

## Polymorphisms of *UGT1A1*\*6, *UGT1A1*\*27 & *UGT1A1*\*28 in three major ethnic groups from Malaysia

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**Background & objectives:** Genetic polymorphisms of uridine diphosphate glucuronyltransferase 1A1 (*UGT1A1*) have been associated with a wide variation of responses among patients prescribed with irinotecan. Lack of this enzyme is known to be associated with a high incidence of severe toxicity. The objective of this study was to investigate the prevalence of three different variants of *UGT1A1* (*UGT1A1*\*6, *UGT1A1*\*27 and *UGT1A1*\*28), which are associated with reduced enzyme activity and increased irinotecan toxicity, in the three main ethnic groups in Malaysia (Malays, Chinese and Indians).

**Methods:** A total of 306 healthy unrelated volunteers were screened for *UGT1A1*\*28, *UGT1A1*\*6 and *UGT1A1*\*27. Blood samples (5 ml) were obtained from each subject and DNA was extracted. PCR based methods were designed and validated for detection of *UGT1A1*\*6, *UGT1A1*\*27 and *UGT1A1*\*28. Direct DNA sequencing was performed to validate the results of randomly selected samples.

**Results:** Malays and Indian have two-fold higher frequency of homozygous of *UGT1A1*\*28 (7TA/7TA) which was 8 and 8.8 per cent, respectively compared to the Chinese (4.9%). However, the distribution of *UGT1A1*\*6 and *UGT1A1*\*27 showed no significant differences among them. *UGT1A1*\*27 which has not been detected in Caucasian and African American population, was found in the Malaysian Malays (3.33%) and Malaysian Chinese (2.0%).

**Interpretation & conclusions:** There was interethnic variability in the frequency of *UGT1A1*\*28 in the Malaysian population. Our results suggest that genotyping of *UGT1A1*\*6, *UGT1A1*\*28 and *UGT1A1*\*27 need to be performed before patients are prescribed with irinotecan due to their high prevalence of allelic variant which could lead to adverse drug reaction.

**Key words** Genetic polymorphism - interethnic - irinotecan - *UGT1A1*

The Food Drug Administration (FDA), USA had first approved irinotecan label with pharmacogenetics information in 2005<sup>1</sup>. In 2010, new pharmacogenetic

information was added to the label regarding the risk of neutropenia in patients who had genetic defect of uridine diphosphate glucuronosyltransferase 1A1

(*UGT1A1*)<sup>2</sup>. Irinotecan is used either in combination with 5-fluorouracil (5-FU) and leucovorin or as a monotherapy for the second-line therapy in the treatment of colorectal cancer<sup>3</sup>. After intravenous (iv) administration, irinotecan is converted to its active metabolite, 7-ethyl-10-hydroxycamptothecin (SN-38) by a carboxylesterase. SN-38 is then detoxified by *UGT1A1* enzyme to its inactive form SN-38 glucuronide (SN-38G) which is excreted into the bile and urine<sup>4</sup>. *UGT1A1* is found to be polymorphic and resulted in wide inter-individual variation in patient's response as well as toxic side effects<sup>4,5</sup>.

More than 30 genetic variants in the promoter region and exon 1 of *UGT1A1* have been reported to decrease enzyme activities and were related to diseases such as Criegler-Najjar (CN) and Gilbert syndrome<sup>6,7</sup>. Under-expression of *UGT1A1* enzyme impaired the metabolism of SN-38 to its inactive form (SN-38G) and caused an excessive accumulation of toxic SN-38. Polymorphism of *UGT1A1* is found responsible for the large inter-individual differences in the pharmacokinetics of irinotecan and risk of severe toxicity<sup>8,9</sup>.

