VOLUME 24 · NUMBER 15 · MAY 20 2006

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

Comprehensive Analysis of *UGT1A* Polymorphisms Predictive for Pharmacokinetics and Treatment Outcome in Patients With Non–Small-Cell Lung Cancer Treated With Irinotecan and Cisplatin

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Submitted June 8, 2005; accepted December 2, 2005.

This study was supported by Grants No. 0210130, 0210140, 0510140, and 0510080 from the National Cancer Center, Goyang, Korea.

Authors' disclosures of potential conflicts of interest and author contributions are found at the end of this article.

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0732-183X/06/2415-2237/$20.00

DOI: 10.1200/JCO.2005.03.0239

ABSTRACT

Purpose
To determine whether uridine diphosphate-glucuronosyltransferase *1A1*, *UGT1A7*, and *UGT1A9* polymorphisms affect the pharmacokinetics (PK) of irinotecan and treatment outcome of Korean patients with advanced non–small-cell lung cancer (NSCLC).

Methods
Eighty-one patients with advanced NSCLC were treated with irinotecan (80 mg/m^2) on day 1 and 8 and cisplatin (60 mg/m^2) on day 1 intravenously of each 3-week cycle. Genomic DNA was extracted from peripheral blood and genotyped using direct sequencing. We analyzed the association of *UGT1A* genotypes with irinotecan PK and clinical outcomes. All statistical tests were two-sided.

Results
In genotype-PK association analysis, *UGT1A1*6/*6* (n = 6), *UGT1A7*3/*3* (n = 6), and *UGT1A9-118(dT)$_{9/9}$* (n = 11) were associated with significantly lower area under the time-concentration curve (AUC) SN-38G to SN-38 (AUC_{SN-38G}/AUC_{SN-38}) ratio (P = .002, P = .009, and P = .001, respectively). In linkage disequilibrium analysis, the *UGT1A7* variants were highly linked with the *UGT1A1*6* (D' = 0.85, r^2 = 0.63) and *UGT1A9*22* (D' = 0.95, r^2 = 0.88), which was substantiated in haplotype analysis. Patients with *UGT1A1*6/*6* had lower tumor response and higher incidence of severe neutropenia. *UGT1A9-118(dT)$_{9/9}$* also showed a trend for high incidence of severe diarrhea, but not tumor response. In survival analysis, patients with *UGT1A1*6/*6* had significantly shorter progression-free survival (P = .001) and overall survival (P = .017).

Conclusion
These findings suggest that *UGT1A1*6* and *UGT1A9*22* genotypes may be important for SN-38 glucuronidation and associate with irinotecan-related severe toxicity. Specifically, *UGT1A1*6* might be useful for predicting tumor response and survival outcome of Korean patients with NSCLC treated with irinotecan-based chemotherapy.

J Clin Oncol 24:2237-2244. © 2006 by American Society of Clinical Oncology

INTRODUCTION

Inherited genetic polymorphisms of the genes involved in the activation and metabolism of certain chemotherapeutic agents can affect the outcome of patients after treatment with such agents. Irinotecan, a semisynthetic camptothecin analog with topoisomerase I–inhibiting activity,[1-3] shows excellent clinical efficacy in such hard-to-treat solid tumors as lung and colorectal cancers.[4-6] It is an excellent candidate for the pharmacogenetic studies because of a well-characterized metabolic pathway and significant, sometimes unpredictable and life-threatening, adverse effects.[7,8]

Irinotecan is a prodrug that is converted by carboxylesterase to an active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38), which has a 100- to 1,000-fold higher cytotoxicity than irinotecan. SN-38 is then further metabolized in the liver by uridine diphosphate-glucuronosyltransferases (UGTs) to an inactive metabolite, SN-38 glucuronide (SN-38G).[1-3] Because glucuronidation is the major route of detoxification and elimination of active metabolite SN-38, inherited differences in irinotecan glucuronidating capacity may have an

Table 1. Primers for *UGT1A* Amplification and Allele Frequency

Gene/Polymorphism	Forward Primer	Reverse Primer	Annealing Temperature (°C)	Allele Frequency (n = 81)	HWE *P*
UGT1A9					
*−118(T)$_{10}$/(T)$_9$ (UGT1A9*22) (rs3832043)*	AGGCGAGCCCCAATTTAG	GGCAAAGCCACAGGTCAG	60	$(T)_{10}$:$(T)_9$ = 0.579:0.424	.232
UGT1A7					
*N129K (UGT1A7*1, *2, *3, *4) (rs17868323)*	CACCATTGCGAAGTGCAT	TTCTTAATGTGCTAAAGGGGAGA	60	T:G = 0.58:0.42	.225
*R131R,Q,K (UGT1A7*1, *2, *3, *4) (rs17863778)*	CACCATTGCGAAGTGCAT	TTCTTAATGTGCTAAAGGGGAGA	60	C:A = 0.58:0.42	.225
*R131R,Q,K (UGT1A7*1, *2, *3, *4) (rs17868324)*	CACCATTGCGAAGTGCAT	TTCTTAATGTGCTAAAGGGGAGA	60	G:A = 0.58:0.42	.225
*W208R (UGT1A7*1, *2, *3, *4) (rs11692021)*	CACCATTGCGAAGTGCAT	TTCTTAATGTGCTAAAGGGGAGA	60	T:C = 0.74:0.26	.911
UGT1A1					
*−3279T > G (UGT1A1*60) (rs4124874)*	AATTCCAAGGGGAAAATG	TCCAAGCCAGCAAGTAAG	52	T:G = 0.765:0.235	.606
*−53∼−42(TA)$_6$/(TA)$_7$ (UGT1A1*28) (rs8175347)*	CAGCCTCAAGACCCCACA	TGCTCCTGCCAGAGGTTC	60	$(TA)_6$:$(TA)_7$ = 0.93:0.07	.999
*211G > A (G71R) (UGT1A1*6) (rs4148323)*	TCCCTGCTACCTTTGTGGA	AGGAAAGGGTCCGTCAGC	65	G:A = 0.76:0.24	.471

