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Identification and Characterization of a Functional TATA Box Polymorphism of the *UDP Glucuronosyltransferase 1A7* Gene

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ABSTRACT

UDP glucuronosyltransferases (UGT) detoxify bilirubin and therapeutic drugs, a process influenced by single nucleotide polymorphisms (SNPs) in their structural genes and promoter elements. UGT1A1*28 is a functional UGT promoter polymorphism associated with Gilbert's disease and severe irinotecan toxicity, which also occurs in the absence of UGT1A1*28. The aim of this study was to identify and characterize UGT promoter variants relevant for irinotecan detoxification. Recombinant UGT1A proteins were analyzed for irinotecan metabolite glucuronidation by UGT activity assays. In 427 healthy blood donors and 71 homozygous UGT1A1*28 carriers, the 5′-untranslated region of the *UGT1A7* gene locus was studied. An SNP was detected by allelic discrimination and characterized by reporter gene experiments. A novel −57 T→G SNP with a gene frequency of 0.39 in healthy blood donors was identified in the putative TATA box of the *UGT1A7* gene, reducing promoter activity to 30%. It is in linkage dysequilibrium with a variant of the UGT1A7 first exon that is present in the reduced-activity UGT1A7*3 and UGT1A7*4 alleles. Homozygous UGT1A1*28 carriers simultaneously carried this variant in 97%. We identified a novel reduced-function TATA box SNP of the *UGT1A7* gene that catalyzes irinotecan metabolite detoxification. Its association with variants of the *UGT1A1* promoter and *UGT1A7* gene may influence irinotecan metabolism. Our finding emphasizes the importance of combinations of structural and regulatory gene polymorphisms that may be useful as markers of drug toxicity.

The UDP-glucuronosyltransferase family of enzymes is a central metabolic system for the glucuronidation of hydrophobic endobiotic and xenobiotic compounds (Tukey and Strassburg, 2000). Glucuronidation leads to the formation of water-soluble metabolites for an array of compounds, including steroid hormones, bilirubin, and bile acids, as well as a vast array of therapeutic drugs, and environmental organic substances, including known human mutagens (Tukey and Strassburg, 2001). Among the most relevant drugs that undergo glucuronidation are morphine (Coffman et al., 1997), acetaminophen (Bock et al., 1993), chloramphenicol (de Wildt et al., 1999), transplant immunosuppressants such as cyclosporine A and tacrolimus (Strassburg et al., 2001), but also the widely used antitumor drug metabolite of irinotecan SN-38 (Haaz et al., 1997; Ciotti et al., 1999). Alterations of glucuronidation activities in the individual are a mechanism by which interindividual profiles of drug metabolism are believed to impact drug efficacy, drug side effects, and the predisposition toward environmental mutagen-associated diseases such as cancer (Tukey and Strassburg, 2001; Tukey et al., 2002).

The human UGT1A proteins have been implicated as risk factors for both the development of cancer and unwanted drug side effects. This risk is determined by three differing features of the *UGT1A* gene locus. First, the *UGT1A* gene locus is regulated and expressed in a tissue-specific fashion, encompassing the hepatic isoforms UGT1A1, UGT1A3, UGT1A4, UGT1A6, and UGT1A9 (Strassburg et al., 1997, 1998, 1999). In extrahepatic tissues such as mouth, esophagus, intestine, pancreas, and colon, nonhepatic enzymes (UGT1A7, UGT1A8, and UGT1A10) have been detected, conferring a tissue-specific profile of glucuronidation to each organ that has been characterized by the analysis of tissue microsomes (Strassburg et al., 1997, 1999; Ockenga et al., 2002; Vogel et al., 2002). Second, the analysis of different

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ABBREVIATIONS: UGT, uridinediphosphate-5′-glucuronosyltransferase; SN-38, 7-ethyl-10-hydroxycamptothecin; HEK, human embryonic kidney; SNP, single nucleotide polymorphism; PCR, polymerase chain reaction; bp, base pair.

tissues in the human gastrointestinal tract has shown that *UGT1A* and *UGT2B* genes are regulated in a polymorphic interindividual fashion, leading to differing steady-state levels of UGT mRNA, protein, and enzymatic glucuronidation activity (Strassburg et al., 1998, 2000). The molecular basis of this feature is presently not completely understood. Third, an increasing number of single nucleotide polymorphisms (SNP) has been identified for all known UGT1A isoforms leading to catalytically altered UGT1A protein variants (Bosma et al., 1993; Ciotti et al., 1997; Guillemette et al., 2000b; Strassburg et al., 2002; Burchell, 2003). Together, this opens the possibility for a considerable number of combinations that represent the biochemical basis of highly interindividual profiles of glucuronidation conserved during evolution (Ehmer et al., 2004). These SNPs mostly lie within the coding regions of the *UGT1A* gene domains, and only one SNP within the promoter region has been identified to date. UGT1A1*28 is characterized by the insertion of a TA into the $A(TA)_6TAA$ element leading to $A(TA)_7TAA$ and a reduction of promoter activity to 30% (Bosma et al., 1995; Monaghan et al., 1996). This SNP is the genetic basis of Gilbert-Meulengracht's disease, leading to unconjugated nonhemolytic hyperbilirubinemia because UGT1A1 is the only efficient metabolic pathway for the elimination of bilirubin from the human body (Strassburg and Manns, 2000). In addition, other variants such as $A(TA)_5TAA$ and $A(TA)_8TAA$ have been described in ethnically distinct populations (Strassburg and Manns, 2000). However, apart from forming the genetic basis of this uncomplicated hepatic disease, UGT1A1*28 carrier status has been linked to the susceptibility toward breast cancer (Guillemette et al., 2000a) and the risk of unwanted intestinal side effects, as well as myelotoxicity in patients with colorectal cancer treated with irinotecan (Ando et al., 1998; Gagne et al., 2002). Irinotecan and its active metabolite SN-38, which has a 100- to 1000-fold higher activity than irinotecan, undergo glucuronidation by UGT1A1 and other UGTs (Ciotti et al., 1999). However, the UGT1A1*28 polymorphism is not capable of explaining all cases of irinotecan-associated toxicity, indicating the existence of additional markers (Innocenti et al., 2004).

