Original Article

UDP-Glucuronosyltransferase 1A7 Polymorphisms Related to Tumor Response in Taiwanese Patients Treated with Irinotecan-Based Chemotherapy

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Background. Many studies have reported an association between genetic variants of the *UGT1A* gene (uridine diphosphate-glucuronosyltransferase, UGT) and the development of toxicities. Considerable ethnic differences in genetic variations of the UGT1A locus have also been observed. The aim of this study was to comprehensively investigate genetic variation in UGT1A1/UGT1A7/UGT1A9, in order to evaluate the clinical influences of toxicities and subsequent outcome in Taiwanese patients undergoing irinotecan-based chemotherapy.
Patients and Methods. One hundred and fifteen patients with metastatic colorectal cancer treated with irinotecan based chemotherapy were recruited. Genomic DNA was extracted from peripheral blood and genotyped using PCR-based methods. We analyzed the association between UGT1A genotypes and development of toxicities and response to chemotherapy.
Results. Only one of the 115 patients (0.9%) was homozygous for UGT1A1*28 in this study. We observed a significant correlation between the low activity UGT1A1*28 genotypes and increasing incidence of neutropenia (odds ratio 2.42, $p = 0.049$). There was also a trend of association between UGT1A1*6 [211G>A] genotype and the incidence of neutropenia (odds ratio 2.18, $p = 0.084$) and between association between UGT1A7 [622T>C] genotype and the incidence of neutropenia (odds ratio 2.05, $p = 0.087$). By genotype, patients with homozygous UGT1A7 [387G/G] showed a significantly lower response rate (31.2% vs. 61.8%, OR = 0.28, $p = 0.049$). Patients with UGT1A haplotype II also showed marginally significantr lower response rates (31.3% vs. 66.7%, OR = 0.23, $p = 0.053$).
Conclusion. Genotyping of UGT1As polymorphisms provides significant predictors for neutropenia occurrence and tumour responses in metastatic CRC patients receiving a FOLFIRI regimen.
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Irinotecan-based chemotherapy has been used worldwide for the treatment of metastatic colorectal cancer for several years. Its efficacy is dependent on activation by carboxylesterases to form the active meta-

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bolite SN-38.[1,2] The major route of SN-38 elimination is via the action of several UDP-glucuronosyltransferase (UGT) including hepatic UGT1A1 and UGT1A9 and extrahepatic UGT1A7.[3-8] Many studies have therefore reported an association between genetic variants of the *UGT1A* gene and the development of toxicities following irinotecan-based chemotherapy. Promoter polymorphisms in the *UGT1A1* gene, such as UGT1A1*28 ($A(TA)_7TAA$ instead of $A(TA)_6TAA$ at nucleotide -53) were first reported to be responsible for irinotecan-induced toxicities in Caucasians.[4-6] Several other polymorphisms of the UGT1A1 enzyme have also been described. In Asia, variants within the coding region of the *UGT1A1* gene, including UGT1A1*6 (G>A at nucleotide 211), UGT1A1*27 (C>A at nucleotide 686) and UGT1A1*60 (T>G at nucleotide -3279), have also been found to be associated with the reduced glucuronidation of SN-38.[4-9] It was thus recommended that these genetic factors be analyzed, in addition to testing for UGT1A1*28 to more accurately predict irinotecan-related toxicity, at least in Asian patients.[10]

Although glucuronidation catalyzed by the UDP-glucuronosyl-transferase 1A enzyme is mainly mediated by hepatic UGT1A1, UGT1A7 and UGT1A9 [3,5,10-12] are also involved. Studies have revealed that polymorphisms of UGT1A7 and UGT1A9, such as UGT1A7*3 (T>G at nucleotide 387/C>A at nucleotide 391/T>C at nucleotide 622); and UGT1A9*22 ((dT)9/9 at nucleotide -118), result in a significant decrease in SN-38 glucuronidating capacity.[13-15] The existence of significant variability of UGT1A genetic variants related to patient variability in response and toxicity may highlight individuals who could be benefit from these pharmacogenetic studies. However, few papers describing studies undertaken in Asian population have been published in this field, except from Japan, and some ethnic differences in distribution of genetic variants have been reported.[16-22]