NOTE. $P < .05$ indicates a lack of HWE.
Abbreviations: *UGT1A*, uridine diphospahte-glucuronosyl transferase 1A; HWE, Hardy-Weinberg equilibrium.

important influence on the pharmacokinetics and toxicity of this drug.[9,10]

To date, 17 human UGTs have been characterized.[11-13] Most of the UGTs are expressed in the liver as well as other extrahepatic tissues; however, some are exclusively extrahepatic.[14] UGT1A1, which is highly expressed in the liver, is the main isoform involved in the formation of SN-38G.[14-17] The clinical significance of the $-53(TA)_{6>7}$ (*UGT1A1*28*) for SN-38 glucuronidation and irinotecan-related toxicity is well established.[14-17] Other *UGT1A1* variants such as *–3279T>G* (*UGT1A1*60*) and *211G>A* (*UGT1A1*6*) have also been suggested to contribute the UGT1A1 enzyme function.[18] In addition to UGT1A1, the hepatic UGT1A9 has been suggested to contribute to the hepatic metabolism of SN-38.[14,19,20] Recently, Yamanaka et al[21] found that one base insertion of thymidine in a promoter region of the *UGT1A9* gene ($-118(T)_{9>10}$, *UGT1A9*22*) has been associated with variable UGT1A9 enzyme expression.

UGT1A7, which is exclusively expressed in extrahepatic sites such as esophagus, gastrointestinal, and orolaryngeal tissues, has been demonstrated to glucuronidate several carcinogens.[22,23] *UGT1A7*3*, a low-activity genotype, was found to be related to the increased risk of oropharyngeal, colorectal, and hepatocellular carcinomas in individuals with the polymorphism.[24-26] Although it is an extrahepatic enzyme, recent in vitro study has demonstrated the effects of UGT1A7 on the glucuronidation of SN-38.[18-20]

Since those functionally important candidate *UGT1A* polymorphisms, including *UGT1A1*6*, *UGT1A1*28*, *UGT1A1*60*, *UGT1A7*3*, and *UGT1A9*22*, may affect the key enzyme activities of irinotecan metabolism, we postulated that those polymorphisms would eventually result in a difference in irinotecan-related toxicity and possibly the tumor response rates. Using DNA samples obtained from a predefined group of Korean patients with advanced non–small-cell lung cancers (NSCLC), who were enrolled in a prospective phase II study of irinotecan plus cisplatin chemotherapy,[27] we examined the association of the genetic polymorphisms of *UGT1A1 (*6, *28,* and *60)*, *UGT1A7 (1, *2, *3, *4)*, and *UGT1A9 (*22)* genes with the pharmacokinetic parameters of irinotecan metabolism and the clinical outcome.

METHODS

Patients and Control Subjects

A total of 81 patients with advanced NSCLC were enrolled onto a phase II study of irinotecan and cisplatin chemotherapy. The study subjects were described in detail elsewhere.[27] Treatment consisted of irinotecan 80 mg/m^2 intravenously on days 1 and 8 and cisplatin 60 mg/m^2 intravenously on day 1 of a 21-day cycle. All patients gave written informed consent approved by the institutional review board of the National Cancer Center Hospital. The study

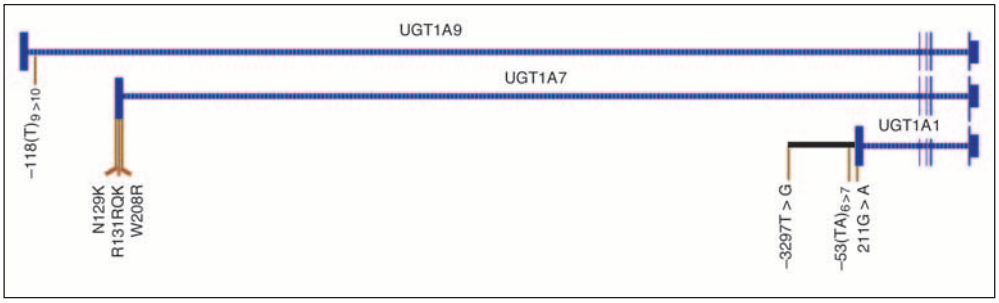

Fig 1. The fine scale single nucleotide polymorphisms map of uridine diphosphate-glucuronosyl transferase (UGT[r]) *1A* gene.

was performed in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines.