In the present study, we identified a functional polymorphism of the promoter region of the human gastrointestinal UGT1A7 and provide evidence for an association with exon SNPs as well as the UGT1A1*28 promoter polymorphism, implicating a possible role for irinotecan toxicity in humans.

Materials and Methods

Determination of Gilbert-Meulengracht Disease. Blood samples were collected from patients worked up for the presence of Gilbert-Meulengracht's disease at the Department of Gastroenterology, Hepatology and Endocrinology of Hannover Medical School (Hannover, Germany). In 200 patients (aged 0.4–71.3 years; average, 17.2 years; 120 male, 80 female) with suspected Gilbert-Meulengracht's disease, genotyping of the UGT1A1*28 promoter polymorphisms was performed using PCR, direct sequencing, and temperature-gradient electrophoresis as described previously (Strassburg et al., 2002).

Healthy Blood Donors. Blood samples were obtained from 427 healthy blood donors from the Department of Transfusion medicine/Blood Bank of Hannover Medical School.

Genomic DNA. Genomic DNA was isolated from full blood samples by the NucleoSpin Blood XL Kit according to the recommendations of the manufacturer (Machery and Nagel, Dueren, Germany).

PCR Analysis. The UGT1A7 promoter sequence was amplified by PCR. The forward primer (5′-GTACACGCCTTCTTTTGAGGGCAG-3′) was located from base pair (bp) −103 to −80 upstream of the ATG start codon (Genbank accession number U39570), whereas the reverse primer (5′-TGCACTTCGCAATGGTGCCGTCCA-3′) was located from bp +292 to +315 downstream of the ATG start codon. Sequencing of both primer regions excluded allelic variants potentially affecting primer binding. The 371-bp product was amplified using the following protocol: hot start at 94°C for 5 min, followed by 32 cycles of 94°C for 30 s, 63°C for 30 s, and a final 7-min elongation reaction at 72°C. PCR was run on a GeneAmp PCR 2400 system (PerkinElmer Life and Analytical Sciences, Juegesheim, Germany).

Sequence Analysis. Sequence PCR was performed using the Dye Terminator Cycle Sequencing Kit 1.1 (Applied Biosystems, Darmstadt, Germany). The nucleotide sequences were determined on an ABI 310 automated sequencer (Applied Biosystems).

Allelic Discrimination Genotyping. Approximately 10 ng of genomic DNA was used as a template in Taqman 5′-nuclease assays. Primers and probes specific for each SNP were designed with Primer Express software (Applied Biosystems) and labeled with either 6-FAM or VIC as reporter dyes and MGB-NFQ (Applied Biosystems) as quencher (Table 1). The Taqman assays were performed using 600 nM primer concentrations and 200 nM probe concentrations (Applied Biosystems) and qPCR Mastermix Plus (Eurogentec, Seraing, Belgium). The run consisted of a hot start at 95°C for 10 min and 35 cycles of 94°C for 15 s and 61°C for 1 min. All assays were performed in 25-μl reactions in 96-well trays using an ABI 7000 instrument (Applied Biosystems).

Construction of UGT1A7 Luciferase Reporter Gene Vectors. A 251-bp DNA fragment was amplified by PCR from two healthy blood donors exhibiting the −57 T and the −57 G variants.

TABLE 1
Primers for luciferase constructs

Primer	Primer Sequence
UGT1A7 −57 C	
Forward	5′-CTATCTGTACTTCTTCCACCTACTATATTATAGGAG-3′
Reverse	5′-CTCCTATAATATAGTAGGTGGAAGAAGTACAGATAG-3′
UGT1A7 −57 A	
Forward	5′-CTATCTGTACTTCTTCCACATACTATATTATAGGAG-3′
Reverse	5′-CTCCTATAATATAGTATGTGGAAGAAGTACAGATAG-3′
UGT1A7 −57 del upstream	
Forward	5′-GAGGGCAGGTTCTATCTGTACTTCTTACTATATTATAGGAGCTTAG-3′
Reverse	5′-CTAAGCTCCTATAATATAGTAAGAAGTACAGATAGAACCTGCCCT C-3′
UGT1A7 −57 del downstream	
Forward	5′-GTTCTATCTGTACTTCTTCCACTGGAGCTTAGAATCCCAGCTGCTGGC-3′
Reverse	5′-GCCAGCAGCTGGGATTCTAAGCTCCAGTGGAAGAAGTACAGATAGAAC-3′
UGT1A7 −57 del TATA box	
Forward	5′-GGGCAGGTGCTATCTGTACTTCTTCCGGAGCTTAGAATCCCAGCTGC-3′
Reverse	5′-GCAGCTGGGATTCTAAGCTCCGGAAGAAGTACAGATAGAACCTGCCC-3′