*UGT1A1\*28* is a genetic polymorphism caused by an insertion repetitions of (TA)<sub>n</sub> in the (TA)<sub>6</sub> TAA-box (-53 to -38 insertion of TA) at the promoter region. An increased number of TA repeats may reduce transcription efficiency, lower the enzyme concentrations and thus lead to the accumulation of SN-38 and irinotecan-induced toxicity<sup>8</sup>. *UGT1A1\*28* has been reported to cause approximately 70 per cent reduction of *UGT1A* enzyme activity<sup>8</sup>. It has been reported that patients with homozygous *UGT1A1\*28* had severe toxicities of irinotecan compared to either wild type *UGT1A1\*1* or heterozygous *UGT1A1\*28*<sup>8</sup>.

Ando *et al*<sup>10</sup> showed that 80 per cent of patients who suffered from life-threatening toxicities have variant sequences due to *UGT1A1\*6* (211G>A) and *UGT1A1\*27* (686C>A). Patients with non-small-cell lung cancer and homozygous *UGT1A1\*6* had significantly lower tumour response rates and shorter progression-free as well as overall survival when treated with irinotecan-based chemotherapy<sup>9</sup>. It was also found that Japanese patients with heterozygous *UGT1A1\*27* experienced severe toxicities such as leucopenia (grade 4) and/or diarrhoea (grade 3 or worst)<sup>10</sup>.

In this study, polymerase chain reaction (PCR) based methods of allele specific PCR (ASPCR) and denaturing high performance liquid chromatography

(dHPLC) were used to investigate the distribution of allele frequencies of *UGT1A1\*28* (-53 to -38 insertion of TA) in the promoter region as well as *UGT1A1\*6* (211G>A) and *UGT1A1\*27* (686C>A) located at the coding region of exon 1 among the three major ethnic groups in Malaysia and other populations.

### Material & Methods

The study was conducted in the Pharmacogenomics Centre, Universiti Teknologi MARA, Malaysia during a period of three years from 2008-2010. This study was approved by the Research Ethics Committee of Universiti Teknologi MARA. Blood samples (5 ml) were collected from unrelated healthy volunteers after obtained a written informed consent. The participants were recruited from staff and students of Universiti Teknologi MARA and others, who were residents of Klang Valley, Malaysia at the point of participation. An advertisement was put up to recruit subjects and participation was voluntary. The participants were inquired about their ancestry up to 3 generations and classified according to Malaysian-Malays, Malaysian-Chinese or Malaysian-Indian. Those unsure of originality were excluded. Genomic DNA was extracted using alkaline lysis method described previously<sup>11</sup>. DNA was stored at -20°C until analysis.

*Genotyping of UGT1A1\*28 by denaturing high performance liquid chromatography (dHPLC)*: dHPLC method was developed to determine the insertion of extra (TA) in the (TA)<sub>6</sub> TAA-box at the promoter region. PCR was carried out to amplify the promoter region using designed primers (forward: 5' GTG AAC TCC CTG CTA CCT TTG 3' and reverse: 5' CTC TGC TGC AGC TGC TGG ATG GC 3') [RefSeq Gene: NG - 002601.2 (Genebank)].

PCR master mix consisted of detergent free buffer (20 mM Tris-HCl (tris-hydrochloride), 100 mM EDTA (ethylenediaminetetraacetic acid), 0.5 mM PMSF (phenylmethylsulfonyl fluoride), 1 mM DTT (DL-dithiothreitol) and 50% glycerol), 0.2 μM of each primers (forward and reverse), 0.2 mM of each dNTPs (deoxyribonucleotide triphosphate) and 1 unit of GoPhorIT™ *Taq* DNA Polymerase (Mbiotech Inc., Seoul, Korea) in a total reaction volume of 25 μl. The preparation of reaction mixtures for PCR was conducted on ice to reduce the chances of primers mis-annealing and artifacts formation.

Touch-down PCR was performed as follows: initial denaturation at 94°C for 3 min followed by 8 cycles of

denaturation at 94°C for 1 min; annealing at 66°C with decrement of 1°C each cycle for 1 min and extension at 72°C for 1 min. Another 25 cycles at 94°C for 1 min, 58°C for 1 min, 72°C for 1 min were performed.