Pharmacokinetic Study

Venous blood (10 mL) for pharmacokinetic analysis was taken into sodium heparinized evacuated tubes on day 1 of cycle 1 before irinotecan infusion, and at 30 (during infusion), 60 (at the end of infusion), 65, 75, 90, and 105 minutes, and 2, 3, 5, 7, and 25 hours after the start of irinotecan infusion. Total irinotecan and its metabolite concentrations in plasma were measured by high-performance liquid chromatography, as described previously.[28] SN-38 and SN-38G were kindly supplied by Aventis Co Ltd (Strasbourg, France). The area under the time-concentration curve (AUC) ratio of SN-38G to SN-38 ($AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$) was calculated as a surrogate marker for *UGT* activity of SN-38 glucuronidation.

UGT1A1, UGT1A7, *and* UGT1A9 *Genotyping Assay*

Genomic DNA was isolated from 81 patients enrolled onto the clinical trial, using DNA Purification Kit (Gentra, Minneapolis, MN). Polymerase chain reaction (PCR) amplifications were performed in a PTC-225 Peltier Thermal Cycler (MJ Research, Waltham, MA) with Taq Gold DNA polymerase. Amplification conditions were 35 cycles of 95°C for 30 seconds, each annealing temperature for 1 minute, and 72°C for 1 minute. A final 10-minute extension at 72°C completed the amplification. Sequences of the PCR primers and the cycling conditions are listed in Table 1.

PCR products were purified using the Montage PCR_{96} Cleanup Kit (Millipore, Billerica, MA) and eluted in 20 μL nuclease-free water. DNA sequencing was carried out with BigDye Terminator V 3.1 Cycle Sequencing Kit (PerkinElmer, Fremont, CA). For dye terminator removal, Multiscreen SEQ 384 well filter plates were used, and sequences were analyzed using an Applied Biosystems (Foster City, CA) 3700 DNA Analyzer. All SNPs and sequence alignments were analyzed by the Polyphred 5.04 (University of Washington, Seattle, WA; http://droog.mbt.washington.edu/PolyPhred.html).

Statistical Analysis

All pharmacokinetic data are presented as a mean ± standard deviation. Associations between pharmacokinetic parameters and the variant genotypes were assessed by a nonparametric Kruskal-Wallis test followed by a comparison of all means with a Tukey-Kramer test using Prism, version 3.02 (GraphPad Software Inc, San Diego, CA). The association between potential variables was assessed using χ^2 test or Fisher's exact test for categoric variables, or Mann-Whitney *U* test for continuous ones. The analysis of a trend of phenotype across the genotype was performed with the Armitage trend test in the SAS system, version 8 (SAS Institute Inc, Cary, NC). Kaplan-Meier estimates and the log-rank test were used in univariate analyses of overall survival.

The genotype frequencies for each SNP were checked for consistency between the observed values and those expected from Hardy–Weinberg equilibrium using Haploview version 3.2 (Massachusetts Institute of Technology, Cambridge, MA; http://www.broad.mit.edu/mpg/haploview/index.php). Haploview based on the expectation-maximization method,[29] were used to estimate the haplotype frequencies, the Lewontin's coefficients D′,[30] and correlation coefficient r^2.[31] The block structures and their haplotype frequencies were estimated using Haploview version 2.05.

RESULTS

UGT1A *Genotypes and Allele Frequencies*

The *UGT1A1*, *UGT1A7*, and *UGT1A9* variants typed in this study and their allele frequencies are listed in Table 1. The fine-scale map of those polymorphisms is shown in Figure 1. Concerning the allele frequencies, the frequencies of *UGT1A9 −118 (*22), UGT1A1-3279T>G (*60)*, and *211G>A (*6, G71R*) were comparable to the data previously reported in Asian patients.[14,18] As expected, the frequency of the *–53(TA)$_7$ (*28)* allele was very low compared with the data in white patients.[14] Interestingly, there was no homozygous *UGT1A1*28* in this study. All those variants were in Hardy-Weinberg equilibrium ($P > .05$).

Table 2. Effects of *UGT1A1*, *UGT1A7*, and *UGT1A9* Genotypes on the Plasma Availability of CPT-11 and Its Metabolites

