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Functional characterization of human UDP-glucuronosyltransferase (UGT) 1A9 variant, D256N, found in Japanese cancer patients

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Abbreviations used are: UGT, UDP-glucuronosyltransferase; irinotecan, 7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin; SN-38, 7-ethyl-10-hydroxycamptothecin; SN-38G, 7-ethyl-10-hydroxycamptothecin 10-*O*-β-D-glucuronide.

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Abstract

SN-38 (7-ethyl-10-hydroxycamptothecin), an active metabolite of the antitumor prodrug irinotecan, is conjugated and detoxified to SN-38 10-*O*-β-D-glucuronide by hepatic UDP-glucuronosyltransferase (UGT) 1A1. Recent studies have revealed that other UGT1A isoforms, UGT1A7 and UGT1A9, also participate in SN-38 glucuronidation. Although several genetic polymorphisms are reported for UGT1A1 and UGT1A7 that affect the SN-38 glucuronidation activities, no such polymorphisms have been identified for UGT1A9. In the present study, *UGT1A9* exon 1 and its flanking regions were sequenced from 61 Japanese cancer patients who were all treated with irinotecan. A novel non-synonymous single nucleotide polymorphism was identified in *UGT1A9* exon 1; heterozygous 766G>A resulting in the amino acid substitution of D256N. The wild-type and D256N UGT1A9s were transiently expressed at similar protein levels in COS-1 cells, and their membrane fractions were characterized *in vitro* for the glucuronidation activities towards SN-38. The apparent K_m values were 19.3 and 44.4 μM, and the V_{max} values were 2.94 and 0.24 pmol/min/mg membrane protein for the wild-type and D256N variant, respectively. The SN-38 glucuronidation efficiency (normalized V_{max}/K_m) of D256N was less than 5% that of wild-type UGT1A9. These results clearly indicate that the D256N variant is essentially non-functional with regard to SN-38 glucuronidation. These findings highlight the importance of further studies into the potential influence of UGT1A9 D256N variant to irinotecan metabolism *in vivo*.

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Irinotecan (7-ethyl-10-[4-(1-piperidino)-1-piperidino]carbonyloxycamptothecin, Fig. 1) is an antitumor prodrug of a potent topoisomerase I inhibitor, SN-38 (7-ethyl-10-hydroxycamptothecin), which is now applied clinically with or without other chemotherapeutic agents such as 5-fluorouracil and cisplatin to a variety of malignant tumors including gastrointestinal and lung cancers (Rothenberg, 2001). Irinotecan is hydrolyzed by carboxylesterases in the human liver, intestinal mucosa and plasma to liberate SN-38 (Rivory et al., 1996; Ahmed et al., 1999; Kehrer et al., 2000), which undergoes subsequent glucuronidation by UDP-glucuronosyltransferases (UGTs) into an inactive 10-*O*-β-D-glucuronide (SN-38G, Fig. 1) and biliary excretion (Guputa et al., 1994; Iyer et al., 1998). Although the precise mechanism remains to be elucidated, the hepatic SN-38 glucuronidation capacity is thought to be an important determinant factor of severe diarrhea, the major dose-limiting side-effect of irinotecan (Ratain, 2002). Therefore, genetic polymorphisms in UGT1A could influence the incidence of the irinotecan-induced diarrhea.

The UGT1A family is known to include 9 functional isoforms (UGT1A1, UGT1A3 to UGT1A10). *UGT1A2P*, *UGT1A11P*, *UGT1A12P* and *UGT1A13P* are thought to be pseudogenes based on their frame-shift mutations. The UGT1A family members have common carboxyl terminal sequences (245 amino acids in length) derived from four exons (exons 2-5) located at the 3' end of the human *UGT1A* gene complex on chromosome 2q37 (Gong et al., 2001). Among these UGT1A isoforms, UGT1A1, UGT1A7 and UGT1A9 have been shown to catalyze the SN-38 glucuronidation *in vitro* (Ciotti et al., 1999; Hanioka et al., 2001; Gagné et al., 2002). For *UGT1A1* and *UGT1A7*, several genetic polymorphisms have

been reported that affect SN-38 glucuronidation activities. A TATA box variant of UGT1A1 (A(TA)6TAA>A(TA)7TAA) is associated with a reduced transcriptional activity, resulting in a corresponding decrease in the level of UGT1A1 protein and SN-38 glucuronidation activity (Iyer et al., 1998). In addition, non-synonymous variations in the coding region of *UGT1A1* (e.g. G71R and P229Q) and *UGT1A7* (e.g. W208R) have been shown to influence their SN-38 glucuronidation kinetics (Gagné et al. 2002, Jinno et al. 2003). In contrast, no such genetic polymorphism of *UGT1A9* has been reported to date.

In the present study, *UGT1A9* exon 1 and its flanking regions were sequenced from 61 irinitecan-treated Japanese cancer patients, and a novel non-synonymous single nucleotide polymorphism was identified in *UGT1A9* exon 1; 766G>A resulting in the amino acid substitution of D256N. The wild-type and D256N variant UGT1A9s were transiently expressed in COS-1 cells and functionally characterized by the glucuronidation of SN-38.

Materials and Methods

Materials

SN-38 (Lot 970507R) and SN-38G (Lot 970507R) were kindly supplied by Yakult Honsha Co. Ltd. (Tokyo, Japan). Human liver polyA$^+$ RNA was obtained form OriGene Technologies (Rockville, MD). COS-1 cells were obtained from the Health Science Research Resources Bank (Osaka, Japan).