Positional cloning was performed using primers containing an XhoI (5′-ACCGCTCGAGCAGAGAACTTCAGCCCAGAGCC-3′, located between −22 and −1 upstream of the UGT1A7 coding region) and a KpnI (5′-GGAGGTACCAGGGCATGATCTGTCCCCAAGG-3′, located between −251 and −228 upstream of the UGT1A7 coding region) restriction enzyme site, and the fragments were inserted into the pGL3 basic vector using the fast-link ligation kit (MBI Fermentas, St. Leon-Rot, Germany). Mutagenesis of the putative box element was performed using specific primers (Table 2) and the QuikChange Site-Directed Mutagenesis Kit (Stratagene, La Jolla, CA). The sequences of all inserts were confirmed by DNA sequencing using the pGL primer 2 rev (5′-CTTTATGTTTTTGGCGTCTTCC-3′).

Luciferase Assays. Human embryonic kidney (HEK) 293 cells, Hepatoma (Hep) G2 cells, human gastric cancer (Kato III), and alveolar epithelial cells (A549) cells were transfected with the pGL3 UGT1A7-TATA box vectors in addition to PhRL-TK plasmid (Dual-Reporter Assay; Promega, Mannheim, Germany) using Lipofectin (Invitrogen, Karslruhe, Germany) following the manufacturer's instructions before harvesting after 72 h. Luciferase was detected using the Lumat LB 9507 (Berthold Technologies, Bad Wildbad, Germany) and normalized relative to *Renilla reniformis* luciferase. The pGL3-basic plasmid served as control in each separate experiment.

Catalytic Glucuronidation Assay. UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, and UGT1A10 were transiently transfected into HEK293 cells, and cells were harvested after 72 h and used as recombinant protein for UGT catalytic activity assays as described previously in detail (Strassburg et al., 1999, 2000). Protein amounts were normalized by Western blot using a previously described rabbit anti-human UGT1A antibody directed against exon 2 (Strassburg et al., 1998). SN-38 (5 mM) (Aventis, Strasbourg, France) was used for each experiment.

Results

Characterization of the UGT1A7 5′-Untranslated Sequence. On the basis of the genomic DNA sequence deposited in GenBank (accession number AF297093) (Gong et al., 2001), primers were designed for the amplification of 315 bp upstream of the ATG start codon of the UGT1A7 exon 1 sequence (Fig. 1A). The analysis of the obtained sequence suggests that a TATA box for polymerase binding is located between base pairs −59 and −44 from the ATG codon, which is in agreement with the structure of other 5′-untranslated regions at the *UGT1A* gene locus (Gong et al., 2001; Tukey and Strassburg, 2001). The analysis of 427 genomic DNA samples from healthy blood donors identified a single nucleotide transversion from T to G at position −57. The homozygous T (−57 T/T) was detected in 160 (37%) persons, a heterozygous T (−57 T/G) was present in 203 (48%), and the homozygous G (−57 G/G) was identified in 64 (14%) samples (Table 3). From these findings, −57 T seems to represent the most prevalent ("wild-type") sequence in our cohort with a gene frequency of 0.61 characterized by a single nucleotide polymorphism with a gene frequency of 0.39. Sequence analysis further indicates that this polymorphism affects the TATA box region of the *UGT1A7* gene.

TABLE 2
Primers for Taqman analysis of UGT1A7 single-nucleotide polymorphisms

UGT1A7 $N^{129}K$ $R^{131}K$	
Forward primer	5′-CACCATTGCGAAGTGCATTT-3′
Reverse primer	5′-AGG ATC GAG AAA CAC TGC ATC A-3′
Probe wild-type	6-FAM-TAATGACCGAAAATT-MGB
Probe homozygous	VIC-TTTAAGGACAAAAAATTAGT-MGB
UGT1A7 $W^{208}R$	
Forward primer	5′-CCAGACTTCTCTTAGGGTTCTCAGAC-3′
Reverse primer	5′-AGACATTTTTGAAAAAATAGGGGCA-3′
Probe wild-type	6-FAM-AGGAGAGAGTATGGAAC-MGB
Probe homozygous	VIC-AGGAGAGAGTACGGAAC-MGB
UGT1A7 −57 T→G	
Forward primer	5′-TTTTGAGGGCAGGTTCTATCTGTA-3′
Reverse primer	5′-GCAGCTGGGATTCTAAGCTCCTA-3′
Probe wild-type	6-FAM-CTTCTTCCACTTACTATATT-MGB
Probe homozygous	VIC-TCTTCCACGTACTATATTA-MGB