	$AUC_{CPT\text{-}11}$ (ng · h/mL)				$AUC_{SN\text{-}38}$ (ng · h/mL)				$AUC_{SN\text{-}38G}$ (ng · h/mL)				$AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ Ratio			
	Mean	SD	Range	*P*	Mean	SD	Range	*P*	Mean	SD	Range	*P*	Mean	SD	Range	*P*
*UGT1A1*6*				.497†				.002†				.325†				.002†
−/− (n = 49)	5,145.1	1,583.9	2,454.6-9,250.2		113.9	42.9	36.8-290.1		1,348.2	780.9	316.0-3,987.8		12.8	7.9	3.5-40.4	
+/− (n = 26)	4,799.0	1,691.2	2,195.0-8,580.1		126.7	60.6	50.7-353.3		1,303.9	815.1	311.7-3,355.5		10.7	5.2	2.5-20.9	
+/+ (n = 6)	5,120.0	978.8	3,534.7-6,315.5		200.4	37.2	141.9-243.2		871.1	254.7	571.5-1,151.1		4.5	1.9	2.5-8.0	
*UGT1A1*60*				.436†				.198†				.282†				.573†
−/− (n = 46)	5,073.7	1,584.9	2,195.0-8,949.8		133.5	57.5	50.7-353.3		1,312.0	713.8	311.7-3,355.5		11.0	6.6	2.5-40.4	
+/− (n = 32)	4,884.2	1,577.5	2,388.7-9,250.2		111.6	46.0	36.7-290.1		1,220.9	825.6	316.0-3,987.8		11.6	7.8	3.5-37.8	
+/+ (n = 3)	5,973.3	1,610.0	4,476.0-7,676.2		121.8	42.2	88.6-169.2		1,923.6	997.5	902.9-2,896.2		16.3	9.3	10.2-26.9	
*UGT1A1*28*				.221‡				.473‡				.457‡				.276‡
6/6 (n = 69)	4,934.8	1,523.5	2,195.0-8,949.8		126.6	55.5	49.6-353.3		1,251.3	706.3	311.7-3,355.5		10.9	6.5	2.5-40.4	
7/6 (n = 12)	5,592.0	1,822.8	2,892.1-9,250.2		111.6	37.6	36.8-177.3		1,570.8	1064.1	416.9-3,987.8		14.9	10.0	3.5-37.8	
UGT1A7				.852†				.006†				.182†				.009†
*1/*1 (n = 24)	4,882.4	1,445.8	2,454.6-7,605.6		107.0	37.5	36.8-204.7		1,201.1	617.0	522.1-3,013.7		12.5	8.0	3.9-40.4	
*1/*2 (n = 21)	5,040.3	1,414.6	2,893.6-7,676.2		118.2	51.0	49.6-290.1		1,520.3	934.8	316.0-3,987.8		13.9	8.7	3.7-37.8	
*1/*3 (n = 25)	5,386.3	1,803.5	2,195.0-9,250.2		132.1	64.2	50.7-353.3		1,389.8	824.0	311.7-3,355.5		11.1	4.7	2.5-18.5	
*2/*3 (n = 5)	3,330.2	976.7	2,388.7-4,645.7		127.5	31.0	104.1-179.8		735.9	487.6	379.4-1,516.0		5.4	2.2	3.6-8.4	
*3/*3 (n = 6)	5,545.2	1,348.6	3,534.7-7,451.5		181.2	47.2	114.9-243.2		1,001.9	271.1	619.5-1,356.3		6.1	3.2	3.5-11.8	
*UGT1A9*22**				.741†				.046†				.05†				.001†
10/10 (n = 23)	5,159.7	1,456.6	2,892.1-7,605.6		107.3	44.7	36.8-209.7		1,297.2	770.5	522.1-3,355.5		13.2	8.0	3.9-40.4	
10/9 (n = 45)	5,118.7	1,656.3	2,195.0-9,250.2		126.2	57.4	49.6-353.3		1,421.5	825.6	311.7-3,987.8		12.2	7.0	2.5-37.8	
9/9 (n = 11)	4,702.3	1,499.7	2,388.7-7,451.5		152.4	47.6	104.1-243.2		811.5	327.4	379.4-1,356.3		5.5	2.5	3.5-11.8	

NOTE. All *P* values are unadjusted for multiple comparisons.
Abbreviations: CPT-11, irinotecan; AUC; area under the time-concentration curve; SD, standard deviation.
*The *UGT1A9*22* genotyping failed in two patients.
†Kruskal-Wallis test.
‡Mann-Whitney test.

UGT1A *Genotype-Dependent Glucuronidation of SN-38*

We examined the association of each *UGT1A* genotype with irinotecan-PK in 81 patients with NSCLC (Table 2). Patients with homozygous *UGT1A1*6* (n = 6), *UGT1A9-118$_{9/9}$* (n = 11), and *UGT1A7*3/*3* (n = 6) had significantly higher AUC_{SN-38} values than the others ($P = .002$, $P = .046$, and $P = .006$, respectively). Those patients also showed a significantly lower AUC_{SN-38G}/AUC_{SN-38} ratio than others ($P = .002$, $P = .001$, and $P = .009$, respectively). However, there was no significant difference in pharmacokinetic characteristics including AUC_{SN-38G}/AUC_{SN-38} ratio in patients with *UGT1A1*28* or *UGT1A1*60*.

Association of UGT1A *Genotypes With Tumor Response, Toxicity, and Delivered Dose of Irinotecan*

Of the 81 patients enrolled, 77 were assessable for response, and 36 (47%) of 77 assessable patients achieved partial responses.[27] By genotype, patients with homozygous *UGT1A1*6* showed a significantly lower response rate (0% *v* 50%, $P = .038$; Table 3). Patients with *UGT1A7*3/*3* also showed a trend for lower response rate ($P = .034$).

We also examined the association of *UGT1A* genotypes with the toxicity profile for all 81 patients enrolled. The most common severe toxicity was National Cancer Institute Common Toxicity Criteria grade 4 neutropenia, which occurred in 22 (27%) patients, whereas grade 3 diarrhea developed in only eight patients (10%). Patients with homozygous *UGT1A1*6* were only associated with higher incidence of grade 4 neutropenia ($P = .044$, Table 3). Patients with *UGT1A9-118(T)$_{9/9}$* or *UGT1A7*3/*3* showed a trend for high incidence of grade 3 diarrhea ($P = .037$ and $P = .028$, respectively). However, *UGT1A1*28* and *UGT1A1*60* did not show any significant association with either objective tumor response or toxicity.