Human genomic DNA samples

All of the 61 subjects in this study were Japanese cancer patients who were treated with irinotecan. The ethics committees of both the National Cancer Center and the National Institute of Health Sciences approved this study. Written informed consent was obtained from all participating subjects. DNA was extracted from blood leukocytes and used for DNA sequence analysis.

Polymerase chain reaction (PCR) conditions for DNA sequencing

Exon 1 of *UGT1A9* was amplified from genomic DNA (150 ng) using 2.5 units of Z-Taq (Takara, Kyoto, Japan) with 0.2 μM of the 1st amplification primers (Table 1). The first PCR conditions consisted of 30 cycles of 98°C for 5 sec, 55°C for 5 sec, and 72°C for 190 sec. The PCR products were amplified by Ex-Taq (0.625 units, Takara) with the 2nd amplification primers (0.2 μM) designed to the intron sequences (Table 1). The second round of PCR was

94°C for 5 min followed by 30 cycles of 94°C for 30 sec, 55°C for 1 min, and 72°C for 2 min. This two-step PCR confers the specific amplification of the *UGT1A9* exon 1. These PCR products were then purified using a PCR Product Pre-Sequencing Kit (USB Co., Cleveland, OH) and were directly sequenced on both strands using an ABI Big Dye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) with the primers shown in Table 1. The excess dye was removed by a DyeEx96 kit (Qiagen, Hilden, Germany). The eluates were analyzed on an ABI Prism 3700 DNA Analyzer (Applied Biosystems).

Construction of Plasmids

Oligo(dT) primed cDNA was synthesized from human liver polyA$^+$ RNA using a SuperScript first-strand synthesis system for RT-PCR (Invitrogen, Carlsbad, CA) according to the manufacture's instructions. The cDNA encoding wild-type UGT1A9 was amplified from the single-stranded stomach cDNA by the *att*B adaptor PCR, and cloned into pDONR201 vector by the Gateway cloning technology (Invitrogen). Briefly, the UGT1A9 cDNA was first amplified by the 10-cycle PCR using the gene specific primers containing the 12-bp partial *att*B sequences at the 5'-end (Table 1). Then an aliquot of the reaction mixture was subjected to the second 25-cycle PCR using the *att*B adaptor primers (Table 1). The resulting *att*B-flanked product was cloned into pDONR201 vector using the Gateway BP reaction, involving a recombination between the *att*B site and the *att*P site. Mutations were introduced into the wild-type UGT1A9 cDNA clone in pDONR201, using a QuikChange multi site-directed mutagenesis kit (Stratagene, La Jolla, CA) with the 5'-phosphorylated

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oligonucleotide primer (Table 1). To ensure that no errors had been introduced during the amplification process, all the plasmid constructs were verified by DNA sequencing of both strands. Subcloning of each UGT1A9 fragment from pDONR201 into pcDNA-DEST40 was performed by the Gateway LR reaction (a recombination between the *att*L site and the *att*R site).

Expression of wild-type and variant UGT1A9s in COS-1 cells

COS-1 cells were grown in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum. The day before transfection, COS-1 cells were plated in 100-mm culture dishes at a density of 5.5×10^4 cells/cm^2. On the following day, the culture medium was replaced with 8 ml of Opti-MEM (Invitrogen) and the expression plasmids were transfected using LipofectAMINE 2000 reagent (Invitrogen). The diluted DNA (14 μg in 810 μl of Opti-MEM) and the diluted LipofectAMINE 2000 reagent (48 μl in 810 μl of Opti-MEM) were combined, and incubated for 20 min at room temperature. The resulting DNA-LipofectAMINE 2000 complex was directly added to each dish.

Forty-eight hours after transfection, the COS-1 cells were washed twice with ice-cold phosphate-buffered saline and harvested in 0.25 M sucrose-5 mM Hepes, pH 7.4 (buffered-sucrose). The cell suspensions were sonicated three times with 10 sec bursts using an ultrasonic processor USP-300 (Shimadzu, Kyoto, Japan), followed by centrifugation at 105,000 *g* for 60 min at 4ºC. The resulting pellets were resuspended in buffered-sucrose and stored at -80ºC.

Western blotting

Twenty μg of the membrane fraction proteins from COS-1 cells were resolved by SDS-PAGE (10% gel) and electrophoretically transferred onto PVDF membranes. Immunochemical detection of each UGT1A9 protein was performed by chemifluorescence using rabbit anti-human UGT1A (diluted at 1:5000; Gentest, Woburn, MA) and donkey anti-rabbit Ig coupled to horseradish peroxidase (diluted at 1:2000). Chemifluorescence (ECL-plus, Amersham Biosciences, Piscataway, NJ) was detected and quantified using the Typhoon 9400 Variable Mode Imager and ImageQuant analysis software (Amersham Biosciences). To confirm that the samples were evenly loaded, the blot was subsequently stripped in a stripping buffer (2% SDS, 100 mM 2-mercaptoethanol and 62.5 mM Tris-HCl, pH 6.7) at 50ºC for 30 min, and re-probed with a polyclonal anti-calnexin antibody (diluted at 1:10000; Stressgen, San Diego, CA).

