Distribution of the *UGT1A1*28* polymorphism in Caucasian and Asian populations in the US: a genomic analysis of 138 healthy individuals

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The hepatic isoform 1A1 of uridine diphosphate glucuronosyltransferase is responsible for glucuronidation and detoxification of SN-38, the active metabolite of irinotecan. The presence of an additional TA repeat in the TATA sequence of the *UGT1A1* promoter leads to a significant decrease in SN-38 glucuronidation. Patients with the *UGT1A1* $(TA)_7$ allele are more likely to experience severe neutropenia and diarrhea following irinotecan chemotherapy. We assessed the distribution of the *UGT1A1* $(TA)_n$ polymorphism in healthy male and female US residents of European and Asian descent. We used a fluorescent polymerase chain reaction-based assay to detect *UGT1A1* $(TA)_n$ polymorphisms in 138 healthy volunteers (56 Caucasians, 37 Chinese, 37 Filipino and eight Japanese) between the ages of 18 and 65 years. The χ^2-test was used to assess between-group differences in the distribution of *UGT1A1* $(TA)_n$ genotypes. The *UGT1A1* $(TA)_{6/6}$ genotype was significantly more common in Asians than in Caucasians (76 vs. 46%), whereas the $(TA)_{6/7}$ (39 vs. 20%) and $(TA)_{7/7}$ (13 vs. 5%) genotypes were more common in Caucasians than in Asians. Genotype distributions did not differ significantly between men and women in either group. The *UGT1A1* $(TA)_{5/5}$ genotype was detected in one Caucasian woman. In conclusion, consistent with previous reports, the *UGT1A1* $(TA)_{7/7}$ genotype was significantly more common in Caucasians than in Asians. *UGT1A1* $(TA)_{n/n}$ genotype distribution did not vary with sex in individuals of European or Asian descent. *Anti-Cancer Drugs* 18:693–696 © 2007 Lippincott Williams & Wilkins.

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Introduction

Chemotherapeutic options for the treatment of colorectal cancer have expanded considerably in recent years. The addition of irinotecan {7-ethyl-10-[4-(1-piperidino)-1-piperidino] carbonyloxycamptothecin (Camptosar; Pfizer, New York, New York, USA) to the standard 5-fluorouracil/leucovorin combination has improved the response rate to 40% and overall survival to 15 months in previously untreated patients with metastatic disease [1]. Irinotecan, however, has dose-limiting toxicities, including grade 3 or 4 delayed diarrhea in 44% of treated patients and neutropenia in 29% [2].

The major metabolite of irinotecan, SN-38 (7-ethyl-10-hydroxycamptothecin), is both a potent topoisomerase I inhibitor and a potentially toxic compound [3]. SN-38 is conjugated to the inactive SN-38 glucuronide by uridine diphosphoglucuronosyl transferase 1A1 (UGT1A1) and then eliminated via the bile [4]. Thus, UGT1A1 is primarily responsible for the glucuronidation and detoxification of SN-38 to its inactive and nontoxic SN-38 glucuronide, and is considered the primary cause of irinotecan-related toxicity.

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*UGT1A1*28* is a common polymorphism in which the TATA sequence of the *UGT1A1* promoter contains an additional TA repeat (seven vs. six repeats). Homozygosity for *UGT1A1*28* is associated with Gilbert's syndrome [5], an inherited condition characterized by mild hyperbilirubinemia. Case reports describing severe neutropenia in Gilbert's syndrome patients who received standard doses of irinotecan [6] suggested a link between the *UGT1A1*28* allele and irinotecan toxicity. Subsequent reports found that longer repeat lengths are associated with decreased UGT1A1 production and SN-38 glucuronidation [7,8] and with increased bilirubin levels [5,9]. Patients carrying the *UGT1A1*28* allele have a decreased SN-38 detoxification capacity, leading to a higher risk of both neutropenia and diarrhea [4,7,10,11]. These observations prompted changes in the irinotecan package label, indicating that a reduced initial dosage should be considered in patients homozygous for *UGT1A1*28* [12], and subsequent findings have supported this position [13].

