1A1 gene have been reported and linked to a variable SN-38 glucuronidation, namely, the −3156G>A and the −3279T>G variations (Innocenti et al., 2002; Sugatani et al., 2002). Genotyping of these variants in the promoter region of UGT1A1, along with the −53 variant, has been suggested to improve the prediction of UGT1A1 status, and specific UGT1A1 haplotypes are associated with altered SN-38 phenotypes (Innocenti et al., 2002, 2005; Sai et al., 2004; Kitagawa et al., 2005).

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ABBREVIATIONS: SN-38, 7-ethyl-10-hydroxycamptothecin; UGT, UDP-glucuronosyltransferase; SN-38G, SN-38-glucuronide; PCR, polymerase chain reaction; HPLC, high-performance liquid chromatography; LD, linkage disequilibrium; MPA, mycophenolic acid.
Because the UGT1A9 protein is involved in the formation of SN-38G (Hanioka et al., 2001; Gagne et al., 2002), we recently identified polymorphic variations in the Caucasian population associated with altered gene expression or catalytic efficiency, namely, the \(-2152C>T, -2757G>A, \text{and} M^\text{N}937C\) (Villeneuve et al., 2003; Girard et al., 2004). Another group reported that the \(-118T\rightarrow C\) repeat was associated with higher reporter gene expression in HepG2 liver cells (Yamanaka et al., 2004), and this variant was recently linked to higher incidence toxicity and increased response to irinotecan in cancer patients. (Carlini et al., 2005). Still, definitive conclusions on the exact role of UGT1A1 and UGT1A9 variations and their influence on irinotecan response in patients cannot be drawn. Additional studies are critical for determining the role of other polymorphisms in UGTs, in addition to UGT1A1, and especially the importance of UGT1 haplotypes.

In this study, we wanted to gain insights into the role of polymorphisms in the UGT1A9 in modulating SN-38G formation in the liver. UGT1A9 was resequenced up to \(-5.5\) kilobase (kb) of the ATG and included intronic sequences. UGT1A1 and UGT1A9 protein content was measured to identify polymorphisms and haplotypes/diplotypes that might influence gene expression and SN-38G formation in liver microsomes.

### Materials and Methods

**Reagents.** SN-38 was prepared by hydrolysis of irinotecan-HCL (McKesson, Mississauga, Canada). Identity of the SN-38 was assessed by mass spectroscopy, and purity was assessed by UV absorbance spectra. Bilirubin, estradiol, and estradiol-3-glucuronide were purchased from Sigma-Aldrich (St. Louis, MO).

**Genomic DNA and Liver Samples.** Human genomic DNA and liver samples were obtained from different sources as described previously (Court et al., 2002) as approved by the respective human research institutional review boards (CHUQ Research Center, Laval University and Tufts University). Available subject information included gender, age, race, ethnicity, and histories of smoking and alcohol use, as described in Hesse et al. (2004). The quality of the liver samples was ascertained by reference to at least 10 other glucuronidation activities measured using the same set of livers. Livers that consistently showed low activity values (>2-fold lower for all the measured activities) relative to the median activity value for the entire liver set had been excluded from study.

**Genotyping of UGT1A1 and UGT1A9 Polymorphisms.** UGT1A1 \(-53T\rightarrow A\), \(-3156G>A\), and \(-3279T>G\) genotypes for all 48 subjects were determined in a previous study by Girard et al. (2005). Genotyping of UGT1A9 exon 1 polymorphisms at codon 3 and 33 and 11 promoter polymorphisms from \(-87\) to \(-2152\) were performed previously (Girard et al., 2004). For the discovery of new variants, primers R503, F506, R507, F838, R840, R841, and R842 (Table 1) were used to amplify the UGT1A9 promoter region located between nucleotides \(-1654\) and \(-5525\) (relative to the ATG). Genotyping of the two new polymorphisms located at positions \(-20549\) and \(-5366\) was performed by direct sequencing. Primers F1159/R1164 and F1154/R1165 were used to amplify regions with polymorphisms \(-4549\) and \(-5366\), respectively. The polymerase chain reaction (PCR) products were submitted to sequencing using primer R1164 and F1154. The UGT1A9 intron polymorphisms at positions \(143C>T, 152G>A, 201A>C, 219T>A, 313A>C, \text{and} 399C>T\) (relative to the end of UGT1A9 exon 1) were genotyped by direct sequencing, with primer F1293, of a PCR product generated with primers F1293 and R1298.