Enzyme assay

The glucuronidation activities of wild-type and variant UGT1A9s were assayed as described previously (Hanioka et al., 2001a). Briefly, the incubation mixtures contained 50 mM Tris–HCl buffer, pH 7.4, SN-38 (final concentration, 2.5-150 μM; dissolved in dimethyl sulfoxide/0.05 N NaOH (1:1)), the membrane fraction of COS-1 cells (100 μg protein), 10 mM $MgCl_2$ and 5 mM UDP-glucuronic acid. Because of the lack of any measurable effect on the enzyme activity, alamethicin was not employed in the assay. After preincubation at

37ºC for 1 min, the reaction was started by the addition of UDP-glucuronic acid. The mixture was incubated at 37º C for 80 min, and the reaction was terminated with 100 µl of 10% (w/v) $HClO_4$. After centrifugation at 12,000 *g* for 10 min at 4ºC, the clear supernatant was filtered using a 0.45 µm PTFE membrane filter, and analyzed by HPLC. Under the assay conditions employed in this study, SN-38 lactone and carboxylate forms are not distinguished, and hence the SN-38 concentrations represent the combined total of lactone and carboxylate forms.

Data analysis

Statistical comparisons were performed using Student's *t* test for unpaired data. Kinetic parameters were calculated with Prism 3.0 (Graph Pad Software, Inc., San Diego, CA), using non-linear regression of the Michaelis-Menten equation. The kinetic parameters were determined from thee independent preparations of UGT1A9 protein.

Results

Genetic variations in the exon 1 of human *UGT1A9*

Among the 61 Japanese cancer patients tested, a novel non-synonymous single nucleotide polymorphism in exon 1 of the *UGT1A9* gene was found in a patient as a heterozygote. As shown in the electropherograms (Fig. 2), the position of the variation was 766 (A of the translational start codon is 1) in exon 1, which was confirmed by repeating the PCR on genomic DNA and sequencing the newly generated PCR products. This genetic variant (766G>A) resulted in the amino acid substitution of D256N. To functionally characterize the non-synonymous D256N variant, wild-type and variant UGT1A9s were expressed in COS-1 cells, and their SN-38 glucuronidation activities were determined.

Expression of wild-type and D256N variant UGT1A9 in COS-1 cells

Wild-type human UGT1A9 cDNA was successfully cloned from liver cDNA by the Gateway recombinational cloning method. The G to A substitution at 766 was introduced to the wild-type UGT1A9 cDNA by PCR-based site-directed mutagenesis. The wild-type and D256N variant were then transiently expressed in COS-1 cells. Fig. 3A shows the representative Western blot of pooled samples from three independent transfections. For each sample preparation, relative UGT1A9 levels were determined using one of the wild-type membrane preparations as a standard. The blots were re-probed with a polyclonal anti-calnexin antibody to confirm that the samples were evenly loaded. As shown in Fig. 3B,

a decrease in the relative expression level of D256N (0.76 ± 0.05) was slight but statistically significant by Student's t test ($p<0.05$). These relative expression levels were used for the normalization of the SN-38 glucuronidation activities of UGT1A9s described below.

SN-38 glucuronidation activities of the wild-type and D256N variant UGT1A9s

Comparison of the functional properties of the wild-type and D256N variant UGT1A9s was made while the SN-38 glucuronidation proceeded linearly within the incubation time (0.1 mg of protein/incubation and 80 min incubation time). The apparent enzyme kinetic parameters (K_m, V_{max}, and V_{max}/K_m) were estimated by fitting the initial velocity of enzymatic reaction, measured as a function of SN-38 concentration (2.5-150 μM), to the Michaelis-Menten equation. The representative non-linear regression curves are depicted in Fig. 4.

Table 2 summarizes the apparent kinetic parameters for SN-38 glucuronidation by the wild-type and variant UGT1A9s. The wild-type UGT1A9 catalyzed SN-38 glucuronidation with an apparent K_m value of 19.3 μM, while that of the D256N variant was 44.4 μM. Their V_{max} values were 2.94 and 0.24 pmol/min/mg membrane protein for the wild-type and D256N variant, respectively. When the V_{max} values were normalized taking the difference in their expression levels into account, the SN-38 glucuronidation efficiency ratio (normalized V_{max}/K_m) were 153 and 7.1 nl/min/mg protein for the wild-type and D256N variant, respectively. These kinetic parameters clearly demonstrate that the D256N variant is nearly inactive towards SN-38 glucuronidation as compared with the wild-type UGT1A9.