Prior to dHPLC analysis, the amplicons from touch-down PCR were subjected to post-PCR denaturing and reannealing steps to ensure the formation of homoduplexes or heteroduplexes. This process was started at 95°C for 3 min, which slowly decreased at a rate of 1.0°C every cycle until a temperature of 65°C was reached. The samples were stabilised at 25°C for 30 sec. Then, 3 µl of the samples were injected to dHPLC Varian system (Variant Inc., San Jose, California, US) at the optimal partial denaturing temperatures. Homozygous samples were re-analysed by mixing the samples with wild-type known sequence which was the positive control at a ratio of 1 : 1. This produced a mix population of heteroduplexes of wild type-mutant or homoduplexes of wild type-wild type during the reannealing step. This is an essential step to differentiate homozygous wild type and homozygous mutant type samples which have different and unique retention times.

The optimised melting temperatures allowed us to detect variations of insertion of extra (TA)<sub>6</sub> TAA-box at the promoter region of *UGT1A1*. Changes in the profiles of the peaks were observed (Fig. 1 A to D). The peaks were verified using the Star Reviewer® software (Variant Inc., San Jose, California, US). The peaks were analyzed according to their unique chromatographic patterns of the DNA elution peaks and retention times (Fig. 2). All samples were screened and these showing different profiles were confirmed with direct sequencing.

*Genotyping of UGT1A1\*6 and UGT1A1\*27 by allele specific polymerase chain reaction (ASPCR)*: Genetic variations of *UGT1A1\*6* and *UGT1A1\*27* were determined using ASPCR method. Allele specific primers which enable the detection of *UGT1A1\*6* and *UGT1A1\*27* in two different tubes, were designed. PCR amplification was performed in two separate tubes with multiplex for wild-type of *UGT1A1\*6* and *UGT1A1\*27* in one, and allele specific primers for detection of variant in another. The expected sizes for *UGT1A1\*6* and *UGT1A1\*27* are 658 and 180 bp, respectively and visualisation of wild type and variant bands in separate lanes enabled the determination of genetic variation. An example of the results is shown in Fig. 2A - D.

The final PCR protocol comprised 25 µl of reaction mixture of 1 x PCR buffer (Biotools®, B & M Labs, S.A., Madrid, Spain), 2.0 mM MgCl<sub>2</sub>, 0.20 mM of each dNTPs (Promega Corporation Madison, WI, USA), 0.6 µM of common reverse primer and 0.2 µM of wild type and variant type primers (patented primer sequences, A PCR Genotyping Kit for Detection of *UGT1A1\*6* and *UGT1A1\*27*'- PI 20094933), 100 to 200 ng (2 µl) genomic DNA as template and 1.0 U DNA *Taq* polymerase (Biotools®, B & M Labs, S.A., Madrid, Spain). PCR amplification was performed with initial denaturation of 80°C for 5 min then 94°C for 2 min; followed by 10 cycles of Touch-down PCR of denaturation at 94°C for 45 sec, annealing and elongation at 72 to 62°C (decrements of 1°C/cycle) for 1 min. The PCR was continued with another 25 cycles of denaturation at 94°C for 45 sec, annealing 62°C for 45 sec, and elongation at 72°C for 1 min. Finally, the PCR process was run at 72°C for 3 min. The PCR was performed using GeneAmp® PCR System 2700 Perkin Elmer (Applied Biosystem, Roche Molecular System Inc., Branchburg, NJ, USA).

The PCR products were documented using 2 per cent agarose gel stained with ethidium bromide (LE, analytical grade; Promega Corporation, USA) in 1 x TBE buffer (Tris, Borate, EDTA) at 110 Volt for 30 min.

Positive control was generated by cloning the homozygous wild-type and homozygous mutant DNA samples into a plasmid. The positive controls were used in each run to avoid false positive results.

*Direct DNA sequencing*: Results obtained from both the dHPLC analysis and ASPCR were validated by direct DNA sequencing. The DNA samples were purified using QIAquick® PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced on ABI 3700 using BigDye® Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA). The DNA sequences were aligned and compared with the gene sequence (accession number: NC\_000002.) from NCBI GenBank.