Association of the −57 T→G Polymorphism with UGT1A7 Exon 1 Polymorphisms. Previous analyses have identified five base pair exchanges at positions 11, 129, 131, and 208 in the first exon of UGT1A7, leading to functionally altered UGT1A7 protein variants designated UGT1A7*1 (wild-type), UGT1A7*2, UGT1A7*3, and UGT1A7*4 (Guillemette et al., 2000b; Zheng et al., 2001; Strassburg et al., 2002). Studies from different laboratories have found that SNPs at positions 129 and 131 as well as at 11 and 208 seem to be in linkage dysequilibrium and always occur in combination (Guillemette et al., 2000b; Strassburg et al., 2002). We therefore studied whether the 5′-untranslated polymorphism at −57 bp was associated with the functional SNPs located within exon 1. Taqman allelic discrimination PCR analysis of 427 healthy blood donors was able to precisely discriminate 5′-untranslated and exon SNPs of the UGT1A7 gene (Fig. 1, B and C). The data show that −57 G was always present when Arg208 (T→C transition at codon 208) was detected and never found together with UGT1A7*1 (exon 1) sequence (Asn129, Arg131, and Trp208). The T→C exchange at codon 208 of the UGT1A7 first exon is present both in the UGT1A7*3 (Lys129/Lys131 and Arg208) and UGT1A7*4 (Asn129/Arg131, Trp208) genotypes (Table 3). The −57 T/G/SNP is therefore in linkage dysequilibrium with W208R and thus associated with the UGT1A7*3 and UGT1A7*4 genotypes, which also explains the coincidence of UGT1A7 −57 G with N129K/R131K (Table 3). The association of −57 G with the Lys129/Lys131 consequently reflects the frequency of the Lys129/Lys131, Arg208 genotype by virtue of its dependence on the presence of Arg208. As a consequence of this finding, −57 G is never present in UGT1A7*1 or UGT1A7*2 unless the patient is compound heterozygous (i.e., UGT1A7*1/*3 etc.) (Table 3). The data were found to be in Hardy-Weinberg equilibrium.

The Novel TATA Box Polymorphism of the UGT1A7 Promoter Is Functional. Both UGT1A7 −57 T and UGT1A7 −57 G promoter sequence carrying 5′-untranslated sequence fragments were functionally tested in luciferase reporter gene experiments. In six parallel experiments, the −57 T UGT1A7 putative TATA box construct exhibited 14-fold activation of luciferase expression over control (empty plasmid) in HEK293 cells (Fig. 2, A and B), confirming the presence of a promoter element in the −250 bp of the *UGT1A7* gene. In contrast, −57 G only showed a 4-fold luciferase expression, indicating a 70% reduction of promoter activity attributable to the T→G exchange. Analogous results were also seen in HepG2, Kato III, and A549 cells.

Additional mutation analyses revealed that a T→C transition and a T→A transversion showed reduced luciferase activity similar to that of −57 G (Fig. 2B). To characterize the putative TATA box deletion, experiments were performed. Deletion of upstream sequence did not change luciferase