**Construction of Plasmid and Transfections.** Primers F1121 and R670 were used to amplify the proximal promoter region of UGT1A9 between \(-152\) and \(+2\) (relative to the ATG). The PCR product was digested with HindIII and Xhol and inserted in the pGL3 vector. Primers F1473 and R1474 were also used to amplify the proximal promoter region of UGT1A9 between \(-171\) and \(-1\) (relative to the ATG). The PCR product was digested with SmaI and inserted in the pGL3 vector. A variant sequence was generated by site-directed mutagenesis for both strategies (QuikChange Site-Directed Mutagenesis Kit, Stratagene, La Jolla, CA) with primers F1155 and R1166.

HepG2 cells were obtained from American Type Culture Collection (Manassas, VA) and were grown in minimum essential medium. Medium was supplemented with 20% fetal bovine serum (Wisent, St-Bruno, Canada), as well as a 0.1 mM mixture of nonessential amino acids, 100 IU/ml penicillin, and 50 \(\mu\)g/ml streptomycin (all from Invitrogen, Burlington, Canada). Sodium pyruvate at a final concentration of 1 mM was added. Transfections were performed as described previously (Duguay et al., 2004a). Cells were harvested 24 h post-transfection and assayed for promoter activity using the dual luciferase reporter assay system according to the manufacturer’s recommendations, including as an internal control, the pRL-null vector (encoding Renilla luciferase) cotransfected in each well (Promega, Madison, WI). Luciferase activity was measured using 40 \(\mu\)l of cell lysates in a 96-well plate on a microplate luminometer LB96V (EG&G Berthold, Bad Wildbad, Germany) and normalized to the Renilla luciferase values. Means of duplicate luciferase activity of four independent experiments were compared between the different constructs with a two-tailed unpaired Student’s t test.

**UGT1A1 and UGT1A9 Protein Quantification and Enzymatic Assays.** UGT1A1 and UGT1A9 protein quantification in all 48 liver microsomes was performed by semiquantitative Western blot analysis using specific antibodies for UGT1A1 and UGT1A9 as described previously (Duguay et al., 2004b; Girard et al., 2004). The relative levels of UGT protein content were compared with the sample with the lowest expression. SN-38 enzymatic assays were performed in a final volume of 50 mM Tris-HCl, pH 7.5, 10 mM MgCl\(_2\), 2 mM UDP-glucuronic acid, 5 \(\mu\)M liver microsomes. Hepatic glucuronidation activities measured using the dual luciferase reporter assay system according to the manufacturer’s recommendations, including as an internal control, the pRL-null vector (encoding Renilla luciferase) cotransfected in each well (Promega, Madison, WI). Luciferase activity was measured using 40 \(\mu\)l of cell lysates in a 96-well plate on a microplate luminometer LB96V (EG&G Berthold, Bad Wildbad, Germany) and normalized to the Renilla luciferase values. Means of duplicate luciferase activity of four independent experiments were compared between the different constructs with a two-tailed unpaired Student’s t test.
reconstituted with 100 μl of 0.1% trifluoroacetic acid in water before HPLC analysis. HPLC (Agilent 1100 system, Agilent Technologies, Palo Alto, CA) was performed using a 4.6 × 250-mm, 10-μm C18 column (Synergi Hydro-RP, Phenomenex, Torrance, CA), and effluent was monitored using a UV absorbance diode array detector set at 450 nm (bilirubin and glucuronide) and 254 nm (internal standard). Mobile phase run at 1 ml/min consisted of 0.1% trifluoroacetic acid with 10% acetonitrile in water initially, with a linear gradient to 100% acetonitrile over 20 min, and held at 100% acetonitrile for a further 10 min before returning to initial conditions over 5 min. Internal standard, bilirubin glucuronide, and bilirubin eluted at 8.15, and 28 min, respectively. Glucuronide peak identity was confirmed by HPLC-mass spectroscopy (ThermoFinnigan DecaXP-Plus, Thermo Electron, Somerset, NJ) and sensitivity to treatment with β-glucuronidase and alkaline pH adjustment. Because bilirubin glucuronide was not available for use as a standard, quantitation was achieved by use of a standard curve generated using known bilirubin concentrations assuming similar UV absorbance of substrate and glucuronide. Results were expressed as nanomole equivalents of bilirubin glucuronide formed per minute per milligram microsomal protein. Preliminary studies confirmed linear metabolite formation for up to 30 min and 0.35 mg/ml microsomal protein concentration. Both within and between-assay variations were less than 15%.