Because *UGT1A* variants with lower enzyme activity were associated with higher incidence of severe toxicity and lower tumor responses, we analyzed the association of each *UGT1A* genotype with the actually delivered dose of irinotecan (mg/m^2/wk). However, there was no significance (Table 3).

Linkage Disequilibrium Analysis

High linkage disequilibrium (LD) was seen among *UGT1A1* variants with D′ values of 1, but low r^2 values (range, 0.01 to 0.09), which included *−3279T/G (*60)*, *−1352A/C*, *−997G/A*, *−53∼−42(TA)$_{6>7}$ (*28)*, and *211G>A (*6)* (Fig 2). Another close linkage across *UGT1A7* and *UGT1A9* was found (D′ values of 1 and r^2 values of 0.52 to 0.94; Fig 2). *UGT1A9*22 (−118(T)$_{9>10}$)* was highly linked with the *UGT1A7* variants (D′ = 0.95, $r^2 = 0.88$). D′ values of 0.49 to 1.0 and r^2 values of 0.05 to 0.26 were observed between *UGT1A9*22* and *UGT1A1* variants. We also found that *UGT1A1*6* was highly linked with *UGT1A7*3* (D′ = 0.85, $r^2 = 0.63$). These findings suggest that the association between *UGT1A7*3* and its specific phenotypes such as low SN-38 glucuronidation in this study may be due to the high LD with *UGT1A1*6* and *UGT1A9*22*.

Association of UGT1A *Genotypes and Survival*

We analyzed the survival outcome according to functionally important *UGT1A1* and *UGT1A9* genotypes. Patients with

Table 3. Association of *UGT1A* Genotypes With Tumor Response, Toxicity, and Delivered Dose of Irinotecan

	Tumor Response				G4 Neutropenia*				G3 Diarrhea*				Delivered Dose of Irinotecan (mg/m^2/wk)			
	Responder†				Yes				Yes							
	No.	%	*P*‡	*P*§	No.	%	*P*‡	*P*§	No.	%	*P*‡	*P*§	Mean	SD	Range	*P*¶
UGT1A9*22‖																
10/10	7/21	33	.109	.677	3/23	13	.108	.203	2/23	9	.058	.037	43.4	6.5	28.9-54.7	.529
10/9	25/44	57			14/45	31			2/45	4			44.8	6.4	30.5-53.3	
9/9	3/10	30			5/11	45			3/11	27			41.9	10.4	24.0-49.2	
UGT1A7			.086	.034			.084	.052			.087	.028				.972
*1/*1, *1/*2, *1/*3	34/67	51			16/70	23			5/70	7			43.7	6.2	28.9-54.7	
*2/*3	2/5	40			3/5	60			1/5	20			44.6	5.9	34.8-49.2	
*3/*3	0/5	0			3/6	50			2/6	33			41.7	10.6	24.0-52.5	
UGT1A1*60			.066	.098			.269	.242			.823	.624				.578
−/−	16/44	36			11/46	24			5/46	11			43.4	6.7	24.0-54.7	
−/+	19/30	63			9/32	28			3/32	9			45.0	6.1	32.0-53.3	
+/+	1/3	33			2/3	67			0/3	0			40.9	12.2	28.9-53.3	
UGT1A1*28			.531	.385			.726	.605			.999	.847				.312
−/−	29/65	45			18/69	26			7/69	10			44.3	6.3	24.0-54.7	
+/−	7/12	58			4/12	33			1/12	8			41.6	8.3	28.9-53.3	
UGT1A1*6			.038	.031			.044	.025			.475	.565				.823
−/− and −/+	36/72	50			18/75	24			7/75	9			44.1	6.0	28.9-54.7	
+/+	0/5	0			4/6	67			1/6	17			40.8	9.6	24.0-47.8	

NOTE. All *P* values are unadjusted for multiple comparisons.
*Toxicity grade by National Cancer Institute Common Toxicity Criteria version 2.0. A total of 81 patients were assessable for toxicity evaluation.
†Responder: complete or partial response, 77 patients were assessable for tumor response-evaluation.
‡Fisher's exact test for all genotypes.
§Exact test of Cochran-Armitage trend test across genotypes.
¶Kruskal-Wallis or Mann-Whitney test.
‖The UGT1A9*22 genotyping failed in two patients.