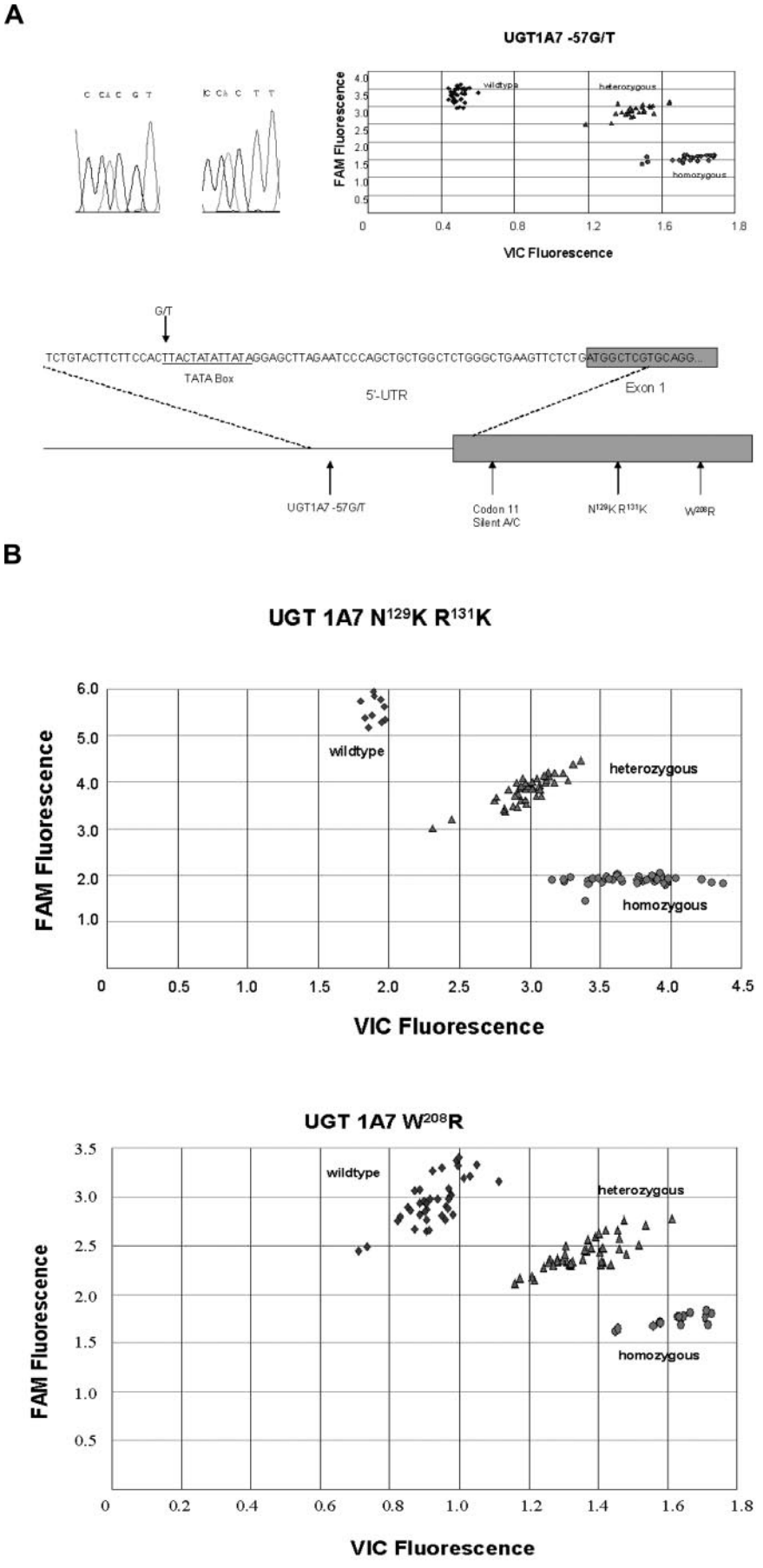

Fig. 1. Schematic representation of the UGT1A7 gene upstream sequence. A, a schematic indicating the localization of five exon polymorphisms and the 5′-untranslated polymorphism located at −57 bp upstream of the *UGT1A7* gene ATG codon. The top left shows fluorographs of the different variant sequences. The top right shows the results of allelic discrimination PCR analysis (Taqman) capable of discriminating putative TATA box variants. B, allelic discrimination of UGT1A7 exon 1 polymorphisms. Shown are typical examples of the allelic discrimination of the SNPs at codon 129/131 (top) and at codon 208 (bottom) of the *UGT1A7* gene by Taqman PCR. 5′-UTR, 5′untranslated region.

transcription, but the elimination of the downstream sequence and the TA-rich region abolished luciferase activity. This suggests the presence of a TATA box.

The Irinotecan Metabolite SN-38 Is a Substrate of the UGT1A1 and UGT1A7 Proteins. Recombinant UGT1A1, UGT1A3, UGT1A4, UGT1A6, UGT1A7, UGT1A9, and UGT1A10 were transfected and expressed in HEK293 cells for use in catalytic activity assays. The radiography of the activity assay in Fig. 3 demonstrates that UGT1A1, UGT1A6, UGT1A7, and UGT1A10 are identified as relevant isoforms for SN-38 glucuronidation. UGT1A7 showed the highest specific activity with SN-38, which was 5-fold higher than that found with UGT1A1, UGT1A6, and UGT1A10, confirming previous findings (Ciotti et al., 1999). UGT1A7 is therefore identified as a relevant SN-38 UGT capable of contributing to its toxicity.

TABLE 3

Association of 5′-untranslated and exon polymorphisms of the *UGT1A7* gene

Genotyping analyses by Taqman allelic discrimination PCR of the 5′-untranslated SNP, N129K/R131K, and W208R variants of the *UGT1A7* gene first exon indicate a linkage dysequilibrium of the 5′-untranslated located UGT1A7 −57 G SNP and the exon 1 located Arg208 SNP. Arg208 was always present when the UGT1A7 −57 G was detected. On the other hand, UGT1A7 −57 T was always simultaneously present with Trp208. A similar linkage dysequilibrium was not found for N129K/R131K and −57 T/G, although homozygous carriers of UGT1A7 −57 G were also homozygous for the UGT1A7 Lys129/Lys131 in all but one person.

UGT1A7 −57 T/G		N129K/R131K (Exon 1)			W208R (Exon 1)		
		Asn129/ Arg131	Asn129/Lys131/ Lys129/Lys131	Lys129/ Lys131	Trp208	Trp208/ Arg208	Trp208
UGT1A7 −57 T	160 (37%)	64 (40%)	75 (47%)	21 (13%)	160 (100%)	0	0
UGT1A7 −57 T/G	203 (48%)	0	118 (58%)	85 (42%)	0	203 (100%)	0
UGT1A7 −57 G	64 (15%)	0	1 (2%)	63 (98%)	0	0	64 (100%)