The study of UGT1A in the Taiwanese population was reported in 2000.[17] Results of that study demonstrated that the frequency of the $A(TA)_7TAA$ allele in the promoter area of the *UGT1A1* gene is substantially lower for Taiwanese people (14.3%) in comparison to Caucasians (35.7-41.5%), while the rate of variation within the coding region of the *UGT1A1* gene is much higher for the Taiwanese than for Caucasians (29.3% vs. 0.1%).[20] It is known that both variations in the promoter area and within the coding region of *UGT1A1* gene are involved in Gilbert's syndrome in Taiwanese people.[18,19] In addition, a recent study reported that Taiwanese patients with combined genotypes carrying UGT1A7 variant alleles and UGT1A1 variant alleles (including UGT1A1*28 and UGT1A1*6) are associated with increased risk of Gilbert's syndrome.[20] Therefore, more comprehensive research on the UGT1A system is necessary for an evaluation of the consequences of irinotecan therapy in Taiwanese populaion. This study attempted to comprehensively investigate genetic variation in genes important in the metabolism of irinotecan, including the promoter area and coding region of UGT1A1/UGT1A7/UGT1A9 to evaluate the clinical influences of toxicities and subsequent outcome in Taiwanese patients undergoing irinotecan treatment as a first-line therapy.

Patients and Methods

Patient selection and treatment course

The patients included in this study were previously treated with biweekly irinotecan (180 mg/m^2) plus 5FU/LV (FOLFIRI) with or without targeted agent (cetuximab, bevacetumab, or sutent), as afirst-line chemotherapy for metastatic CRC. All patients (1) had been diagnosed with pathology-proven colorectal adenocarcinoma, either metastatic or non-resectable locoregional relapse; (2) were age between 18 and 70 years; (3) had an Eastern Cooperative Oncology Group (ECOG) performance status of 0-2, and life expectancy of 3 months or more; (4) had serum creatine $\leqq$ 1.5 mg/dl, serum bilirubin $\leqq$ 2.0 mg/dl, WBC > 3000/cmm, and platelet count > 10^5/cmm at baseline; (5) had no bowel obstruction; and (6) received irinotecan plus 5-FU and LV as first-line treatment regimens. The responses were evaluated on the basis of standard World Health Organization criteria. Treatment-related toxicities were evaluated according to the National Cancer Institute Common Toxicity Criteria, with particular attention to neutropenia and diarrhea. Dose modification was made during the

treatment course. All these data were collected from the reviewing medical chart records. The phamacogenetic study protocol was approved by the IRB committee of Chang Gung Memorial Hospital.

Determination of the *UGT1A* genes

Total genomic DNA was isolated from peripheral blood cells using a blood DNA isolation kit (Maxim Biotech Inc. San Francisco, CA, USA). For the *UGT1A1* gene, the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method was applied to detect the four known variants in the *UGT1A1* gene in the Taiwanese: UGT1A1*28 ($A(TA)_7TAA$ instead of $A(TA)_6TAA$ at nucleotide -53), UGT1A1*6 (G>A at nucleotide 211), UGT1A1*27 (C>A at nucleotide 686), and UGT1A1*60 (T>G at nucleotide -3279), as described previously.[17,23] The DNAs of the known variations at promoter area and nucleotides 211, 686, and -3279, which were discovered in the Taiwanese and identified by the DNA sequencing method,[17,23] were run as positive controls in each performance of the PCR-RFLP genotyping assay. The primers and restriction enzymes used for determination of the four known SNPs in the *UGT1A1* gene have been described previously.[26] For the *UGT1A7* gene, the UGT1A7 genotypes were identified by determining variations at nucleotides 622 and 387 of the *UGT1A7* gene, using RFLP with the enzymes Rsa and Afl, as described previously.[10,11] The DNAs of UGT1A7*1/*1 (wild-type), *1/*2, 1/*3, *2/*2*, *2/*3 and *3/*3, determined and identified using DNA sequencing,[10] were run as controls for each RFLP genotyping assay. For the *UGT1A9* gene, heterozygous variation and homozygous variation at nucleotide -118 in the *UGT1A9* gene [A(T)10AT instead of A(T)9AT, were analyzed by direct sequencing.