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Discussion

It is increasingly apparent that an individual susceptibility to adverse drug reaction can be attributed to genetic factors. In this regard, polymorphism of *UGT1A1* in irinotecan toxicity is one of the most well-documented examples (Pirmohamed and Park, 2001; Tukey et al., 2002). Additional TA repeat in the $A(TA)_6TAA$ sequence of the *UGT1A1* promoter (*UGT1A1*28*) has been shown to result in 30-80% reduction in transcriptional activity compared with wild-type *UGT1A1* (Bosma et al., 1995; Beutler et al., 1998). The decrease in the level of UGT1A1 protein, and hence glucuronidation activity results in an aberrantly high metabolic ratio of SN-38 to SN-38G. Recent pharmacogenetic studies (Ando et al., 2000; Iyer et al., 2002) have shown a positive correlation between the *UGT1A1* promoter genotype and the severe toxicity of irinotecan, including diarrhea and leukopenia. To date, more than thirty polymorphic variations have been reported in the coding region of the *UGT1A1* gene (Tukey and Strassburg, 2000). Some of these variants such as G71R and P229Q are also known to influence the SN-38 glucuronidation kinetics (Gagné et al., 2002; Jinno et al., 2003). Besides UGT1A1, recent kinetic studies *in vitro* have revealed that UGT1A7 and UGT1A9 participate in the glucuronidation of SN-38 (Ciotti et al., 1999; Hanioka et al., 2001b; Gagné et al., 2002). Guillemette et al. (2000) have identified three variant *UGT1A7* alleles, *UGT1A7*2* (N129K/R131K), *UGT1A7*3* (N129K/R131K/W208R) and *UGT1A7*4* (W208R). Among these variants, *UGT1A7*3* and *UGT1A7*4* have been shown to exhibit decreased SN-38 glucuronidation activity (20-40% of the wild-type allele

*UGT1A7*1*) (Gagné et al., 2002). However, the clinical importance of *UGT1A7* polymorphism in irinotecan toxicity remains to be determined because of the rather limited tissue distribution of UGT1A7 in orolaryngeal and gastric tissues (Strassburg et al., 1997; Zheng et al., 2001; Vogel et al., 2001). In this regard, no significant association has been found between the presence of *UGT1A7* variant alleles and the occurrence of severe toxicity of irinotecan (Ando et al., 2002). In contrast to UGT1A7, UGT1A9 has been shown to be expressed in both liver and extrahepatic tissues such as colon and kidney (Strassburg et al., 1998; McGurk et al., 1998). Therefore polymorphism in the *UGT1A9* gene could potentially influence the pharmacokinetics of irinotecan/SN-38 and irinotecan-induced toxicity, as addressed by Gagné et al. (2002).

Taking these previous studies into account, we have determined the nucleotide sequence of exon 1 of the *UGT1A9* gene from cancer patients treated with irinotecan, and identified a novel non-synonymous variant 766G>A (D256N). Functional characterization revealed that the SN-38 glucuronidation efficiency (normalized V_{max}/K_m) of the D256N variant was less than 5% of wild-type UGT1A9. The apparent K_m value of wild-type UGT1A9 (19.3 μM) in this study is nearly equal to that obtained using recombinant UGT1A9 expressed in insect cells (13.4 μM) (Hanioka et al., 2001b), and comparable with that of UGT1A1 expressed in COS-1 cells (11.5 μM) (Jinno et al., 2003). However, Gagné et al. (2002) reported a K_m value of 0.7 μM for the HEK293 cell-expressed UGT1A9. We have no explanation for this discrepancy at present, except for pointing to the difference in experimental conditions such as phosphatidylcholine and saccharolactone in the glucuronidation assay medium, which are

absent in the present study.

The physiological significance of *UGT1A9* polymorphism in irinotecan toxicity, in any case, mostly depends on the expression levels of UGT1A1 and UGT1A9 in the liver, although no quantitative information is available at present. A quite large inter-individual variability has been reported for hepatic UGT1A1 level: more than a 30-fold variability was found both for UGT1A1 mRNA levels (Congiu et al., 2002) and estradiol 3-*O*-glucuronidation activities catalyzed by UGT1A1 (Fisher et al., 2000). These variations are explained, to some extent, by the *UGT1A1* promoter polymorphism, *UGT1A1*28* (Fisher et al., 2000). Environmental factors are also thought to be involved in the inter-individual variability of UGT1A1 expression levels (Ritter et al. 1999). Recently, a phenobarbital-responsive enhancer module (PBREM) (Sugatani et al., 2001) and xenobiotic response element (XRE) (Yueh et al., 2003) have been found in the *UGT1A1* promoter region, which is activated by human constitutive active/androstane receptor (hCAR) and aryl hydrocarbon (Ah) receptor, respectively. In contrast, *UGT1A9* has been identified as a PPARα and PPARγ target gene (Barbier et al., 2003). Therefore, exposure to xenobiotic chemicals such as polycyclic aromatic hydrocarbons and flavonoids could influence the relative expression levels of UGT1A1 and UGT1A9 in the liver.

Another potential factor affecting the relative contribution of UGT1A9 to SN-38 glucuronidation may come from competitive binding of SN-38 and bilirubin to UGT1As. When the concentration of SN-38 is low, competitive binding with bilirubin to UGT1A1 may inhibit SN-38 glucuronidation and prolong circulation times of this active metabolite (Kehrer

et al., 2000). Indeed, we have shown that bilirubin (2 μM) effectively inhibits SN-38 glucuronidation activities of human liver microsomes and recombinant UGT1A1 by 40-60%, while the inhibitory effect of bilirubin is weak for UGT1A9 (Hanioka et al. 2001b). Under physiological conditions, therefore, UGT1A9 may play a more important role in the hepatic SN-38 glucuronidation than expected from the kinetic studies *in vitro*. To clarify the potential role of this UGT1A9 D256N variant in irinotecan metabolism, a detailed pharmacokinetic study is now under way including the patient carrying this variant.

In addition to the glucuronidation of SN-38, UGT1A9 plays important roles in the metabolism of clinically relevant drugs including an analgesic and antipyretic drug acetaminophen, an anxiolytic drug oxazepam, an anaesthetic drug propofol and a β-blocker propranolol (Ebner and Burchell, 1993; Court et al., 2001, 2002). The UGT1A9 D256 variant found in this study may also influence the metabolism of these drugs.