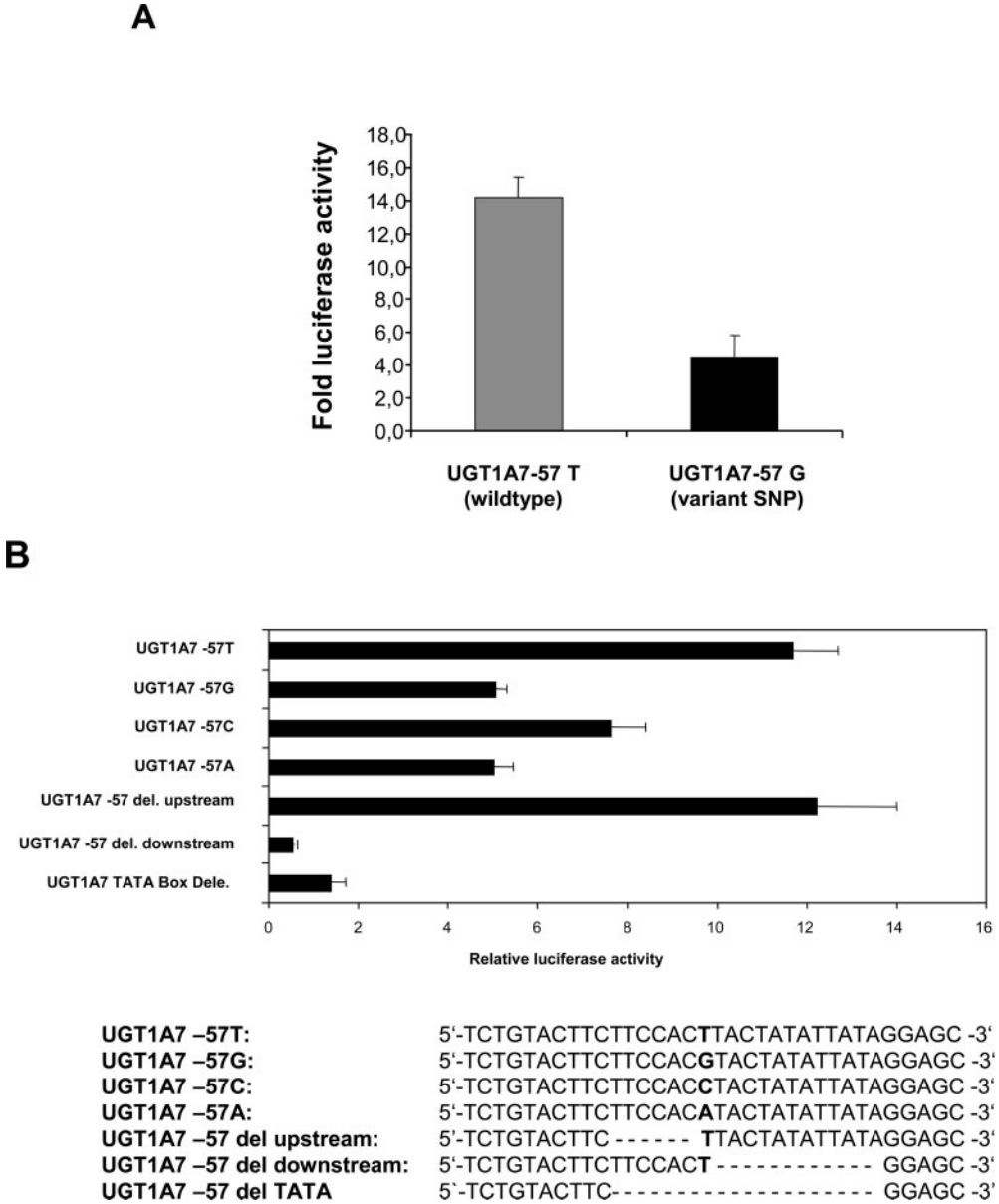

Fig. 2. Promoter activity by luciferase reporter gene analysis. A, graphic representation of six parallel and independent experiments characterizing the ability of UGT1A7 −57 T and UGT1A7 −57 G promoter sequence to drive luciferase expression in transiently transfected HEK293 cells. Luciferase expression is reduced to 30% in the UGT1A7 −57 G promoter sequence construct. Results are given as means; error bars indicate standard deviations, and all results are normalized for *R. reniformis* activity and are derived from experiments with empty vector as controls. B, mutational analysis of the putative TATA box element representing two individual and parallel experiments. A T→C transition or a T→A transversion leads to reduced luciferase activity comparable with UGT1A7 −57 G. Deletion of the upstream sequence retains luciferase activity, but deletion of the putative TATA box or downstream sequence eliminates luciferase transcription. This suggests the presence of an active TATA box element.

Association of the UGT1A7 and the UGT1A1*28 Promoter Polymorphisms. To elucidate an association of UGT1A1*28 as a known risk factor for SN-38 toxicity and then UGT1A7 5′-untranslated variant, a cohort of 200 DNA samples from patients who were genotyped previously for the presence of Gilbert-Meulengracht's disease was analyzed (Table 4). In this cohort, sequencing and temperature-gradient gel electrophoresis identified 71 patients who were homozygous for UGT1A1*28, 65 patients heterozygous for UGT1A1*28, and 64 patients with the UGT1A1*1 promoter. Of the 71 patients who were homozygous for UGT1A1*28, only two displayed UGT1A7 −57 T. One allele of UGT1A7 −57 G was therefore present in 97% of subjects. On the other hand, persons with a UGT1A1*1 promoter had a UGT1A7 −57 T promoter variant in 73%. These data provide evidence for an association of the Gilbert-Meulengracht promoter UGT1A1*28 with the newly identified functional UGT1A7 promoter polymorphism. Both represent the only known examples of functional promoter polymorphisms at the human *UGT1A* gene locus. The high activity of UGT1A7 toward SN-38 may indicate a role of this finding for irinotecan efficacy and toxicity in anticancer therapy.