Statistical analysis

We evaluated the association between UGT1A1/UGT1A7/UGT1A9 genotypes/haplotypes and risk of severe toxicities and chemotherapy responses. Each polymorphism was tested to ensure that it fitted the Hardy-Weinberg equilibrium. Simple logistic regression was utilized to calculate odds ratio (OR) and the 95% confidence interval (CI) for severe toxicities for each genotype and haplotype observed. The frequencies of complete remission, partial remission, overall response, stable disease and progressive disease for the patients carrying the different genotypes/haplotypes were also compared with those bearing the wild-type in the *UGT1A1*/*UGT1A7*/*UGT1A9* genes using the Cox logistic regression test.

All data described in this study were analyzed using the Statistical Package for Social Sciences [version 10.0 software SPSS for Windows Inc., Chicago, IL, USA]. A p value <0.05 and/or a 95% CI of the OR above, or below 1.0, were defined as constituting statistical significance. However, only categories of UGT1A1/UGT1A7/UGT1A9 haplotypes with a number of patients is > 5 were included in the statistical analysis for the haplotypes of combination of the *UGT1A1*/*UGT1A7*/*UGT1A9* genes.

Results

Information on the patients included in this study is summarized in Table 1 including age, gender, and chemotherapy regimens. In general, we did not observe significant differences in toxicity and response rate amongst different combination therapy subgroups (Table 1). Among the different therapies there was a total of 25 patients (21.8%) treated with dose reduction due to toxicities, as shown in Table 1. However, we did not observe any significant difference between different combination regimens and responses here either (Table 1).

UGT1A allele, genotype and haplotype frequencies

The UGT1A1, UGT1A7, and UGT1A9 variants analyzed in this study are listed in Table 2. Variants of UGT1A7 have been designated in previous reports as follows: UGT1A7*1 (N129K131R208), UGT1A7*2 (K129K131W208), UGT1A7*3 (K129K131R208) As expected, the frequency of the $-53(TA)_7$ (*28) allele was very low compared with the data in white patients. Only one of the 115 patients (0.9%) was homozygous for UGT1A1 (*28) in this study. Table 3

Table 1. Patients demography, toxicities, responses and different irinotecan based regimens

	Treatment regimen			
	FOLFIRI only	FOLFIRI with Cetuximab	FOLFIRI with Bevacetumab	FOLFIRI with Sunitinib
Total patient No.	90	6	8	11
Gender (M/F)	48/42	3/3	5/3	5/5
Age (mean/range)	62 (34-84)	56 (31-80)	59 (50-69)	54 (37-83)
Dose reduction/ No. (%)	22 (24.4)	1 (16.7)	0 (0)	2 (18.2)
Diarrhea/No. (%)	10 (11.1)	1 (16.7)	1 (12.5)	0 (0)
Neutropenia/No. (%)	36 (40.0)	2 (33.3)	1 (12.5)	6 (54.5)
PR/No. (%)	27 (30.0)	1 (16.7)	4 (50.0)	8 (72.7)
SD/No. (%)	24 (26.7)	2 (33.3)	0 (0)	3 (27.3)
PD/No. (%)	11 (12.2)	2 (33.3)	1 (12.5)	0 (0)

PR: Partial response; SD: Stable disease; PD: Progression disease.

Table 2. Genotype and allele frequencies of UTG1A in 115 Taiwanese

UGT1A Genotype	Variant	No	Frequency%	Allele	No	Freq.%
UGT1A1*28	*1/*1	88	76.5	*1	202	87.8
	*1/*28	26	22.6	*28	28	12.2
	*28/*28	1	0.9			
UGT1A1*6	G/G	89	77.4	G	204	88.7
	G/A	26	22.6	A	26	11.3
UGT1A1*27	C/C	109	94.8	C	224	97.4
	C/A	6	5.2	A	6	2.6
UGT1A1*60	T/T	45	39.1	T	148	64.3
	T/G	58	50.4	G	82	35.7
	G/G	12	10.5			
UGT1A7*3	*1/*1	42	36.5	*1	141	61.3
	*1/*2	35	30.4	*2	54	23.5
	*1/*3	22	19.1	*3	35	15.2
	*2/*2	5	4.3			
	*2/*3	9	7.8			
	*3/*3	2	1.7			
UGT1A9*22	*1/*1	38	33.0	*1	136	59.1
	*1/*22	60	52.2	*22	94	40.9
	*22/*22	17	14.8			