The frequencies of *UGT1A1* $(TA)_{n/n}$ variants have been reported as 43% for the $(TA)_{6/6}$ genotype, 48% for the

$(TA)_{6/7}$ genotype and 9% for the $(TA)_{7/7}$ genotype in a series of US patients receiving chemotherapy for colorectal cancer [7]. Genotype frequencies, however, vary greatly among ethnic groups. The potentially deleterious $(TA)_{7/7}$ genotype is most common in individuals of African descent and least common in those of Asian descent [9,14]. A high $(TA)_7$ allele frequency has also been reported in individuals of Indian descent [15,16]. Within ethnic groups, an increasing number of $(TA)_7$ alleles are associated with increased bilirubin levels.

In this study, we examined the frequency of the *UGT1A1*28* allele in healthy US residents of European and Asian descent. Asian participants included those of Chinese, Japanese and Filipino heritage.

Materials and methods

Study participants

Analysis of the number of TA repeats in the *UGT1A1* gene promoter was performed using DNA samples collected from individuals of different ethnic groups. All of the individuals studied were healthy (i.e. not under a physician's care for any acute or chronic health problems) volunteers between 18 and 65 years of age who worked at Quest Diagnostics Nichols Institute (San Juan Capistrano, California, USA) and provided written informed consent for genotype analysis at the time of the blood draw. Individuals who were taking prescription medication, over the counter medication or herbal remedies on an ongoing basis were excluded from the study.

Whole blood (5 ml) was collected in an ethylenediaminetetraacetic acid lavender-top tube. DNA was extracted using a BioRobot EZ1 System (Qiagen, Valencia, California, USA) and EZ1 DNA Blood kit (Qiagen; cat. no. 951054). Following genomic DNA extraction from whole blood, DNA was amplified with fluorescent polymerase chain reaction using primers developed in our laboratory. The polymerase chain reaction products were denatured in Hi-Di formamide and size-fractionated by capillary electrophoresis on an ABI 3100 Genetic Analyzer (Applied Biosystems, Foster City, California, USA). The data were then analyzed using GeneMapper software (Applied Biosystems). Genotype assignment was based on fragment size. The χ^2-test was used to determine the statistical significance of the distribution of *UGT1A1* $(TA)_{n/n}$ genotypes among patient groups.

Results

We studied a total of 138 healthy individuals who volunteered for genotype analysis, including 56 Caucasians (28 men, 28 women), 37 Chinese (17 men and 20 women), 37 Filipinos (13 men, 24 women) and eight Japanese (three men, five women). None of these individuals had colorectal cancer to our knowledge. The genotype distributions from all ethnic groups analyzed are summarized in the Table 1. The $(TA)_{6/6}$ genotype was the most common overall (64%), followed by $(TA)_{6/7}$ (28%) and $(TA)_{7/7}$ (8%). The frequency of each genotype differed significantly between Caucasian and Asian individuals as a group (Table 1). The $(TA)_{6/6}$ genotype was significantly more common in Asians than in Caucasians, but was still the most frequently identified genotype in Caucasians. The $(TA)_{6/7}$ and $(TA)_{7/7}$ genotypes, however, were significantly more common in Caucasians than in the Asian group (Table 1). The homozygous $(TA)_{5/5}$ genotype was detected in one Caucasian woman. No significant differences were observed in genotype frequencies between men and women in any of ethnic groups studied (data not shown).

Table 1 Distribution of *UGT1A1* genotypes in Caucasian and Asian groups

Ethnicity	*n*	M : F	Genotype [*n* (%)]			
			5/5	6/6*	6/7**	7/7***
Caucasian	56	28 : 28	1 (2)	26 (46)	22 (39)	7 (13)
Asian	82	33 : 49	0	62 (76)	16 (20)	4 (5)
Chinese	37	17 : 20	0	29 (78)	5 (14)	3 (8)
Filipino	37	13 : 24	0	28 (76)	9 (24)	0
Japanese	8	3 : 5	0	5 (63)	2 (25)	1 (13)
Total	138	61 : 77	1 (2)	88 (64)	38 (28)	11 (8)

No significant sex differences were observed in genotype frequencies in any of the groups (data not shown). When analyzed by Asian subgroup, genotype distributions differed significantly for Caucasian vs. Chinese ($P=0.01$) and Filipino ($P=0.009$) groups but not for Caucasian vs. Japanese ($P=0.69$) groups. Genotype frequencies did not differ significantly among the three Asian groups ($P=0.286$).
$^*P<0.001$ Caucasian vs. Asian; $^{**}P=0.004$ Caucasian vs. Asian; $^{***}P=0.046$ Caucasian vs. Asian (χ^2).