Discussion

The precise understanding of human hepatic and extrahepatic drug metabolism is essential for the prediction of disease susceptibility and the efficacy and toxicity of drug therapy (Tukey and Strassburg, 2000; Ehmer et al., 2004). In this study, we identify a functional polymorphism in the upstream region (TA-rich element) of the human *UGT1A7* gene, leading to a 70% reduction of promoter activity. Mutational analyses suggest that −57 T is part of a TATA box element. The identified T→G transversion at position −57 is the second TATA box polymorphism of a human UGT identified to date and provides evidence for yet another mechanism by which human organ-specific glucuronidation is modulated.

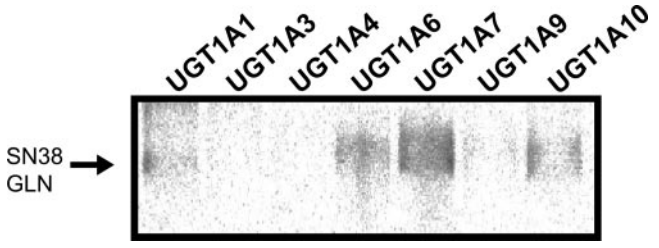

Fig. 3. UGT1A7 is the principal SN-38 UGT. Autoradiography of a catalytic UGT activity assay using recombinant UGT proteins transiently expressed in HEK293 cells and the irinotecan metabolite SN-38 as substrate. Specific UGT activity is strongest for UGT1A7, which is 5-fold higher than the other activities. Protein amounts were normalized by Western blot (data not shown). SN38 GLN, glucuronide of the irinotecan metabolite SN-38.

TABLE 4

Association of UGT1A1*28 promoter polymorphisms with the novel UGT1A7 promoter polymorphism

Genotyping of 200 patients referred for suspected Gilbert-Meulengracht's disease. Among these patients, 71 were homozygous for the UGT1A7*28 TATA box polymorphism of the *UGT1A1* bilirubin transferase gene. Taqman allelic discrimination PCR analysis of all 200 patients demonstrated that in persons homozygous for the UGT1A1*28 TATA box polymorphism, 73% carried the homozygous UGT1A7 −57 G promoter polymorphism, and only 2 (3%) had the wild-type UGT1A7 promoter, indicating that among patients with homozygous Gilbert-Meulengracht's disease, 97% carry the reduced activity UGT1A7 promoter polymorphism. However, UGT1A7 −57 T→G is also present in persons who carry a UGT1A1*1 promoter.

UGT1A1*28 ($A(TA)_7TAA$)	UGT1A7 −57 G/T		
	UGT1A7 −57 G	UGT1A7 −57 T/G	UGT1A7 −57 T
UGT1A1*28 (71)	53 (75%)	16 (22%)	2 (3%)
UGT1A1*28/*1 (65)	6 (9%)	50 (77%)	9 (14%)
UGT1A1*1 (64)	5 (8%)	12 (19%)	47 (73%)

