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ORIGINAL ARTICLE

Is there diversity among *UGT1A1* polymorphism in Japan?

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Abstract

AIM: To investigate into the diversity of *UGT1A1* polymorphism across three different districts in Japan and highlight genetic differences among the population in Japan.

METHODS: We enrolled 50 healthy volunteers from each of the Yamaguchi (western part of Japan), Kochi (southern part of Japan) and Akita (northern part of Japan) prefectures. Blood samples (7 mL) were collected from each participant and stored in EDTA for subsequent genotyping by fragment size analysis, direct sequencing and TaqMan assay of *UGT1A1**28, *UGT1A7**3/*UGT1A9**22 and *UGT1A1**93/*UGT1A1**6/*UGT1A1**27/*UGT1A1**60/*UGT1A7* (-57), respectively.

RESULTS: The only statistically significant differences in allele polymorphisms among the group examined were for *UGT1A1*6*. The Akita population showed more *UGT1A1*6* heterozygosity (P = 0.0496).

CONCLUSION: Our study revealed no regional diversity among *UGT1A1*, *UGT1A7* or *UGT1A9* polymorphisms in Japan.

Key words: *UGT1A1* gene; Polymorphism; Diversity

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INTRODUCTION

Irinotecan with fluoropyrimidine is approved worldwide as a first-line chemotherapeutic agent for metastatic colorectal cancer[1-5]. Although prolonged survival has been reported with the use of this drug, severe diarrhea and neutropenia have also been reported as dose-limiting

toxicities in 20%-35% of patients treated by the agent. Recent studies revealed that the risk of such severe toxicities might be associated with genetic variation in irinotecan metabolism, indicating a possible predictive factor.

Irinotecan is activated by hydrolysis to SN-38, a potent topoisomerase I inhibitor[6] that is primarily inactivated through biotransformation into SN-38 glucuronide (SN-38G) by the enzyme uridine diphosphate glucuronosyltransferase isoform 1A1 (*UGT1A1*)[7]. In addition, the toxicity of irinotecan has been correlated with polymorphisms in the number of TA repeats in one of the promoter regions of the *UGT1A1* gene (*UGT1A1 *28*), which affects transcriptional efficiency[8]. Because of the clinical importance of the glucuronidation pathway in irinotecan treatment, *UGT1A1 *28* was proposed as a potent predictor for severe toxicity[9-11]. Recently, a novel prospective dose-finding study of irinotecan alone based on *UGT1A1*6* and **28* genotyping was reported[4,12]. These results showed that the *UGT1A1 *6* or **28* genotype status could be used to determine RD (recommended doses) of irinotecan. We conducted a prospective phase Ⅱ study of FOLFIRI for metastatic colorectal cancer in Japan, analyzed the *UGT1A1*28* and **6* polymorphisms and demonstrated that the combination of the *UGT1A1*28* and **6* polymorphism is important to predict the adverse event of the CPT-11[5].

The role of *UGT1A1*28* alleles in the toxicity and pharmacokinetics of irinotecan is considerably different between Asians and Caucasians. Only homozygotes of **28* have been associated with neutropenia in Caucasians[11,13-15], whereas both homozygote and heterozygote **28* patients have shown severe toxicity with irinotecan in Japan[4,9]. Other results revealed that SN-38 glucuronidation was highly impaired in heterozygotes, as previously reported[9,16]. Such ethnic differences may be associated with other genetic variants of UGT1A family polymorphisms, such as *UGT1A1*60*, **6*, *UGT1A7*3* and *UGT1A9*22*, which were demonstrated in linkage disequilibrium experiments with *UGT1A1*28*[17-22]. Such genotype variation could affect SN-38 glucuronidation and also the severe irinotecan-related toxicity. This study aimed to clarify the regional differences in *UGT* enzyme polymorphisms among three different districts in Japan that are widely different, both geographically and culturally.

MATERIALS AND METHODS

The 50 volunteers from Akita, Kochi and Yamaguchi prefectures comprised of 8 males and 42 females, 6 males and 44 females, and 11 males and 39 females, respectively, with an average age of 37.5, 43.8 and 38.4 years, respectively. The examinee demographics are shown in Table 1.

Blood samples (7 mL) were collected from each participant and stored in EDTA for subsequent analysis. Examinees were limited to those whose parents and grandparents came from the same region.

Written informed consent was obtained from all participants.

Table 1 Examinee characteristics

	Akita	Kochi	Yamaguchi
Sex			
Male	8	6	11
Female	42	44	39
Age (yr)	37.4 (23-55)	43.8 (24-66)	38.4 (18-67)

Table 2 Primers, probes used for genotyping

Gene	Variant	Primers and probes[1]	
*UGT1A1*28*	-53 TA6/TA7	F-FAM	5'-gtgacacagtcaaacattaacttgt-3'
		R	5'-gcctttgctcctgccagaggtt-3'
*UGT1A7*3*	N129K	F	5'-tacactctggaggatcagga-3'
	W208R	R	5'-tattgggcatcacgggtttg-3'
*UGT1A9*22*	-118 T10/T9	F	5'-acttaacattgcagcacagg-3'
		R	5'-atgggcaaaagccttgaact-3'
*UGT1A1*93*	-3156 G/A	F	5'-cagaagggctagagaggaggaa-3'
		R	5'-cttgctctcaaaactctgggataga-3'
		FAM	5'-cctgtccaagctca-3'
		VIC	5'-cacctgtctaagctca-3'
*UGT1A1*6*	211 G/A	C 559715 20	
*UGT1A1*27*	686 C/A	C 2307598 20	
*UGT1A1*60*	-3279 T/G	C 1432134 10	
UGT1A7 (-57)	-57 T/G	C 287265 10	