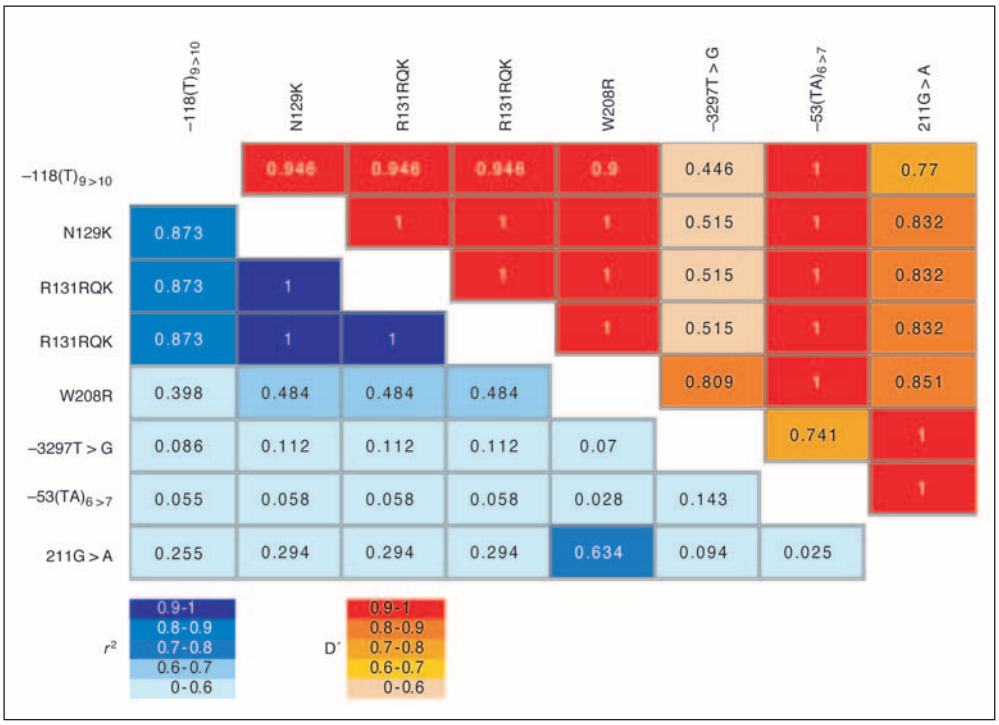

Fig 2. Linkage disequilibrium analysis for UGT1A1[r], *7*, and *9* single nucleotide polymorphisms. D′ (upper, red) and r^2 (lower, blue) values are shown in each square.

*UGT1A1*6/*6* showed significantly shorter progression-free survival ($P = .001$; Fig 3A) and overall survival ($P = .017$; Fig 3B) when compared with patients with other genotypes. Meanwhile, no significant difference in survival outcome was observed according to *UGT1A1*28*, *UGT1A1*60*, and *UGT1A9*22* genotypes.

Haplotype Analysis

We constructed haplotypes using those five polymorphisms including *UGT1A9*22*, *UGT1A7**, *UGT1A1*60*, *UGT1A1*28*, and *UGT1A1*6* to clearly show the effect of those key SNPs, and found 16 haplotypes. The three most common haplotypes were 1, 2, and 3, which account for 82.7% of all haplotypes (Table 4). A total of 23 diplotypes were found, and the commonly observed diplotypes were 1,3 (n = 16); 1,2 (n = 16); 1,1 (n = 16); 2,2 (n = 5); and 2,3 (n = 4; Table 5).

We analyzed the effects of the most commonly observed haplotypes, 1, 2, 3, and 4, on the $AUC_{SN\text{-}38G}$-$AUC_{SN\text{-}38}$ ratio. The Kruskal-Wallis test showed that only haplotypes 1 ($P = .008$, Fig 4A) and 2 ($P = .01$, Fig 4B) were significantly associated with the $AUC_{SN\text{-}38G}$/$AUC_{SN\text{-}38}$ ratio. The presence of haplotype 1 (+/− or +/+) showed significantly higher $AUC_{SN\text{-}38G}$-$AUC_{SN\text{-}38}$ ratios than the absence of

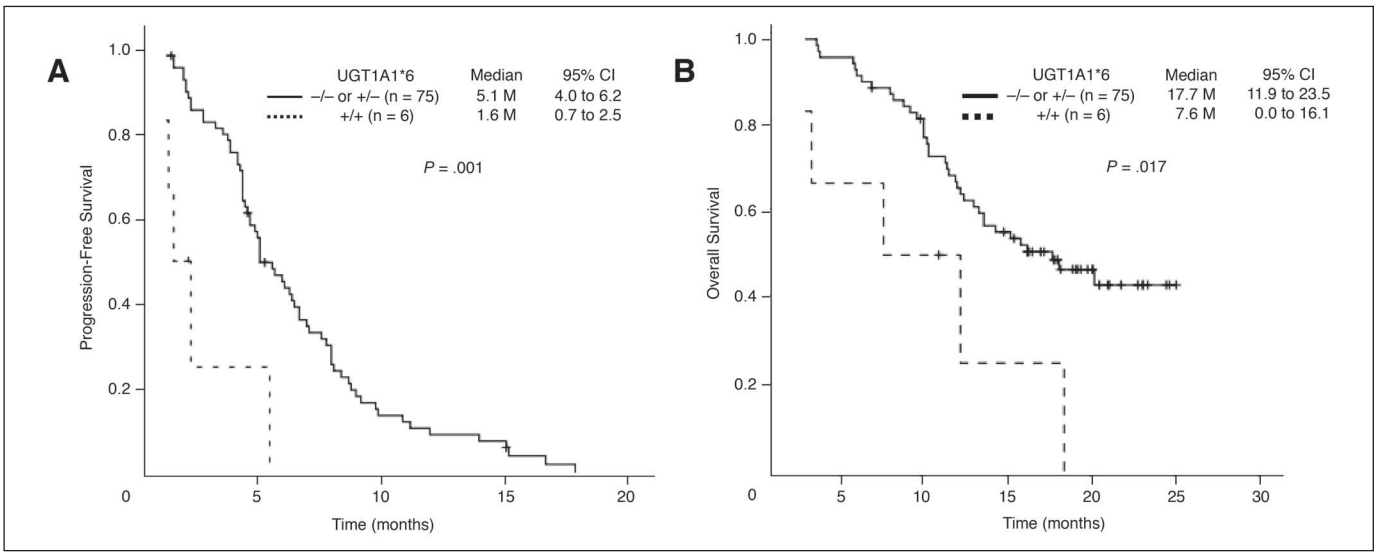

Fig 3. Progression-free survival (A) and overall survival (B) according to UGT1A1*6[r] diplotypes. Patients with *UGT1A1*6/*6* (n = 6) diplotypes showed significantly reduced progression-free and overall survival.