Haplotypes and Linkage Analyses. Haplotypes for UGT1A1 and UGT1A9 were determined using Phase v2.1 program (Stephens et al., 2001), and analysis was performed with Caucasian subjects only (n = 42). The linkage between the different polymorphisms was determined with the linkage disequilibrium (LD) plotter tool program found at https://innateimmunity.net/. All the statistical analyses were done using the JMP v4.0.2 program (SAS Institute, Cary, NC) and the SigmaStat program (v3.1; SPSS Inc., Chicago, IL). Correlation analyses between activity values were done using the Spearman’s test. R values higher than 0.50 and p values less than 0.05 were considered significant. One-way analysis of variance and a comparison for each pair using Student’s t test were used to determine the relationship between genotypes/diplotypes and protein levels or glucuronosyltransferase activities. Only diplotypes with more than three subjects were included in the analysis. The normal distribution of all the expression and activity values was evaluated with the Shapiro-Wilk W test (p > 0.05). Raw data that were not normally distributed were transformed with a logarithm function to achieve a normal distribution that is essential for parametric test. For all the analyses, a p value less than 0.05 was considered significant.

Results

To generate a comprehensive basis for investigating the role of UGT1A9 polymorphisms on SN-38 glucuronidation, we first extended the search for genetic polymorphisms to 5.5 kb of upstream sequence and included intronic sequences. We report two novel promoter single-nucleotide polymorphisms, −5366G>T and −4549T>C (Fig. 1), in strong LD with the previously reported functional variations −2152C>T (LD, r² = 1.0) and −275T>A (LD, r² = 0.65). All four polymorphisms are associated with significantly higher hepatic UGT1A9 protein expression in heterozygous subjects (1.6-fold) (Table 2). The previously reported functional UGT1A9 −118T>C variant (Yamanaka et al., 2004) was also observed at a frequency of 0.4. Intronic variants recently identified at positions I152A, I219A, and I313C (Carlini et al., 2005) were found in the population tested at an allelic frequency between 0.13 and 0.4, whereas the I143T and I201C were not observed. A novel UGT1A9 intronic variant at position I399C>T was found at a frequency of 0.44.

Correlation analyses were then performed with enzymatic activities, UGT protein content in livers, and UGT1A9 polymorphisms. Compared with individuals with the UGT1A9 I399C/C genotype, subjects with the UGT1A9 I399T/T genotype had a significant increase in SN-38G formation (p < 0.05) (Fig. 2A). In addition, UGT1A1 and UGT1A9 hepatic protein content was elevated in this group of subjects (p < 0.05) (Fig. 2B and C). This elevation in UGT protein content was reflected at the level of glucuronidation activities. Glucuronidation of UGT1A1 substrates, namely, bilirubin and estradiol-3, as well as UGT1A9 substrates MPA and propofol (Fig. 2D), were significantly increased in subjects with the UGT1A9 I399T allele (p < 0.05) (Table 2).

In the population tested, the intronic UGT1A9 I399T variant was not linked to the UGT1A1 C53, −3156, and −3279 variants (p² <
**TABLE 2**

*UGT1A9 and UGT1A9 protein expression and glucuronosyltransferase activities in human livers genotyped for functional polymorphisms*

Data are expressed as mean ± S.D.