shows UGT1A haplotypes; only haplotypes I, II, III, and IV with a frequency > 5%, were included in toxicity and response analysis.

Genotype correlation with toxicity

Twelve of the 115 patients developed grade 3 or 4 diarrhea (10.4%) and forty-five developed grade 4 neutropenia (39.1%, Table 1). Therefore, the predominant toxicity observed was neutropenia (45 patients) as opposed to diarrhea (12 patients). We observed a significant correlation between the low activity UGT1A1*28 genotypes (UGT1A1*28/*28, and UGT1A1*1/*28) and increasing incidence of neutropenia (OR = 2.42, $p = 0.049$, Table 4). There was also a trend of association between UGT1A1*6 [211G >A] genotype and incidence of neutropenia (OR = 2.18, $p = 0.084$) and between UGT1A7 [622T>C] genotype and incidence of neutropenia (odds ratio 2.05, $p = 0.087$). However, there was no statistically significant association between UGT1A1*27 [686 C>A] and UGT1A1*60 [-3279T>G] genotypes and toxic events (Table 4). Moreover, we did not find other statistically significant association of UGT1A7*3 and

Table 3. Haplotype of UGT1A frequencies in 115 Taiwanese

Haplotype	UGT1A1				UGT1A7		UGT1A9	No.	%
	211	686	-3279	A(TA)nTAA	387	622	-118		
I	G	C	T	6	T	T	(T)10	27	23.5
II	G	C	T/G	6	T/G	T	(T)9/10	27	23.5
III	G/A	C	T	6	T/G	T/C	(T)9/10	11	9.56
IV	G/A	C	T/G	6, 7	T/G	T/C	(T)9/10	10	8.69
V	G	C	T/G	6, 7	T	T	(T)10	5	4.34
VI	G/A	C	T/G	6	G	T/C	(T)9	4	3.48
VII	G	C	G	6	G	T	(T)9	4	3.48
VIII	G	C	T	6	T/G	T/C	(T)9/10	3	2.60
IX	G	C	G	6	T/G	T	(T)9/10	2	1.73
X	G	C	G	6, 7	T/G	T	(T)9/10	2	1.73
XI	G/A	C	T	6	G	T/C	(T)9	2	1.73
XII	G/A	C	T/G	6, 7	G	C	(T)9	2	1.73
XIII	G	C/A	T/G	6, 7	T	T	(T)10	2	1.73
XIV	G	C	T/G	6	T	T	(T)10	2	1.73
XV	G	C	T/G	6	G	T/C	(T)9	1	0.86
XVI	G	C	T/G	6	G	T	(T)9	1	0.86
XVII	G	C	T/G	6, 7	G	T/C	(T)9	1	0.86
XVIII	G	C	T/G	6	T/G	T/C	(T)9/10	1	0.86
XIX	G/A	C	T	6	T	T	(T)10	1	0.86
XX	G	C/A	T/G	6, 7	T/G	T/C	(T)9/10	1	0.86
XXI	G	C/A	T/G	6, 7	T/G	T	(T)9/10	1	0.86
XXII	G	C/A	T/G	6, 7	G	T/C	(T)9	1	0.86
XXIII	G	C/A	G	6, 7	G	T	(T)9	1	0.86
XXIV	G/A	C	T/G	6	T/G	T/C	(T)10	1	0.86
XXV	G	C	G	7	T	T	(T)10	1	0.86
XXVI	G	C	T	6	T/G	T	(T)10	1	0.86

UGT1A9 -118$(dT)_{9/9}$ genotypes and toxic events (Table 4).