Table 4. Haplotype Frequency

Haplotype No.	Haplotype: UGT1A9*22 −118(T)$_{9>10}$	Haplotype: UGT1A7 (*)	Haplotype: UGT1A1*60 −3279T >G	Haplotype: UGT1A1*28 −53(TA)$_{6>7}$	Haplotype: UGT1A1*6 211G > A	Frequency (%; N = 162)
1	10	TCGT (*1)	T	6	G	47.5
2	9	GAAC (*3)	T	6	A	20.4
3	9	GAAT (*2)	G	6	G	14.8
4	10	TCGT (*1)	G	7	G	4.9
5	9	GAAC (*3)	T	6	G	3.1
6	10	TCGT (*1)	T	6	A	2.5
7	9	TCGT (*1)	T	7	G	1.2
8	9	GAAC (*3)	G	7	G	0.6
9	9	GAAT (*2)	G	7	G	0.6
10	9	GAAC (*3)	G	6	G	0.6
11	9	TCGT (*1)	T	6	G	0.6
12	10	GAAC (*3)	G	6	G	0.6
13	10	GAAC (*3)	T	6	A	0.6
14	10	GAAT (*2)	T	6	G	0.6
15	10	TCGT (*1)	G	6	G	0.6
16	9	TCGT (*1)	G	6	G	0.6

haplotype 1 (−/−; $P = .016$ and 0.003, post hoc analysis by the Mann-Whitney test, Fig 4A), whereas the homozygous haplotype 2 (+/+) showed a significantly lower $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ ratio than the absence of haplotype 2 (−/−; $P = .001$, post hoc analysis by the Mann-Whitney test, Fig 4B). Haplotypes 1 and 2 differ by the presence of *UGT1A1*6*, *UGT1A7*3*, and *UGT1A9*-118(T)$_9$. Because *UGT1A7* variants were highly linked with *UGT1A1*6* or *UGT1A9*22*, these findings suggest that *UGT1A1*6* and *UGT1A9-118* may be functionally important for SN-38 glucuronidation.

Table 5. Diplotype Frequencies

Diplotype	Frequency (%; N = 81)
1,1	19.80
1,2	19.80
1,3	19.80
2,2	6.20
2,3	4.90
3,4	3.70
1.4	2.50
1,5	2.50
1,7	2.50
4,5	2.50
1,6	1.20
1,8	1.20
1,9	1.20
1,11	1.20
1,12	1.20
1,13	1.20
1,15	1.20
2,6	1.20
2,10	1.20
2,14	1.20
3,6	1.20
4,6	1.20
5,16	1.20

DISCUSSION

To elucidate the most important functional polymorphism that determine the clinical outcome of NSCLC patients treated with irinotecan-based chemotherapy, we examined the interaction of various candidate polymorphisms of the *UGT1A1*, *UGT1A7*, and *UGT1A9* genes. In our study, *UGT1A1*6/*6* and *UGT1A9-118(dT)$_{9/9}$* were associated with significantly lower $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ ratios ($P = .002$ and $P = .001$, respectively). This was also substantiated in haplotype analysis. Although the *UGT1A7*3* allele was related to lower SN-38 glucuronidation activity, high LD was observed across *UGT1A7*3*, *UGT1A1*6*, and *UGT1A9*22*, which attributed to the specific phenotype of *UGT1A7*3* in this study. Furthermore, those patients with homozygous *UGT1A1*6* or *UGT1A9-118(T)$_{9/9}$* had higher incidences of irinotecan-related severe toxicities. Specifically, patients with homozygous *UGT1A1*6* had lower tumor response rates and shorter progression-free and overall survival than the patients with other genotypes. These findings suggest that *UGT1A1*6* and *UGT1A9*22* genotypes might be important for SN-38 glucuronidation and also could predict the clinical outcome of Korean patients treated with irinotecan-based chemotherapy.