In 1995, a TA insertion into the TATA box of the *UGT1A1* gene was identified, leading to the reduced function $A(TA)_7TAA$ promoter designated UGT1A1*28. This variant has been found to be associated with unconjugated nonhemolytic hyperbilirubinema in Gilbert-Meulengracht's disease (Bosma et al., 1995; Monaghan et al., 1996). In subsequent studies, UGT1A1*28 was linked to the susceptibility toward breast cancer (Guillemette et al., 2000a). Moreover, it was discovered that one of the principal anticancer therapeutics in colorectal cancer, irinotecan, undergoes glucuronidation which involves UGT1A1 as well as UGT1A6, UGT1A7, and UGT1A9 (Ciotti et al., 1999; Gagne et al., 2002). Therefore, the regulation of UGT1A1 as well as structural gene SNPs of the involved isoforms were hypothesized to influence the efficacy and gastrointestinal as well as myelotoxicity of irinotecan therapy. A number of studies have since associated the presence of UGT1A1*28 with irinotecan toxicity (Iyer et al., 1999; Gagne et al., 2002; Innocenti et al., 2004). In addition, the combination of UGT1A1*28 with other coding SNPs such as UGT1A6 were reported (Peters et al., 2003). However, these analyses have also shown that UGT1A1*28 is not sufficient to explain myelotoxicity and gastrointestinal toxicity in irinotecan-treated patients, which also occurs in carriers of the *UGT1A1*1* gene. A recent analysis of the human *UGT1A* gene locus has shown that at least 17 SNPs are detectable in the *UGT1A3*, *UGT1A4*, *UGT1A6*, *UGT1A7*, *UGT1A8*, *UGT1A9*, and *UGT1A10* genes (Ehmer et al., 2004) in addition to 36 variants known for UGT1A1 and the common exon 2 to 5 domains of the *UGT1A* genes (Strassburg and Manns, 2000). The combinations of these variants are likely to represent the genetic and biochemical basis for individual drug metabolism by glucuronidation. This study introduces a novel variant by identifying an SNP of the UGT1A7 promoter with an allelic frequency of 39% in healthy blood donors. The *UGT1A7* gene has been identified as a risk gene for cancer because of its ability to detoxify benzo(α)pyrene metabolites and heterocyclic amines (Strassburg et al., 1998) as well as the association of its SNP variants with gastrointestinal and hepatic cancer (Vogel et al., 2001; Zheng et al., 2001; Wang et al., 2004). It is expressed in the proximal gastrointestinal tract in the mouth, esophagus, stomach, and lung, in which it establishes contact with airborne, dietary, or pharmaceutical xenobiotic compounds, including the anticancer drug irinotecan and its metabolite SN-38. Recombinant protein experiments (Fig. 3) show that SN-38 undergoes significant glucuronidation by UGT1A7, which was found to be 5-fold higher than the catalytic rates detected with UGT1A1, UGT1A6, and UGT1A10. The UGT1A7 promoter variant will reduce this activity by 70%. To complicate the picture, the UGT1A7 variant is in linkage dysequilibrium with the W208R SNP of the UGT1A7 first exon, which is present in two UGT1A7 genotypes showing reduced catalytic activity: UGT1A7*3 (N129K/R131K and W208R) and UGT1A7*4 (W208R) (Guillemette et al., 2000b; Strassburg et al., 2002). Therefore, the presence of the promoter −57 T→G SNP leads to an even further reduction of UGT1A7 activity in its carriers. This finding illustrates that

the detection of structural gene SNPs in the *UGT1A7* gene and possibly also in other drug-metabolizing enzymes is not sufficient to predict the true extent of functional variability, which can also be influenced by the presence of SNPs altering the expression of the (wild-type or variant) gene product. In view of the known association of irinotecan toxicity with carriers of the UGT1A1*28 genotype, we expanded our analysis to patients who exhibit this genotype and found that among carriers of the UGT1A1*28 allele, 97% exhibited at least one allele of the UGT1A7 −57 T→G SNP and 75% were homozygous for both UGT1A1*28 and UGT1A7 −57 T→G. These data indicate that because of the high specific activity of UGT1A7 for the irinotecan metabolite SN-38 and the association of UGT1A1 and UGT1A7 reduced function promoter polymorphisms, the here-identified SNP may also play an important role for the understanding and prediction of irinotecan toxicity. At present, the frequency of −57 T→G in cancer patients alone or in combination with UGT1A1*28 is not known, and additional prospective studies will be required to elucidate its potential role as a risk factor for drug-associated toxicity. The findings of this study also indicate that search and characterization of SNPs located within the coding exons of drug-metabolizing enzymes alone is not sufficient to predict the true picture of altered drug metabolism. Carriers of the most prevalent (wild-type) alleles may have regulatory variants that determine their individual metabolic activities. In summary, drug metabolism is altered by 1) individual or combination of individual SNPs located within coding exons, 2) individual or combination of individual SNPs located in promoter elements, and 3) the combination of both. The individual prediction of metabolic predisposition will therefore only be achieved through the analysis of genetic variant patterns (Ehmer et al., 2004). To this end, relevant candidates such as the promoter SNP identified in this study need to be detected, characterized, and assembled into a complex network of determinants of human metabolism.

Beyond the issue of drug therapy, the novel promoter SNP offers an explanation for the previously found polymorphic gene regulation detectable for various UGT1A and UGT2B gene products in human stomach (Strassburg et al., 1998) and human small intestine (Strassburg et al., 2000). In these tissues, individual patterns of UGT mRNA and protein expression were detected. From our data, interindividual expression patterns detected at the mRNA steady-state level and at the protein level in the presence of normal function DNA variants may be the consequence of SNPs located in the 5′-untranslated regions of the respective genes. Further studies will be required to elucidate the impact of promoter SNPs on the inducibility of drug-metabolizing enzymes in different tissues to understand the interaction of genetic predisposition and environmental exposure on human metabolism.

In summary, we provide evidence for the second functional promoter polymorphism of a human phase II drug-metabolizing enzyme that may influence the clinical finding of irinotecan toxicity. A reduction of promoter activity to 30% of the UGT1A7 −57 T gene, in addition to the high number and frequency of coding exon SNPs at the human *UGT1A* gene locus (Ehmer et al., 2004) conserved during evolution, predicts that human glucuronidation is highly individual and requires the establishment of genetic pattern analysis for the prediction of disease predisposition and pharmacogenomically tailored drug therapy. Human glucuronidation can be altered even in the absence of detectable coding region variants of the respective *UGT* genes studied. The identified UGT1A7 −57 T/G TATA box SNP fills a gap in the understanding of UGT regulation and may contribute a useful, facile, and inexpensive tool for the prediction and identification of individual disposition.

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