## Results

A total of 306 healthy unrelated volunteers from three major ethnic groups (Malay= 100; Chinese= 104 and Indian= 102) in Malaysia were recruited for the determination of *UGT1A1\*6*, *\*27* and *\*28*.

*UGT1A1\*28* variants were detected in all three Malaysian races. About 24.5 to 39.2 per cent

of Malaysian were heterozygous of *UGT1A1*\*28 (6TA/7TA) variant. In this study, Malays and Indian have two fold higher frequency of homozygous of *UGT1A1*\*28 (7TA/7TA) which is 8 and 8.8 per cent, respectively compared to only 4.9 per cent in the Chinese. The predicted frequencies of *UGT1A1*\*28 genotypes among the three major ethnic groups in Malaysia were calculated according to Hardy-Weinberg equation with 95% confidence intervals (95% CI) (Table). All the genotypes were found to follow Hardy-Weinberg equilibrium (HWE). In comparison to the allele frequencies reported among the Caucasians and some other ethnic groups in Asian countries such as Taiwan and Hong Kong<sup>14-16</sup>, *UGT1A1*\*28 variant was found to be higher in the Malays and Indians with allele frequency of 25 and 28 per cent, respectively.

Interestingly, a combination of heterozygous insertion of 8TA genotype and also a SNPs located at -63 (G>C) or known as *UGT1A1*\*68 was detected in 0.02 per cent of the Malaysian-Chinese population. *UGT1A1*\*68 have been reported previously in Caucasian, Indian and African-American population<sup>7,17</sup>. Sample with combination of these 2 alleles showed different peak profile (Fig. 1).

The genotype and allele frequencies of *UGT1A1*\*6 were higher than *UGT1A1*\*27 in the studied populations (Table). The genotype and allele frequencies of *UGT1A1*\*6 and *UGT1A1*\*27 were not statistically significant among the Malaysian Malay, Chinese and Indian. *UGT1A1*\*27 was found in this population where the frequencies were less than 3 per cent in Malay and Chinese Malaysian. However, it was not detected in Indian subjects in this study.

### Discussion

*UGT1A1*\*6 and *UGT1A1*\*27 alleles were commonly reported in Asians<sup>10,12,17,18</sup>. We failed to validate an ASPCR method initially to determine the insertion of extra (TA) in the (TA)<sub>6</sub> TAA-box at the promoter region of *UGT1A1* using the same platform. The detection of *UGT1A1*\*28 was however, successful with the use of dHPLC. The results from both ASPCR and dHPLC were re-confirmed by direct DNA sequencing.