Genotype correlation with tumor response

Of the 115 patients enrolled, 84 were assessable for response evaluated by imaging studies, and 40 of these 84 assessable patients (48%) achieved partial responses. By genotype, patients with homozygous UGT1A7 [387G/G] showed a significantly lower response rate (31.2% vs. 61.8%, OR = 0.28, $p = 0.049$; Table 5). Among individuals with low-activity UGT1A7*2/*2* and UGT1A7*3/*3 genotypes, the response rate was 33.3% (5/15 patients) compared with 62% (21/34 patients) with UGT1A7*3 wild-type (p = 0.072) Table 5. Furthermore, the -118 $(dT)_{9/9}$ genotype showed a significant association with lower tumor response when compared with all other genotypes (31.3% or 5 of 16 patients with -118 $(dT)_{9/9}$ versus 59.4% or 19 of 32 patients with -118 $(dT)_{10/10}$ genotypes, $p = 0.072$). Patients with UGT1A haplotype II also showed marginal significance for lower response rate (31.25% vs. 66.7%, OR = 0.23, $p = 0.053$).

Linkage disequilibrium analysis

We observed that the low-activity UGT1A7*2, and UGT1A7*3 alleles were completely associated with the UGT1A9-118 $(dT)_9$ allele, whereas the high activity UGT1A7*1 allele was linked with the UGT1A9-118 $(dT)_{10}$ allele (likelihood ratio test of association $p < 0.001$).

Discussions

In this study, we reported results from a follow-up UGT1A genotyping study of 115 metastatic colorectal

Table 4. UGT1A polymorphism and haplotype related to severe toxicity by univariate analysis

Polymorphism	Total No.	Severe toxcity during the entire course of cycles									
		Neutropenia					Diarrhea				
		No	%	OR	95%CI	*p*	No	%	OR	95%CI	*p*
UGT1A1											
*1											
*1/*1	88	30	34.0	1.00			10	11.4	1.00		
*1/*28, *28/*28	27	15	55.6	2.42	1.00-5.81	0.049	2	7.4	0.62	0.13-3.04	0.559
*6 [211G>A]											
GG	89	31	34.8	1.00			8	9.0	1.00		
GA, AA	26	14	53.8	2.18	0.90-5.29	0.084	4	15.4	1.84	0.51-6.68	0.354
*27 [686C>A]											
CC	109	41	37.6	1.00	-	-	11	10.1	1.00	-	-
CA, AA	6	4	66.7	3.32	0.58-18.9	0.177	1	16.7	1.78	0.19-16.6	0.613
*60 [-3279T>G]											
TT	45	16	35.6	1.00	-	-	6	13.3	1.00	-	-
TG	58	23	39.7	1.19	0.53-2.67	0.671	4	6.9	0.48	0.13-1.82	0.282
GG	12	6	50.0	1.81	0.50-6.56	0.365	2	16.7	1.30	0.23-7.44	0.768
UGT1A7											
387T>G											
TT	52	17	32.7	1.00	-	-	2	3.8	1.00	-	-
TG	56	20	35.7	0.82	0.36-1.86	0.631	8	14.3	3.33	0.67-16.6	0.142
GG	17	8	47.1	1.31	0.42-4.06	0.643	2	11.8	2.67	0.34-20.67	0.348
622T>C											
TT	82	28	34.2	1.00	-	-	7	8.5	1.00	-	-
TC, CC	33	17	51.5	2.05	0.90-4.66	0.087	5	15.2	1.91	0.56-6.53	0.300
UGT1A7*3											
0	42	17	40.4	1.00	-	-	2	4.8	1.00	-	-
1	57	21	63.2	0.86	0.38-1.94	0.713	8	14.0	3.27	0.66-16.2	0.148
2	16	7	56.2	1.14	0.36-3.66	0.821	2	12.5	2.86	0.37-22.2	0.316
UGT1A9											
*1/*1	38	17	44.7	1.00	-	-	2	5.2	1.00	-	
*1/*22	60	20	33.3	0.59	0.20-1.77	0.348	8	13.3	1.20	0.23-6.27	0.829
*22/*22	17	8	47.1	0.83	0.27-2.60	0.751	2	11.8	0.39	0.05-3.06	0.374
UGT1a haplotype											
I	27	9	33.33	1.00	-	-	2	7.4	1.00	-	-
II	27	5	18.52	0.45	0.13-1.60	0.219	3	11.1	1.56	0.24-10.2	0.641
III	11	6	54.55	3.00	0.67-13.4	0.150	3	27.3	5.36	0.74-38.6	0.096