Regarding the effects of *UGT1A* genotypes on clinical outcome, Carlini et al[32] reported that *UGT1A9-118(dT)$_{9/9}$* and *UGT1A7*2/*2* and **3/*3* were significantly associated with better response and less severe toxicity in patients with colorectal cancer. In this study, we found that patients with *UGT1A9-118(dT)$_{9/9}$* or *UGT1A7*3/*3* were significantly associated with a decreased $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ ratio and had a trend for higher incidence of severe toxicities. Because glucuronidation is the major route of detoxification and elimination of active metabolite SN-38, inherited differences in irinotecan glucuronidating capacity is known to have an important influence on the pharmacokinetics and toxicity of irinotecan.[3,9,33] Furthermore we found that *UGT1A9-118(dT)$_{9>10}$* is highly linked with *UGT1A7* variants. These findings with the well-balanced association of a decreased $AUC_{SN\text{-}38G}/AUC_{SN\text{-}38}$ ratio and higher incidence of severe toxicity in

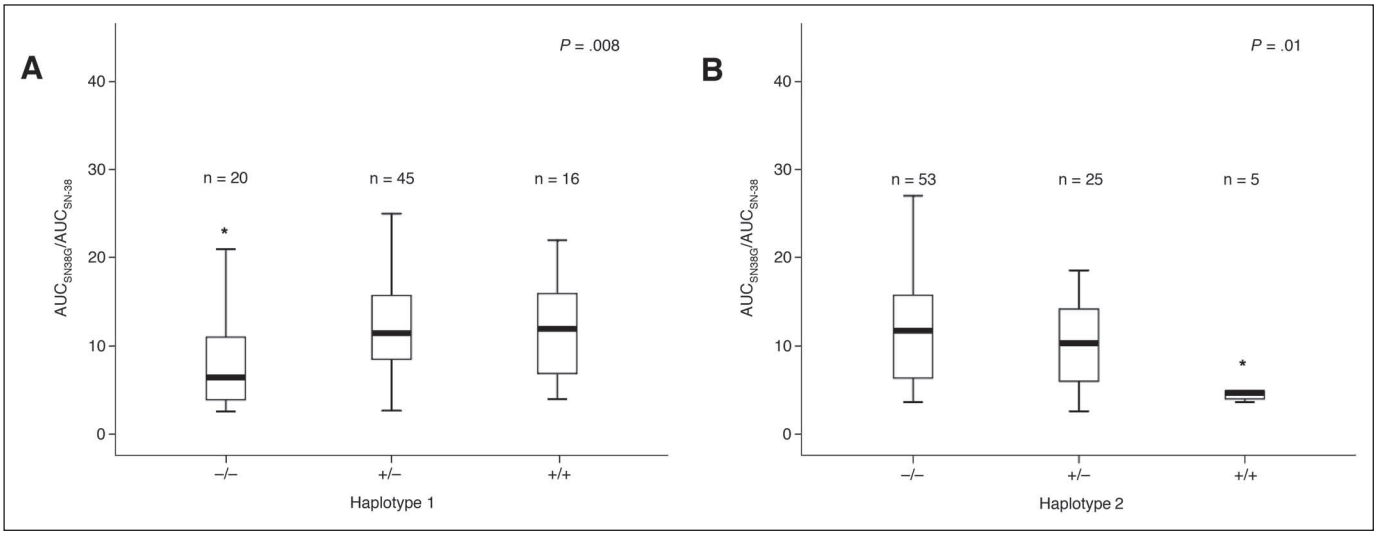

Fig 4. SN-38 glucuronidation activities according to haplotypes 1 (A) and 2 (B). Data are expressed as AUC_{SN-38G}-AUC_{SN-38} chromatographic peak heights. Bars represent the mean value. Group data were compared using Kruskal-Wallis analysis. Bonferroni adjustment was performed to determine the level of significance ($P < .017$). (*) $P < .017$; P was obtained by post hoc analysis by the Mann-Whitney test.

our study support the adverse pharmacogenetic effects of *UGT1A9-118(dT)$_{9/9}$* in irinotecan-based chemotherapy.

Several studies have demonstrated an association between *UGT1A1*28*, a low-activity phenotype, and the increased incidence of irinotecan-related severe toxicity.[15-17,33] Indeed, we observed a decreased AUC_{SN-38G}/AUC_{SN-38} ratio in patients with the *UGT1A*28* allele; however, it was not statistically significant when compared with the wild type in this study. In fact, *UGT1A1*28* is highly prevalent in white individuals, with reported frequencies of 0.29 to 0.47,[34,35] while it has much lower frequency (0.08 to 0.19) in Asians.[36] In this study, the allele frequency of *UGT1A1*28* was 0.07. Furthermore, there was no homozygous *UGT1A1*28*. Therefore, we could not completely rule out the functional importance of *UGT1A1*28* in this study. Meanwhile, *UGT1A1*6* is more prevalent (0.11 to 0.13) than *UGT1A1*28* among the Asian population. It is also most commonly associated with Gilbert's syndrome among Asians.[37] In our study, the allele frequency of *UGT1A1*28* was lower than that of *UGT1A1*6* (0.148 *v* 0.395, $P = .001$). This finding suggests that *UGT1A1*6* may be more important than *UGT1A1*28* in predicting the outcome of irinotecan-containing treatment among Korean patients.

In summary, comprehensive analysis of *UGT1A1*, *UGT1A7*, and *UGT1A9* genotypes showed that theses genes are strongly linked to each other and the interaction among functional polymorphisms are related to the alteration in the activity of these enzymes. Although it is still hypothetical, we suggest that *UGT1A1*6* and/or *UGT1A9*22* genotypes might be important for predicting severe toxicity and treatment outcome after irinotecan-based chemotherapy. To confirm the data observed in this study, further larger studies are needed in an independent data set, preferably in a group of patients of similar ethnicity.

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Authors' Disclosures of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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