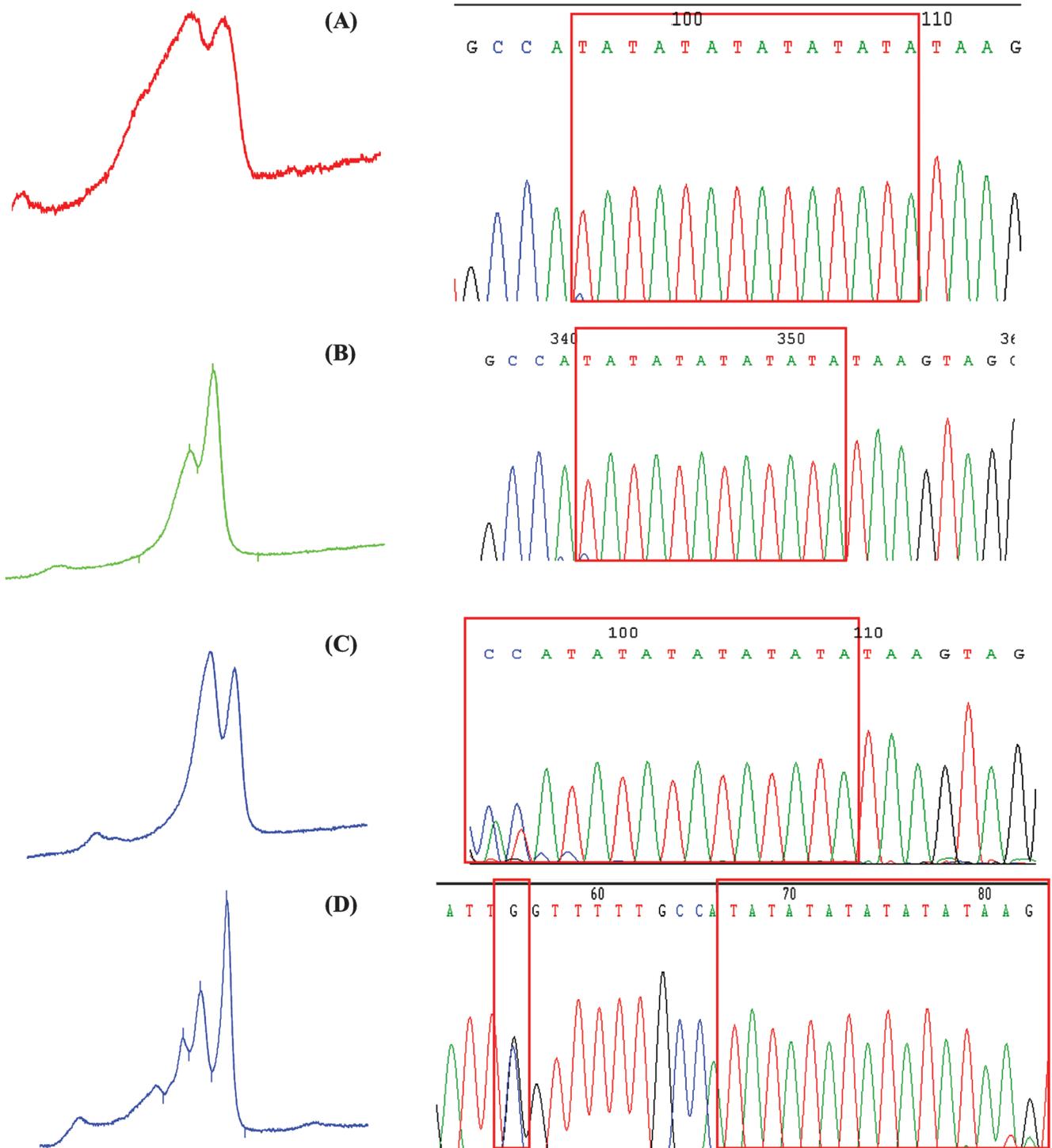
*UGT1A1*\*6 was found to be present at high frequencies among the three major ethnics in Malaysia. This is in accordance with the 5 to 15 per cent frequencies reported on other studies in Asian population<sup>14,15</sup>. The Caucasians have a lower frequency of *UGT1A1*\*6 (5%) and reported to be rare variant among this

population<sup>19</sup>. Previous study showed a correlation between *UGT1A1*\*6 genotype and adverse reaction of irinotecan whereby patients with *UGT1A1*\*6 variant had higher level of SN-38<sup>13</sup>. The occurrence of adverse effects have been reported in patients with *UGT1A1*\*6 genotypes in Asians<sup>9,13,14</sup>. The distribution of *UGT1A1*\*6 and *UGT1A1*\*27 was found to be similar among the three major ethnic groups in Malaysia. Similar results were observed among the Malay, Chinese and Indian in Singapore<sup>14</sup>. *UGT1A1*\*27 was found to be higher in Malaysian-Malays (3.0%) followed by Malaysian-Chinese (2.0%). Lower allele frequency of *UGT1A1*\*27 was found in the healthy Japanese population 0.67 per cent<sup>13</sup>. None of the Malaysian-Indians had this genotype. Similarly, *UGT1A1*\*27 was not detected in Caucasian and African-American population<sup>19</sup>.

Patients with *UGT1A1*\*28 genotype have been reported to have seven times higher risk of side effects from irinotecan than people who do not have it<sup>10</sup>. Some studies showed that combination of two polymorphic variations in the same gene may increase the risk of adverse effects. Higher incidence of neutropenia has been observed in patients who had combination of *UGT1A1*\*28 and *UGT1A1*\*6 genotype with lower glucuronidation capacity for SN-38 thus leading to the accumulation of SN38 toxic metabolites<sup>20</sup>. This suggests a gene dose effect and patients are recommended to be genotyped for the commonly encountered variants.