<table>
<thead>
<tr>
<th>Functional Polymorphisms</th>
<th>Number of Subjects</th>
<th>UGT Activities</th>
<th>UGT Protein Content</th>
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<td></td>
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<td>SN-38G</td>
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<td>pmol/min/mg</td>
<td>pmol/min/mg</td>
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<td>63.2 ± 43.4</td>
<td>0.79 ± 0.46</td>
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<td>36.6 ± 42.9*</td>
<td>0.47 ± 0.68*</td>
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<td>36.6 ± 42.9*</td>
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<tr>
<td>p value*</td>
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* Significantly different (Student’s t test) from the corresponding homozygote reference group (p < 0.05).
1 Significantly different (Student’s t test) from the corresponding homozygote mutant group (p < 0.05). All other genotype-phenotype relationships were nonsignificant (p > 0.05).
2 Analysis of variance.
3 Student’s t test.
4 Alleles with −2152T also included polymorphisms at positions −275T>A, −4549T>C, and −5366G>T.
5 Alleles with I399 also included polymorphisms at position I219 and I313.

![Fig. 2. Haplotype structure of UGT1A9 determined with the Phase program in Caucasian subjects only (n = 42). Haplotypes with at least one genotype with less than 90% confidence were excluded. * represents previously unreported polymorphisms.](https://example.com/fig2)

![Fig. 3.](https://example.com/fig3)

In addition, a very low degree of LD exists between **UGT1A9 I399T** and all the other UGT1A9 functional variants (r² < 0.18) in the subjects tested (Fig. 4). When restricted to subjects with the **UGT1A1 −53(TA),906** genotype, individuals with the **UGT1A9 I399T** genotype presented 1.7-fold higher UGT1A1 protein content and 2.5-fold higher SN-38 glucuronidation activity compared with UGT1A9 I399C/C carriers (p < 0.05) (Fig. 4). Because of the limited sample size, we could not verify the effect of the **UGT1A9 I399** genotype within homozygous subjects for the **UGT1A1 −53(TA),77** genotype. Similar results were observed for bilirubin and estradiol glucuronide levels (1.8-fold and 2.0-fold; p < 0.05; data not shown). These observations suggest that the **UGT1A9 I399T** variant may be functional or linked to an unknown functional variant in the **UGT1** gene affecting SN-38 glucuronidation levels.

**The UGT1A9 −118T** variant was also significantly associated with higher rates of SN-38G formation (p = 0.05) (Table 2 and Fig. 5A). Despite the fact that this polymorphism was not significantly associated with variation of hepatic UGT1A9 protein content (p > 0.05), homozygous **−118T**, individuals showed significantly higher levels of UGT1A1 protein compared with individuals **−118T** (p < 0.05) (Fig. 5C). Consistent with this observation, the **−118T** variant was associated with higher glucuronidation of UGT1A1 substrates estradiol and bilirubin (p < 0.05) (Table 2). Conversely, the **UGT1A9 −118T** was not associated with significant alteration of the glucuronidation of UGT1A9-specific substrates.
(propofol and MPA) (Girard et al., 2004), suggesting an indirect effect of this variation on UGT1A1 expression and UGT1A1-mediated glucuronidation activity. To further evaluate this hypothesis, HepG2 cells derived from human liver cells were transfected with various constructs of the UGT1A9 promoter containing 9 or 10 T repeats at position −118. Results revealed a modest functional impact of 1.4-fold higher expression associated with the −118T10 compared with the −118T9 (p = 0.004) (Fig. 5D). This modest increase may explain the lack of significant alteration of the UGT1A9 protein content and UGT1A9-mediated glucuronosyltransferase activities in liver microsomes.

In the population studied and when restricted to Caucasian subjects (n = 42), a low degree of LD was observed between the UGT1A9 I399C>T and the −118T10/9 variant (r² = 0.19). In contrast, the UGT1A9 −118T10 was found to be linked to the −3279G variant of UGT1A1 (r² = 0.40) (Fig. 3). This linkage may explain part of the effect of the UGT1A9 −118T10 allele on UGT1A1 protein and activity levels. Of interest, LD was relatively low between UGT1A1 −53 and UGT1A9 −118T (r² = 0.26).