In conclusion, we identified a novel genetic variant of *UGT1A9* in Japanese cancer patients; 766G>A results in the amino acid substitution of D256N, which is essentially non-functional for SN-38 glucuronidation. These findings highlight the importance of carrying out further investigations into the possible influence of the UGT1A9 D256N variant with regard to irinotecan metabolism *in vivo*.

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Footnotes

Hideto Jinno and Mayumi Saeki contributed equally to this article.

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Legend for figures

Fig. 1 Chemical structures of irinotecan and its major metabolites, SN-38 and SN-38 glucuronide.

Fig. 2 Electropherograms of *UGT1A9* gene sequences at nucleotide 751-780 for wild-type (top) and heterozygous 766G>A (bottom) individuals. Arrows indicate the variant nucleotide positions.

Fig. 3 Expression of the wild-type and D256N variant UGT1A9s in COS-1 cells.

(A) Aliquots (20 μg) of the pooled membrane fraction from three independent preparations were subjected to SDS-PAGE, electrophoretically transferred to a PVDF membrane and detected immunochemically with rabbit anti-human UGT1A antiserum (diluted at 1:5000) as described in Materials and Methods. To confirm that the samples were evenly loaded, the blot was subsequently stripped and re-probed with a polyclonal anti-calnexin antibody (diluted at 1:10000). (B) The stained UGT1A9 bands of each Western blot of three independent preparations were quantified using the ImageQuant analysis software, and the expression levels of UGT1A9 proteins were normalized to the average of wild-type. The results are expressed as the mean ± SD from three independent preparations.

Fig. 4 Representative Michaelis-Menten kinetics for SN-38 glucuronidation by recombinant wild-type and D256N variant UGT1A9s.

SN-38 glucuronidation assays were performed in the presence of the membrane fractions (100 μg) at a substrate concentration of between 2.5 and 150 μM. The solid line indicates fitting of data to the Michaelis-Menten equation by the non-linear regression.

Table 1 Primers used for sequencing exon 1 of the *UGT1A9* gene and plasmid construction

Reactions	Primer Name	Sequences
1st Amplification of exon 1s from *UGT1A9* to *UGT1A7*	UGT1A9-7ZF	5’-TCTTGATTGTCCTCCATTGAGT-3’
	UGT1A9-7ZR	5’-ACCAAGCAACCATACTCATAGG-3’
2nd Amplification of *UGT1A9* exon 1	UGT1A9-1stF	5’-CCAAGGCAAAGACCATAAGC-3’
	UGT1A9-1stR	5’-TTGCTACTGACGAGTACACGC-3’
Sequencing	UGT1A9F1	5'-GGTTTTGTGCTGGTATTTCTCC-3'
	UGT1A9R1	5'-GCAAAAGCCTTGAACTCCC-3'
	UGT1A9F2	5'-GGCAACTGGGAAGATCACTG-3'
	UGT1A9R2	5'-CCGTACTCTCTCCTTGAAAGTC-3'
	UGT1A9F3	5'-TGCTCCTCTTTCCTATGTCC-3'
	UGT1A9R3	5'-TGTCAAATCACAGTTCAGTAAAGA-3'
cDNA cloning	*att*B1_UGT1A9F	5'-AAAAAGCAGGCTGCAGTTCTCTGATGGCTTGCA-3' *[1]
	*att*B2_UGT1AR	5'-AGAAAGCTGGGTCTCAATGGGTCTTGGATTTGTGGG-3' *[1]
	*att*B1_Adaptor	5’-GGGGACAAGTTTGTACAAAAAAGCAGGCT-3’
	*att*B2_Adaptor	5’-GGGGACCACTTTGTACAAGAAAGCTGGGT-3’
Site-directed mutagenesis	UGT1A9_766A	5'-phospho-TTGGTTGTTGCGAACGAACTTTGTTTTGGACTATC-3’ *[2]

*[1] The 12-bp partial *att*B sequences at the 5’-end are underlined.

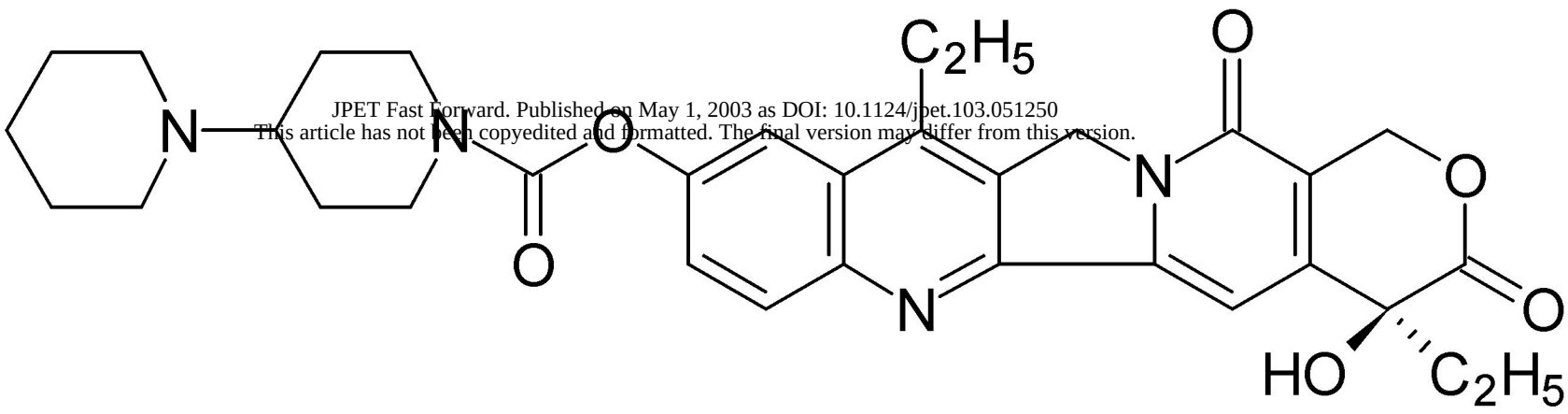
*[2] The base exchanged is underlined.