The allelic types and frequency of *UGT1A1*\*28 among the Malaysian Indians in this study showed similarity to Caucasian population<sup>19</sup>. On the other hand, lower allele frequency of *UGT1A1*\*28 was observed in Malaysian Chinese (17%), similar to those reported in Chinese in Singapore, Taiwan and Japan at 16, 14.3 and 9.7 per cent, respectively<sup>14,15,19</sup>. In our previous studies on genetic polymorphism of *CYP2C9* and *CYP2D6*, the Malaysian Indians showed similar pattern of high frequencies for alleles that are found common in Caucasians<sup>21,22</sup>; while the Malaysian Malays and Chinese were intermediate between East Asians and Caucasian with respect to allelic frequencies of *CYP2D6*<sup>11</sup>. In this study, combination of TA8 genotype and *UGT1A1*\*68 (G>C) was found in 0.02 per cent of Malaysian Chinese population. Interestingly, *UGT1A1*\*68 (G>C) has been reported in Indian population<sup>7</sup>. This suggests heterogeneity and inter-ethnic difference for *UGT1A1*\*28 in Malaysia.

*UGT1A1* variants with reduced functionalities are highly prevalent in Asia and thus irinotecan-induced adverse effects among the patients are



**Fig. 1.** dHPLC peak profiles and sequencing results for the confirmation of insertion of TA box at promoter region of *UGT1A1*\*28. (A) Homozygous for *UGT1A1*\*28; (B) wild-type for *UGT1A1*\*1; (C) heterozygous for *UGT1A1*\*1/\*28 (TA<sub>6</sub>/TA<sub>7</sub>), (D) combination of heterozygous for *UGT1A1*\*28 TA<sub>6</sub>/TA<sub>8</sub> and SNPs at -63 (G>C) known as *UGT1A1*\*68.



**Table.** Allele frequencies with the observed and expected genotype frequencies of *UGT1A1*\*28, *UGT1A1*\*6 and *UGT1A1*\*27 variants in three major races in Malaysia and other populations

Populations	n	Allele frequency		Genotype	Genotype frequency % (95% CI)		* P value	References
		G	A		Observed	Expected		
<i>UGT1A1</i> *28								
Pooled healthy volunteers	306	0.76	0.24	6TA/6TA	59.9 (64.5 - 65.5)	58.2 (52.5 - 63.5)	0.116	Current study
				6TA/7TA	32.9 (27.7 - 38.3)	36.1 (30.6 - 41.4)		
				7TA/7TA	7.2 (4.1 - 9.9)	5.6 (3.02-8.2)		
Malay	100	0.75	0.25	6TA/6Ta	57.0 (47.3 - 66.7)	55.5 (46.3 - 65.7)	0.431	Current study
				6TA/7TA	35.0 (25.7 - 44.5)	38.0 (28.5 - 47.5)		
				7TA/7TA	8.0 (2.68 - 13.32)	6.5 (1.67 - 11.3)		
Chinese	104	0.83	0.17	6TA/6TA	70.6 (62.3 - 79.7)	68.6 (60.1 - 77.9)	0.164	Current study
				6TA/7TA	24.5 (16.7 - 33.3)	28.4 (19.4-36.6)		
				7TA/7TA	4.9 (0.75 - 9.1)	2.9 (0.00 - 6.13)		
Indian	102	0.72	0.28	6TA/6TA	52.0 (42.3 - 61.7)	51.2 (41.3-60.7)	0.713	Current study
				6TA/7TA	39.2 (29.5 - 48.5)	41.0 (31.5-50.5)		
				7TA/7TA	8.8 (3.45 - 14.6)	8.1 (2.74-13.3)		
Chinese (Thai)	76	0.88	0.12	6TA/6TA	78.9 (69.8 - 88.2)	77.7 (68.7 - 87.3)	0.305	13
				6TA/7TA	18.4 (9.36 - 26.6)	20.9 (11.8 - 30.2)		
				7TA/7TA	2.6 (0.00 - 6.18)	1.4 (0.00 - 4.0)		
Chinese (Singapore)	90	0.83	0.17	6TA/6TA	70.8 (61.6 - 80.4)	71.0 (61.6 - 80.4)	0.872	10
				6TA/7TA	26.9 (17.8 - 36.2)	26.5 (17.8 - 36.2)		
				7TA/7TA	2.25 (0.00 - 5.23)	2.5 (0.00 - 5.73)		
Chinese (Hong Kong)	50	0.87	0.13	6TA/6TA	76 (64.2 - 87.8)	75.7 (64.2 - 87.8)	0.846	13
				6TA/7TA	22.0 (10.5 - 33.5)	22.62 (10.5 - 33.5)		
				7TA/7TA	2.0 (0.00 - 5.88)	1.69 (0.00 - 5.28)		