Figure 6 shows the results of UGT1A9 diplotype analyses, restricted to polymorphisms significantly associated with altered protein expression or glucuronosyltransferase activities (−5366, −4549, −2152, −275, −118, and I399). The haplotype UGT1A9_I (GTCT9C) is the most frequent (0.46) and corresponds to the reference UGT1 sequence (AF297093) (Fig. 1). Haplotype UGT1A9_II (GTCT10T), with the variant −118T10 and I399T, is the second most frequent haplotype (0.33). Haplotype UGT1A9_III (GTCT9T), characterized I399T, is also common in the Caucasian population (0.13). Three other haplotypes (UGT1A9_IV, V, and VI) were found but at relatively low frequencies (0.05, 0.03, and 0.01). There was no significant association between UGT1A9 diplotypes and UGT1A9 hepatic protein content (Fig. 6A). However, compared with subjects with the UGT1A9_I × UGT1A9_I reference diplotype, subjects with the UGT1A9_II × UGT1A9_III diplotype presented higher SN-38 glucuronidation activities (p < 0.05) (Fig. 6B). The impact of the
haplotypes characterized by the presence of the $-5366T$, $-4549C$, $-2152T$, and $-275A$ polymorphisms could not be assessed because of their low frequencies.

We then evaluated the impact of the $UGT1A1$ diplotypes (Fig. 7). First, we observed that the $UGT1A1$ variants $-53 (UGT1A1*28, TA_6), -3156G>A$, and $-3279T>G$ were individually associated with lower $UGT1A1$ protein content and decreased glucuronidation activities for SN-38 and for $UGT1A1$ probe substrates bilirubin and estradiol (Table 2). Compared with diplotype $UGT1A1_I$ (TG6) × $UGT1A1_I$, a significant association of diplotype $UGT1A1_{II}$ (GA7) × $UGT1A1_{II}$ with $UGT1A1$ protein content and SN-38 glucuronidation was shown, consistent with previous reports ($p < 0.05$) (Fig. 7, B and C) (Innocenti et al., 2004). This relationship was also significant for bilirubin and estradiol (data not shown). A gene-dosage effect could be appreciated for $UGT1A1$ protein content and SN-38G with an intermediate level of protein or glucuronide for livers with diplotype $UGT1A1_{I} × UGT1A1_{II}$.

Finally, although our samples size was limited, these results led us to explore the haplotype structure of $UGT1A1$ and $UGT1A9$ variants in the Caucasian subjects tested. Ten unambiguous $UGT1A1-1A9$ haplotypes were inferred. Livers with diplotype $UGT1A1-1A9_{II}$ (GTCTCGA7) × $UGT1A1-1A9_{II}$ had a lower, but not significant, $UGT1A1$ protein level and significantly lower glucuronidation activities for SN-38 ($p < 0.05$) (Fig. 8), bilirubin, and estradiol ($p < 0.05$) (data not shown), with intermediate levels for livers with diplotype $UGT1A1-1A9_{I} × UGT1A1-1A9_{II}$. No significant association of diplotypes with $UGT1A9$ protein content was detected.

**Discussion**

Findings show that the $UGT1A9$ $I399$ and $-118$ polymorphisms have an influence on the hepatic glucuronidation of SN-38 in vitro and that their effects are likely indirect or through their linkage to unknown functional $UGT1$ variant(s).

The presence of a previously unreported variation in the intron 1 of $UGT1A9$ ($I399C>T$) is highly predictive of SN-38 glucuronidation rates by human liver microsomes. In fact, this variant was associated with the most significant and most dramatic increase in SN-38G activity (2.64-fold; $p = 0.0007$) compared with all the other genotypes, including those of the $UGT1A1$ promoter. The presence of the $UGT1A9$ $I399T$ allele is also highly predictive of $UGT1A1$ protein content in the liver, rates of glucuronidation of specific probe substrates of the $UGT1A1$ protein, in addition to $UGT1A9$ protein expression and $UGT1A9$-mediated activities. Furthermore, that would
indicate that this variation might be an independent predictor of SN-38 glucuronidation because within subjects with UGT1A1 
-53(TA)_{66} genotype, those with the UGT1A9 -118T_{10} allele presented significantly higher levels of UGT1A1 protein and glucuronidation activities of UGT1A1-specific substrates, sustaining the hypothesis of an indirect effect.