Table 2 Kinetic parameters for SN-38 glucuronidation by wild-type and D256N variant human UGT1A9s.[a]

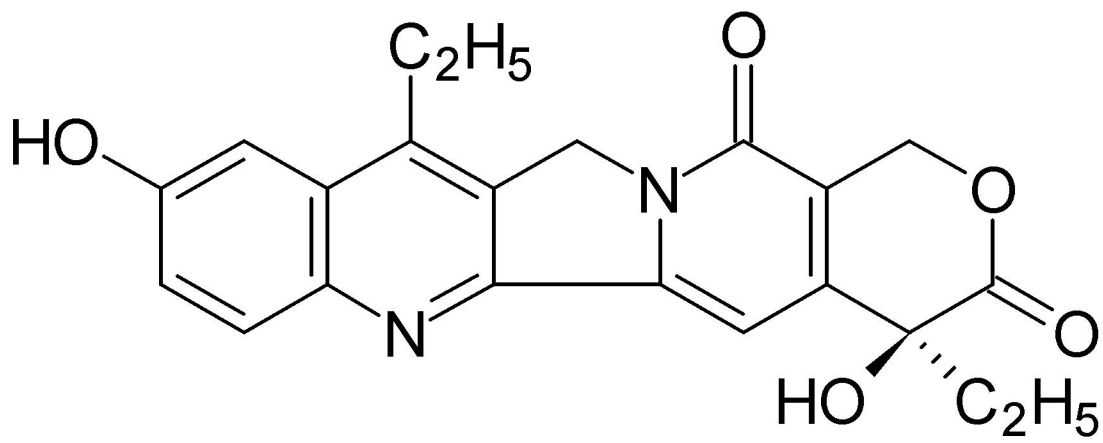
UGT1A9	Apparent K_m (μM)	V_{max} (pmol/min/mg protein)	V_{max}/K_m (nl/min/mg protein)	Normalized V_{max} [b] (pmol/min/mg protein)	Normalized V_{max}/K_m [b] (nl/min/mg protein)
Wild-type	19.3 ± 0.7	2.94 ± 0.22	152 ± 10	2.95 ± 0.29	153 ± 20
D256N	44.4 ± 6.9‡	0.24 ± 0.01‡	5.4 ± 0.7‡	0.31 ± 0.01‡	7.1 ± 1.4‡

[a]: The results were expressed as the mean ± SD from three independent transfection experiments. ‡ signifies data significantly different from that of wild-type at the level of $p < 0.01$.

[b]: V_{amx} values were normalized by the relative protein expression level, 0.76 ± 0.05 for the D256N variant.



Irinotecan



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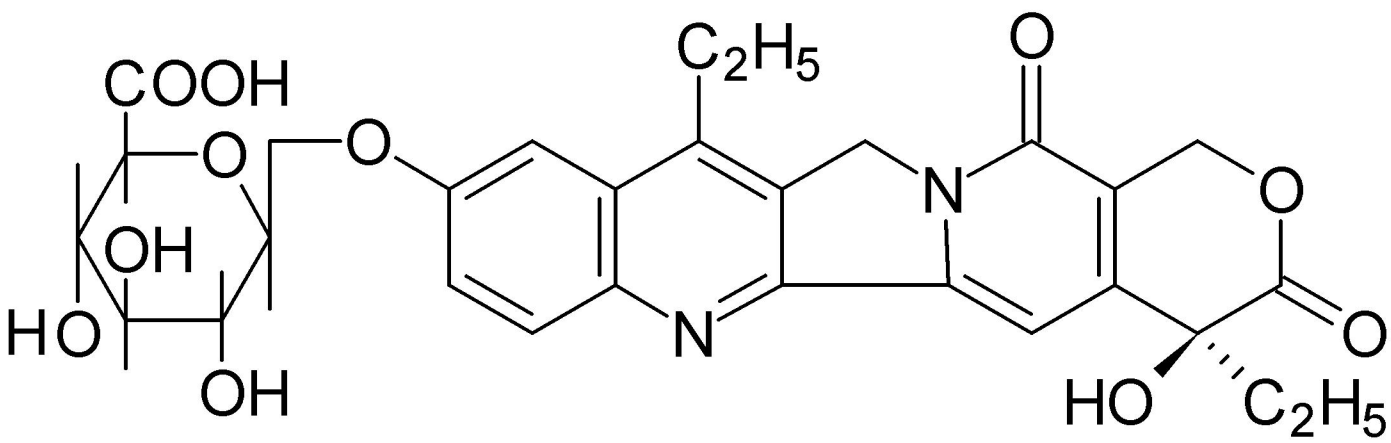