Contd....

Populations	n	Allele frequency		Genotype	Genotype frequency % (95% CI)		* P value	References
		G	A		Observed	Expected		
Japanese	150	0.90	0.10	6TA/6TA	82.7 (76.9 - 89.0)	81.6 (75.9 - 88.2)	0.135	11
				6TA/7TA	15.3 (9.3 - 20.7)	17.5 (11.9 - 24.2)		
				7TA/7TA	2.0 (0.00 - 4.24)	0.9 (0.00 - 2.41)		
Caucasians (Europe)	248	0.68	0.32	6TA/6TA	44.4 (37.8 - 50.2)	46.2 (39.8 - 52.2)	0.191	13
				6TA/7TA	47.1 (40.8 - 53.2)	43.6 (37.8 - 50.2)		
				7TA/7TA	8.5 (5.0 - 11.9)	10.3 (6.3 - 13.7)		
<i>UGT1A1*6</i>								
Pooled healthy volunteers	306	0.86	0.14	GG	74.2 (69.1 - 78.9)	73.6 (69.1 - 78.9)	0.395	Current study
				GA	23.2 (18.3 - 27.7)	24.4 (19.2 - 28.8)		
				AA	2.6 (0.82 - 4.38)	2.0 (0.43 - 3.57)		
Malay	100	0.88	0.12	GG	78 (69.9 - 86.1)	75.0 (66.5 - 83.5)	0.154	Current study
				GA	18 (10.5 - 25.5)	21.9 (13.9 - 30.1)		
				AA	3.0 (0.00 - 6.34)	1.6 (0.00 - 4.06)		
Chinese	104	0.86	0.14	GG	74.0 (65.6 - 82.4)	74.0 (65.6 - 82.4)	0.506	Current study
				GA	18.2 (10.6 - 25.4)	24.7 (16.7 - 33.3)		
				AA	3.0 (0.00 - 6.28)	2.1 (0.0 - 4.86)		
Indian	102	0.84	0.16	GG	79.6 (72.2 - 87.8)	71.1 (62.2 - 79.8)	0.103	Current study
				GA	18.4 (10.5 - 25.5)	26.5 (18.4 - 35.6)		
				AA	2.0 (0.00 - 4.72)	2.5 (0.00 - 5.53)		
Malay (Singapore)	95	0.85	0.15	GG	91.8 (88.5 - 97.5)	90.8 (85.3 - 96.8)	0.050	10
				GA	7.1 (3.37 - 10.63)	9.0 (3.25 - 14.8)		
				AA	1.2 (0.00 - 2.8)	0.2 (0.00 - 1.1)		

Contd.....