In addition, results of in vitro gene reporter assays in liver cells support a limited impact of the UGT1A9 -118T_{10} variation on transcriptional gene activity (1.4-fold increase; \( p = 0.004 \)) that may not be sufficient to influence levels of UGT1A9 protein in the liver. This is in contrast with a previous study reporting a 2.6-fold greater transcriptional activity associated with a UGT1A9 -118T_{10} promoter construct compared with -118T_{9} in HepG2 liver cells. These inconsistencies between studies are somehow difficult to explain because the transfected cell line and the UGT1A9 promoter constructs were identical in both studies. Therefore, with the aim to gain further information from this in vitro approach, we subcloned a slightly different promoter region including the UGT1A9 -118 variation and obtained identical results with a 1.4-fold elevation of the reporter gene expression associated with the -118T_{9} allele. These in vitro observations are in agreement with our previous observation of the lack of association of the presence of the UGT1A9 -118T_{10} allele with UGT1A9 protein content in liver microsomes (Girard et al., 2004).

When evaluating combined UGT1A1/UGT1A9 haplotypes, we observed a trend toward lower formation of SN-38G in livers with the diplotype I/III (9/10 at -118) compared with those with I/I (10/10 at -118), whereas Innocenti et al. (2005) observed higher, but not significant,
Significant results are shown. The horizontal bars indicate mean values. Only diplotypes having \( n \geq 3 \) measurements are reported. Significant results are shown.

SN-3G formation in livers with the UGT1A1/IA9 haplotype (diplotype I/III) compared with those with diplotype I/I. In both studies, the sample size was rather limited to draw definitive conclusions. Nevertheless, overall in vitro data would point to a limited involvement of this variant in the control of UGT1A9 gene expression and raise the possibility of an effect on SN-38 glucuronidation through linkage with UGT1A1 variants or to unknown polymorphism(s). However, this deserves to be verified because the experimental data do not currently support this hypothesis.

A lack of LD between UGT1A1 \(-53(TA)_{n}\) and UGT1A9 \(-118T_{n}\) (\( r^2 = 0.26 \)) and with the UGT1A1 I999 (\( r^2 = 0.05 \)) was observed in Caucasians. This is consistent with results of a recent study by Innocenti et al. (2005). In this study, the LD between the UGT1A1 \(-3279\) and UGT1A9 \(-118T_{n}\) was higher (\( r^2 = 0.39 \)). When considering additional variants such as the UGT1A9 \(-331/-440\), a strong LD (\( r^2 = 0.79 \)) is observed between UGT1A9 and UGT1A1 \([-53(TA)_{n}]\). This indicates that linkage among the common UGT1 variants extends from UGT1A1 through to at least UGT1A9, corresponding to a genetic distance of about 88 kb. A significant correlation between expression of both proteins in the liver was observed (\( R_s = 0.48; \ p = 0.0006 \)). We also observed that among the common UGT1A1/IA9 haplotypes, polymorphisms associated with lower levels of UGT1A1 and UGT1A9 proteins appear on the same haplotype, indicating that subjects with low levels of UGT1A9 are also susceptible to lower expression of UGT1A1.

Clearly, our genotyping results in limited human livers are exploratory in nature; therefore, our results should be interpreted with caution while awaiting replication and further investigation through clinical study. Nevertheless, this study indicates that the novel intronic UGT1A9 I999 and the \(-118T\) promoter variation may represent additional candidates in combination with UGT1A1 promoter haplotypes to predict SN-38 glucuronidation profile in vivo.

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**References**


Address correspondence to: Chantal Guillemette, Canada Research Chair in Pharmacogenomics, Laboratory of Pharmacogenomics, CHUL Research Center, T3-48, 2705 Bou!, Laurier, Quebec, QC, Canada, G1V 4G2. E-mail: chantal.guillemette@crchul.ulaval.ca.