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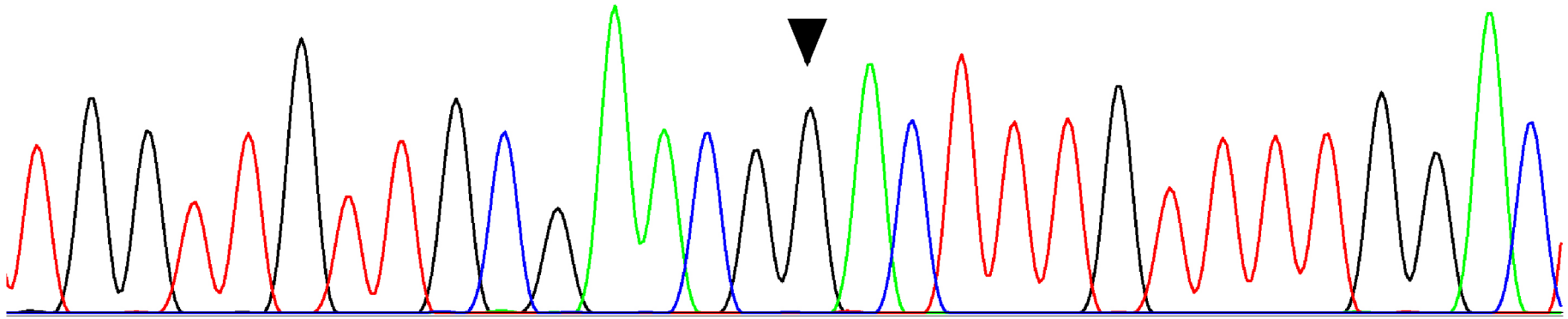

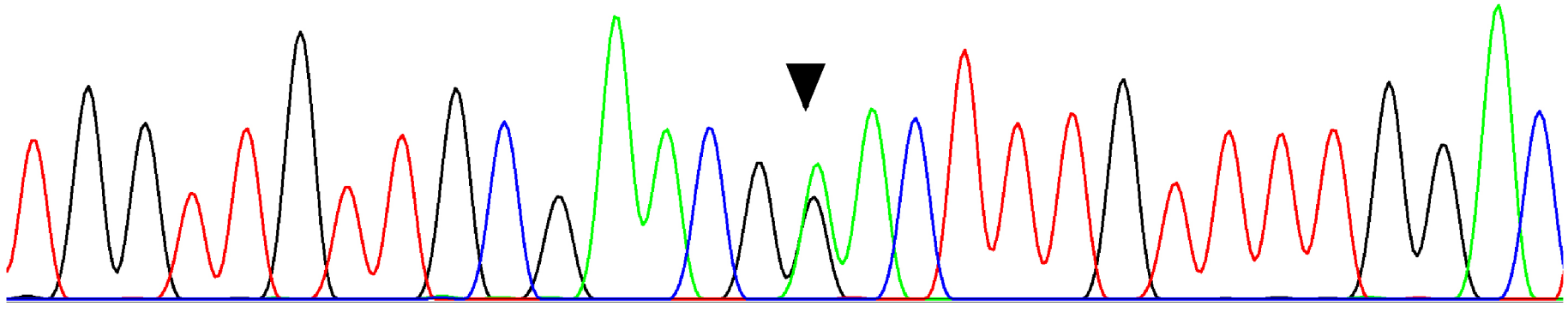

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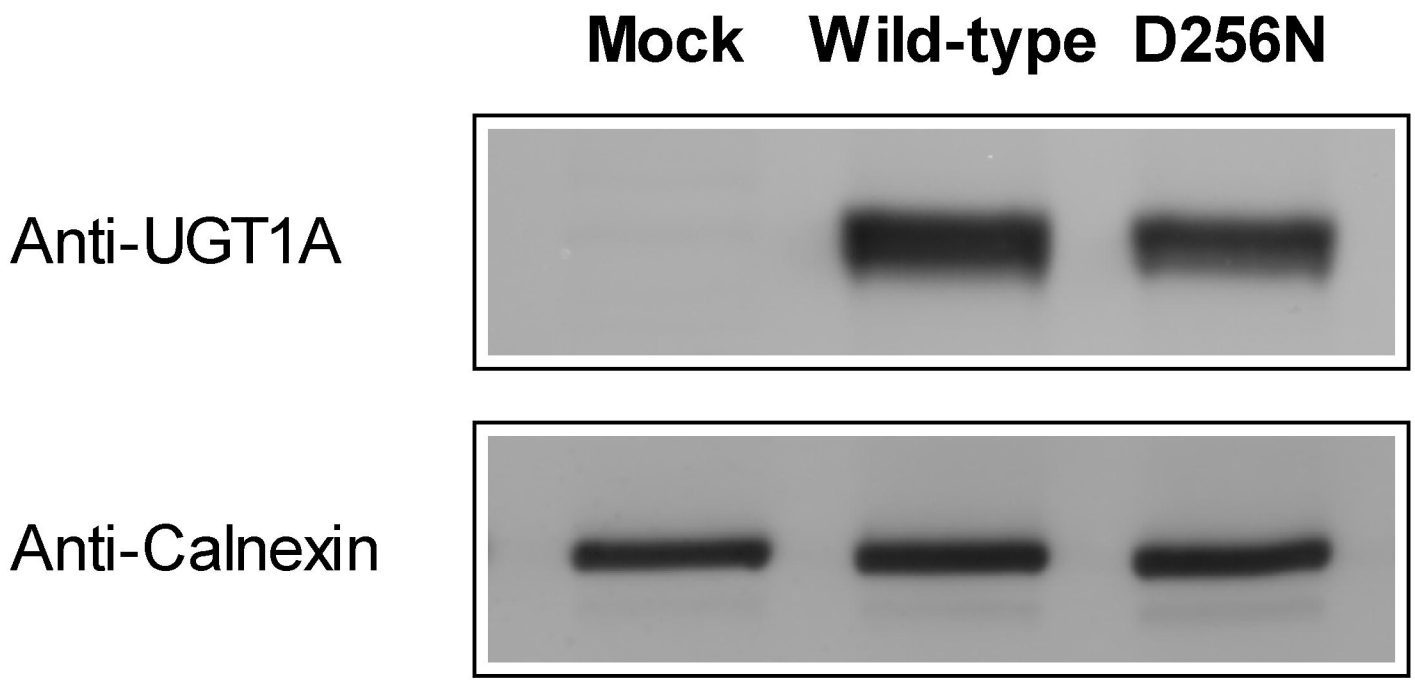
A