cancer patients treated with FOLFIRI with/without targeted agent, as the first-line therapy. The frequencies of UGT1A1, UGT1A7, and UGT1A9 alleles and genotypes in this study of the Taiwanese population were found to be similar to those reported previously for Asian populations.[16-22] However, a significant association was found only between the UGT1A1*28 genotypes and the severe neutropenia caused by irinotecan (34% vs. 55.6%, OR = 2.42, $p = 0.049$; Table 4) was found. Surprisingly, patients with homozygous UGT1A7 [387G/G] showed a significantly lower response rate (31.25% vs. 61.76%, OR = 0.28, $p = 0.049$; Table 5). Patients with UGT1A haplotype II also showed a marginally significant lower response rate (31.3% vs. 66.7 %, OR = 0.23, $p = 0.053$).

Concerning the allele or genotype frequencies, East Asian have a lower allelic frequency of UGT1A*28 than Caucasians.[24] The frequency of the homozygous variant genotype (*28/*28) in this study was only 0.9% (1/115 patients). This is comparable with previous studies of UGT1A1 *28/*28 polymorphism in population of Asian origin, which showed an

Table 5. UGT1A polymorphism and haplotype related to chemotherapy response

Polymorphism	Total No.	Respose to chemotherapy				
		No	%	OR	95%CI	*p*
UGT1A1						
*1						
*1/*1	64	30	46.88	1.00	-	-
*1/*28, *28/*28	20	11	55.00	1.39	0.51-3.80	0.527
*6 [211G>A]						-
GG	66	34	51.52	1.00	-	
GA, AA	18	7	38.89	0.60	0.21-1.73	0.345
*27 [686C>A]						
CC	78	36	46.15	1.00	-	
CA, AA	6	5	83.33	5.83	0.65-52.2	0.115-
*60 [3279T>G]						
TT	34	18	52.94	1.00	-	
TG	40	18	45.00	0.73	0.29-1.82	0.496
GG	10	5	50.00	0.89	0.22-3.64	0.870
UGT1A7						
[387T>G]						
TT	34	21	61.76	1.00	-	-
TG	34	15	44.12	0.49	0.19-1.29	0.147
GG	16	5	31.25	0.28	0.08-1.00	0.049
[622T>C]						
TT	60	32	53.33	1.00	-	-
TC, CC	24	9	37.50	0.53	0.20-1.38	0.193
UGT1A7*3						
0	34	21	61.76	1.00	-	
1	35	15	42.86	2.15	0.18-1.22	0.118
2	15	5	33.33	3.23	0.09-1.11	0.072
UGT1A9						
*1/*1	16	5	31.25	1.00	-	-
*1/*22	36	17	47.22	1.97	0.57-6.82	0.286
*22/*22	32	19	59.38	3.22	0.90-11.4	0.072
UGT1a haplotype						-
I	21	14	66.67	1.00	-	
II	16	5	31.25	0.23	0.06-0.92	0.053
III	6	2	33.33	0.25	0.04-1.71	0.158
IV	6	3	50.00	0.50	0.08-3.15	0.460

average frequency of 2% (0-6%).[24] The genotype frequencies observed for UGT1A1*6 also agree with those previously reported in an Asian population, although we excluded patients with elevated bilirubin, a phenotype associated with these two genotypes in East Asian patients.[25] This genetic variability might contribute to the impaired SN-38 glucuronidation phenotype in patients heterozygous for UGT1A1*6 (G/A). In this study (22.6%) is considerably higher than other East Asian groups (Taiwan Chinese 0.15 and Japanese 0.26) and whites (0.36). Considerable ethnic differences in other genetic variations of the UGT1A locus were also observed such as the frequency of the UGT1A7*3/*3 allele (1.7% in this study) and the frequencies of UGT1A9 -118 (*22/*22) (14.8% in this study). These result are comparable to the data previously reported in Asian patients.[26]