Genotype distributions also differed significantly between Caucasian and Chinese individuals and between Caucasian and Filipino individuals; the number of Japanese individuals studied precludes a meaningful comparison with the Caucasian group (Table 1). The genotype distribution did not differ significantly among the three Asian groups studied (Table 1).

Discussion

We evaluated the distribution of *UGT1A1* TATA polymorphisms among healthy individuals of European and Asian descent, with no history of colorectal cancer, residing in the US. The overall frequencies of the $(TA)_{6/6}$, $(TA)_{6/7}$ and $(TA)_{7/7}$ genotypes were consistent with previously reported results. Homozygosity for *UGT1A1*28* among Asians was somewhat higher in our study (5%) than in previous reports [9,14], but was significantly lower than among Caucasians (13%). We did not note a significant difference in the genotype distribution among the Asian subgroups, although the relatively small sample sizes may have obscured minor intergroup differences. As expected, the *UGT1A1* $(TA)_{n/n}$ genotype distributions did not differ significantly

between men and women in any of the ethnic groups studied.

One Caucasian woman in our study was homozygous for the $(TA)_5$ allele. Reported primarily in black populations, this allele has been associated with increased glucuronidation by UGT1A1 [9]. To the best of our knowledge, this is the first report of the homozygous $(TA)_{5/5}$ genotype among Caucasians. In that our study only examined individuals without a history of colorectal cancer, a selection bias may have been introduced resulting in an underestimation of the prevalence of the *UGT1A1*28* polymorphism if it is in some way linked to colorectal cancer.

*UGT1A1*28* is the main polymorphism associated with increased risk of severe toxicity from irinotecan. Other variations in the UGT1A1 coding region, however, are associated with altered bilirubin or metabolism or irinotecan toxicity or metabolism including 211G > A (G71R; *UGT1A1*6*) [17], 1457T > G (Y486D) [18] and 686C > A (P229Q) [19], have also been described. As with the *UGT1A1*28* allele, the frequencies of these polymorphisms tend to vary across ethnicities. In a study of peripheral blood samples, Kinawa *et al.*, detected homozygosity for *UGT1A1*6* in *23%* (35/150) of Japanese participants but in no Caucasian (0/150) or African–American (0/150) participants [14]. In the same study, only *2%* of Japanese participants were homozygous for the *UGT1A1*28* allele, whereas *22%* of African-American and *12%* of Caucasian participants were homozygous for this allele. *UGT1A1*6*, rare in Caucasians but associated with Gilbert's syndrome in Asian populations, appears to decrease UGT1A1 levels, as well [18]. In Korean patients with advanced non-small-cell lung cancer receiving irinotecan, this polymorphism was associated with reduced SN-38 glucuronidation, increased risk of severe neutropenia, and shorter disease-free and overall survival [20]. Ando *et al.*, however, found this allele not to be a significant risk factor for toxicity in Japanese patients treated with irinotecan, but also noted that its effect might be more pronounced in combination with *UGT1A1*28* [21]. Some researchers have commented that *UGT1A1*6* may be the allele primarily responsible for irinotecan toxicity in Asian populations and that testing for this allele, in addition to *UGT1A1*28*, may be necessary in patients of Asian descent [22].

Variations in other pathways of irinotecan clearance could also potentially affect toxicity risk. Hepatic transport enzymes such as ABCB1 may be involved in transporting irinotecan and its metabolites to the bile, and the homozygous T allele of the *ABCB1* 1236C > T polymorphism has been associated with increased SN-38 levels [23]. Cytochrome P450 3A4 (CYP3A4) can directly inactivate irinotecan. Analysis of CYP3A4 phenotype, in combination with *UGT1A1* promoter polymorphisms, may help predict variations in irinotecan pharmacokinetics [24,25].

In summary, genetic studies of UGTs are designed to characterize an individual's predisposition to certain diseases and their risk of adverse outcomes to drug treatment. Functional genomic polymorphisms in drug-target genes, metabolizing enzymes and DNA-repair enzymes may have important implications for drug efficacy [21]. Therefore, identifying significant associations between genomic polymorphisms and defined clinical endpoints such as overall survival, response to therapy and drug toxicity may improve the prediction of treatment success, and assist the clinician in individualizing patient chemotherapy. Although knowledge of the effects of different *UGT1A* polymorphisms on irinotecan metabolism and toxicity is still evolving [26], *UGT1A1*28* clearly plays an important role, and is the first such polymorphism widely used to assess toxicity risk and the need for initial dosage reductions.

Acknowledgments

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