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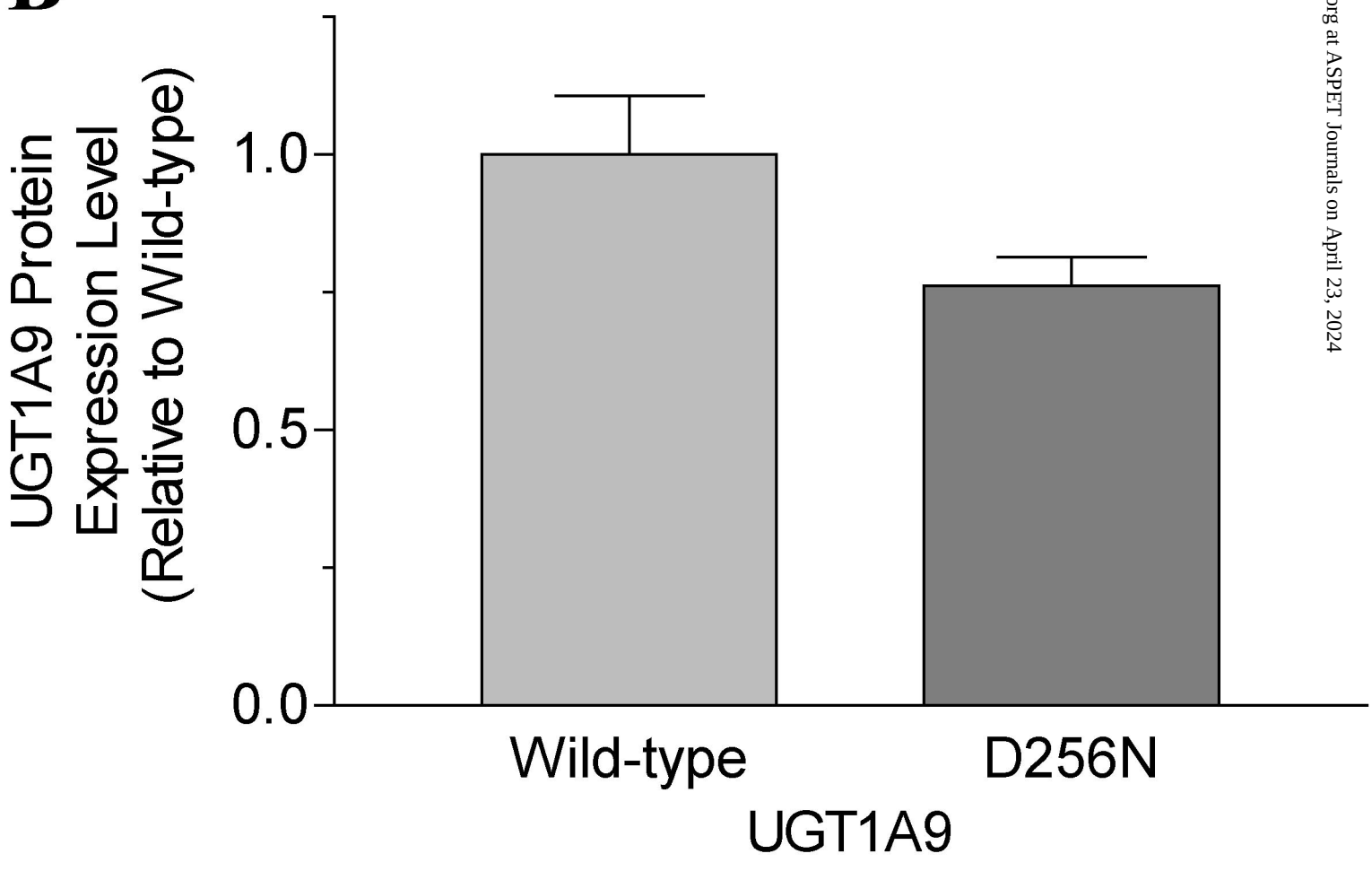

JPET #52150 Figure 4