Several issues need to be considered in the investigation of the relationship between toxicities and genotypes of *UGT1A* gene. Firstly, it has been thought that UGT1A1 is the predominant enzyme responsible for the metabolism of SN-38 because of significant

associations between the homozygous genotype and in vivo SN-38 pharmacokinetics and irinotecan-related toxicities. However, the association between genotype and hematologic toxicity is influenced not only by genotype but also by the dose of irinotecan. A recent meta-analysis demonstrated that although UGT1A1*28/*28 genotype confers a high probability of hematologic toxicity at high irinotecan doses (> 250 mg/m^2), a comparatively low incidence of toxicity at low doses (<180 mg/m^2) is observed between these patients, and it is only moderately predictive of severe irinotecan-induced hematologic toxicity at intermediate doses (180-250 mg/m^2) of irinotecan.[27] Previous studies regarding UGT1A1 pharmacogenetics also suggests[8-10] an association between these alleles and the development of diarrhea has been much weaker. In this study, using a 180 mg/m^2 dosage, we did not observed a significant association between genotypes and diarrhea. We only confirmed the association of neutropenia with UGT1A*1/*28 and UGT1A*28/*28 genotypes. The relationships between other UGT1A polymorphisms and irinotecan-related toxicities warrant additional investigation in high-dosage irinotecan-containing regimens, to determine whether the relationships hold in Asian population such as the Taiwanese.

Secondly, some other genetic variants such as UGT1A7 and UGT1A9 have been reported to be additional markers for the prediction of severe toxicities. For instance, Cecchin et al found that the UGT1A7*3/*3 genotype is more predictive of hemoatologic toxicity than UGT1A1*28 after the first cycle of therapy.[28] The results of Cecchin et al imply that UGT1A7 might be an important player in the metabolism of SN-38. This enzyme is extrahepatic with expression observed in lung, esophagus, stomach, and pancreas, thus making its role in the disposition of irinotecan, an IV-administered drug unclear. However, some Japanese studies fail to observe a significantly different distribution of UGT1A7 genotypes between those with and without severe toxicity (grade 4 neutropenia and grade 3 and 4 diarrhea).[29] This therefore suggested that determination of UGT1A7 genotypes would not be useful for predicting severe toxicity of irinotecan. In this study, we only observed increased association of UGT1A7 related to neutropenia, but this was not statistically significant (UGT1A7 [622T>C], OR = 2.05, $p = 0.087$).

Thirdly, glucuronididation detoxicifies SN-38 in the liver, contributing to a lower systemic circulation of SN-38, and hence protection against systemic toxicities such as neutropenia. However, the toxic SN-38 molecule can be regenerated within the mucosa of the gastrointestinal tract, which may predispose for irinotecan-induced diarrhea. UGT1A7 is not expressed in the liver, but is expressed in the proximal gastrointestinal tract and therefore may influence the disposition of SN-38 within the gut.[30] However, in this study, we did not observe that different regimens of irinotecan predispose patients to different toxicities in relation to these extrahepatic genotypes.

Low UGT activity would predict higher tumor concentration of SN-38; therefore, low-activity UGT genotypes, such as UGT1A1*28 or UGT1A7*3, have been associated with better tumor response.[30,31] We were surprised that we did not observe this phenomenon. Furthermore, there is a lack of empirical evidence that dosing irinotecan on the basis of the genotype improves the safety of irinotecan without compromising the efficacy of the therapy. In theory, exposure to active metabolite of irinotecan, SN-38, is increased among the UGT1A1*28/*28 genotype patients. Therefore, adequate dose reduction has no deleterious consequences, since it will equalize drug exposure to the same level as in wild-type (UGT1A1*1/*1) patients. A previous paper by Ruzzo et al. did not report an association between UGT1A1*28 and progression-free survival in 75 colorectal cancer patients treated with FOLFIRI.[32] However, some studies have also shown that low-intensity chemotherapy regimens can lead to a decreased survival in patients with various solid tumor types.[32] The possible mechanism explaining the worse outcome of UGT1A7 387T>G patients would be the increased intratumoral inactivation of SN-38 in UGT1A7 387T >G patients, as previous studies have not shown any strong association with SN-38 AUC, ruling out a systemic antitumor effect of increased exposure to SN-38 in UGT1A7 387G/G patients. However, UGT1A7*3 and haplotype II should be studied additionally to assess whether they are useful markers for predicting response to irinotecan-based chemotherapy.