Populations	n	Allele frequency		Genotype	Genotype frequency % (95% CI)		* P value	References
		G	A		Observed	Expected		
Chinese (Singapore)	90	0.85	0.15	GG	71.1 (61.6 - 80.4)	72.3 (62.7 - 81.3)	0.397	10
				GA	27.78 (17.8 - 36.2)	25.5 (16.1 - 34.0)		
				AA	1.1 (0.00 - 3.06)	2.25 (0.00 - 4.89)		
Indian (Singapore)	94	0.97	0.03	GG	94.7 (90.5 - 99.4)	93.7 (87.8 - 98.2)	0.003	10
				GA	4.3 (0.04 - 7.96)	6.2 (1.2 - 10.8)		
				AA	1.1 (0.00 - 3.01)	0.1 (0.00 - 0.74)		
Japanese	150	0.88	0.12	GG	73.7 (65.9 - 80.1)	72.1 (64.8 - 79.2)	0.124	11
				GA	22.4 (15.4 - 28.6)	25.6 (18.98 - 33.02)		
				AA	3.8 (0.8 - 7)	2.3 (0.0 - 4.7)		
Chinese (Taiwanese)	290	0.89	0.11	GG	78.6 (74.3 - 83.7)	79.4 (75.4 - 84.6)	0.142	12
				GA	21.03 (16.3 - 25.7)	19.4 (14.5 - 23.5)		
				AA	0.3 (0.00 - 0.93)	1.2 (0.00 - 2.45)		
Caucasians	150	0.99	0.01	GG	98.7 (97.4 - 100.6)	98.7 (97.4 - 100.6)	0.934	11
				GA	1.3 (0.00 - 3.11)	1.3 (0.00 - 3.11)		
				AA	0.00	0.0		
<i>UGT1A1*27</i>								
Pooled healthy volunteers	306	0.98	0.02	CC	97.4 (95.1 - 98.9)	97.4 (95.1 - 98.9)	0.816	Current study
				CA	2.6 (0.86 - 4.38)	2.6 (0.86 - 4.38)		
				AA	0.00	0.02		
Malay	100	0.97	0.03	CC	93 (89.5 - 96.5)	93.1 (86.5 - 96.5)	0.717	Current study
				CA	7 (3.5 - 10.5)	6.8 (89.5 - 96.5)		
				AA	0.00	0.10		

Contd.....

Populations	n	Allele frequency		Genotype	Genotype frequency % (95% CI)		* P value	References
		G	A		Observed	Expected		
Chinese	104	0.98	0.02	CC	99.0 (97.1 - 100.9)	99.0 (97.1 - 100.9)	0.961	Current study
				CA	0.97 (0.00 - 2.91)	0.97 (0.00 - 2.91)		
				AA	0.00	0.00		
Indian	102	1.00	n.d	CC	100.00	0.00	n.a	Current study
				CA	0.00	0.00		
				AA	0.00	0.00		
Japanese	150	0.99	0.11	CC	99.3 (97.4 - 100.5)	99.3 (97.4 - 100.5)	0.967	19
				CA	0.6 (0.00 - 1.84)	0.7 (0.00 - 2.03)		
				AA	0.00	0.0		
Chinese (Taiwanese)	290	0.97	0.13	CC	94.5 (91.3 - 96.7)	94.6 (91.3 - 96.7)	0.629	12
				CA	5.5 (2.9 - 8.1)	5.4 (2.8 - 9.0)		
				AA	0.00	0.08		
Caucasians	150	1.00	0.00	CC	0.00	0.00	n.a	11
				CA	0.00	0.00		
				AA	0.00	0.00		

\*Expected genotype frequencies are calculated from the allelic frequency according to Hardy-Weinberg equation.  $P \geq 0.05$  was regarded as in equilibrium with Hardy-Weinberg. \*Chi-squared test was used to compare the genotype frequencies in different population.  $P < 0.05$  or less was considered as significant

expected. Genotyping of this variant prior to initiation of irinotecan among patients is relevant in our local population.

With the high prevalence of genetic variants of *UGT1A1* among the three ethnic groups in Malaysia and increasing colorectal cancer cases in Malaysia<sup>23</sup>, genotyping of *UGT1A1*\*28, *UGT1A1*\*6 and *UGT1A1*\*27 is relevant among patients prescribed with irinotecan in Malaysia. As there may be inter-ethnic differences in the distribution of allelic variant in *UGT1A1*, it is important to know its prevalence to predict the population likely to be at risk of adverse effects.

#### Acknowledgment

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