Regarding the haplotypes, theoretically, this study and our previous studies of the *UGT1A1* gene[17,20,26] demonstrated that the actual category number of haplotypes observed for the *UGT1A1* gene is only approximately 20 in the Taiwanese population (Table 3). Moreover, some sample size may be too small to make convincing interpretations. Therefore, only haplotypes that were present in more than 5 patients were included for analysis (Table 4 and 5) considering clinical relevance, because of cost-effective considerations of haplotype as an efficient type of predictor. Nevertheless, only haplotype II was marginally significant in relation to response was observed (p = 0.053). To some extent, pharmacogenomic studies[21] have demonstrated that SNPs considered together may provide better prediction of those patients who are at the highest risk of severe hematologic toxicity rather than individual SNPs. However, our sample size was too large to allow us to distinguish the clinical effects of several UGT1A variants from those of individual variants and to determine whether UGT1A haplotypes might be better predictors of the outcome of FOLIRI therapy than individual variants.

In summary, our data demonstrated UGT1A1*28 genotypes among Taiwanese patients related to severe neutropenia caused by irinotecan. Patients with homozygous UGT1A7 [387G/G] showed a significantly lower response rate and patients with UGT1A haplotype II also showed marginal significance for lower response rate.

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原　著

在台灣以抗癌妥為基礎的化療治療患者 UDP-葡萄糖醛酸轉移酶 1A7 基因多態性與腫瘤反應的相關性

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背景　尿核甘雙磷酸葡萄糖醛酸基轉移酵素 (uridine diphosphate-glucuronosyltransferase, UGT) 具有基因多型性，已有許多研究報告指出，UGT1A 基因的差異性與化療引起的毒性有關聯，而 UGT1A 基因在不同種族間有極大的差異。我們的目的在於研究台灣地區大腸直腸癌病人中，UGT1A1/UGT1A7/UGT1A9 基因的多型性是否會影響以 Irinotecan 為主的化學治療之毒性和臨床療效。

對象與方法　總共有 115 位罹患轉移性大腸直腸癌並接受以 Irinotecan 為主之化學治療的病人進入本臨床試驗，從這些病患的周邊血液萃取其基因組去氧核糖核酸 (genomic DNA) 並以聚合酵素連鎖反應為基礎的方式 (PCR-based) 作基因型的分析。我們藉此分析 UGT1A 基因型與化療毒性以及臨床治療結果之間的關連。

結果　115 位病人中只有一位 (0.9%) 具有 UGT1A1*28 基因。我們發現低活性的 UGT1A1*28 基因和嗜中性白血球低下的發生率增加有顯著的相關性 (Odd ratio 2.42, p = 0.049)。結果也顯示基因型 UGT1A1*6 [211G>A] 與嗜中性白血球低下的發生有相關趨勢 (odds ratio 2.18, p = 0.084) 且 UGT1A7 [622T>C] 也有相同趨勢 (odds ratio 2.05, p = 0.087)。根據基因型，具有同質性 UGT1A7 [387G/G] 的病患有較低的腫瘤反應 (31.2% v 61.8%, OR = 0.28, p = 0.049)。在 UGT1A haplotype II 的病患也有較低的腫瘤反應，呈現邊際的顯著相關性 (31.3% v 66.7%, OR = 0.23, p = 0.053)。

結論　在接受 FOLFIRI 化學治療之轉移性大腸直腸癌的病患，UGT1A 的基因多型性可作為預測嗜中性白血球低下的發生率和腫瘤對化療的反應性的顯著指標。

關鍵詞　大腸癌、抗癌妥、毒性、多態性。