[1]Primers for fragment size assay: F-FAM: Forward primer labeled FAM; R: Reverse primer. Primers for Sequence assay: F: Forward primer; R: Reverse primer. TaqMan assay: F: Forward primer; R: Reverse primer; FAM: Reporter 1 probe; VIC: Reporter 2 probe. Number: TaqMan SNP genotyping assays number.

Genotyping

Genomic DNA was extracted from peripheral blood anti-coagulated with EDTA-2Na, using a conventional NaI method[23]. *UGT1A1**28, *UGT1A7**3/*UGT1A9**22 and *UGT1A1**93/*UGT1A1**6/*UGT1A1**27/*UGT1A1**60/*UGT1A7* (-57) were genotyped by fragment size analysis, direct sequencing and TaqMan assay, respectively. Primers and probes used in this study are shown in Table 2.

For fragment size analysis, PCR reactions were performed in a total volume of 10 μL containing template DNA (80 ng/μL) according to the manufacturer's instructions (Ex Taq; Takara, Tokyo, Japan). The amplification was carried out with a Gene Amp PCR System PC808 (ASTEC, Tokyo, Japan), with an initial denaturation at 95 ℃ for 2 min followed by 27 cycles of denaturation at 94 ℃ for 30 s, annealing at 60 ℃ for 20 s, and extension at 72 ℃ for 30 s. The PCR products of TA6 and TA7, whose sizes were 94 *bp* and 96 *bp*, respectively, were mixed with Hi-Di formamide, including the internal size standard (GeneScan 500, Applied Biosystems, CA, USA) at a 1:10 (*vol*/*vol*) ratio. Then, samples were run in the ABI Prism 3100 Genetic Analyzer (Applied Biosystems). Fragment sizes were determined by comparison with the internal size standard (GeneScan LIZ-500) using the local Southern algorithm and the data were analyzed by GeneMapper™ software version 3.5 (Applied Biosystems).

For direct sequencing, PCR amplifications were performed using the Gene Amp PCR System PC808

Table 3 Polymorphisms of *UGT1A1* *n* (%)

	*UGT1A1**28 (*P* = 0.663)			*UGT1A1**6 (*P* = 0.0496)			*UGT1A1**27 (*P* = 1.000)			*UGT1A1**60 (*P* = 0.766)			*UGT1A1*-3156		
	6/6	6/7	7/7	A/A	G/A	G/G	A/A	C/A	C/C	G/G	T/G	T/T	A/A	G/A	G/G
A	41 (82)	8 (16)	1 (2)	1 (2)	20 (40)	29 (58)	0 (0)	0 (0)	50 (100)	2 (4)	19 (38)	29 (58)	1 (2)	8 (16)	41 (82)
K	37 (74)	13 (26)	0 (0)	0 (0)	14 (28)	36 (72)	0 (0)	1 (2)	49 (98)	1 (2)	25 (50)	24 (48)	0 (0)	13 (26)	37 (74)
Y	37 (74)	12 (24)	1 (2)	3 (6)	9 (18)	38 (76)	0 (0)	0 (0)	50 (100)	2 (4)	22 (44)	26 (52)	1 (2)	12 (24)	37 (74)

A: Akita prefecture; K: Kochi prefecture; Y: Yamaguchi prefecture.

Table 4 Polymorphisms of *UGT1A7* and *UGT1A9* *n* (%)

	UGT1A7 N129K (*P* = 0.853)			*UGT1A7* W208R (*P* = 0.409)			*UGT1A7* -57 (*P* = 0.409)			*UGT1A9**22 (*P* = 0.993)		
	G/G	T/G	T/T	C/C	T/C	T/T	G/G	T/G	T/T	9/9	9/10	10/10
A	7 (14)	24 (48)	19 (38)	2 (4)	23 (46)	25 (50)	2 (4)	23 (46)	25 (50)	5 (10)	24 (48)	21 (42)
K	8 (16)	20 (40)	22 (44)	4 (8)	17 (34)	29 (58)	4 (8)	17 (34)	29 (58)	6 (12)	22 (44)	22 (44)
Y	5 (10)	23 (46)	22 (44)	4 (8)	14 (28)	32 (64)	4 (8)	14 (28)	32 (64)	5 (10)	23 (46)	22 (44)

A: Akita prefecture; K: Kochi prefecture; Y: Yamaguchi prefecture.

(ASTEC, Tokyo, Japan) with Ex Taq polymerase. Amplification conditions were 30 cycles of 95 ℃ for 30 s, each annealing temperature for 20 s, and 72 ℃ for 30 s. PCR products were purified using ExoSAP-IT (Amersham Bioscience, Tokyo, Japan) for 20 min at 37 ℃ and then for 20 min at 80 ℃. Sequencing reactions were carried out using a BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Tokyo, Japan). After purification with ethanol, the reaction products were analyzed using an ABI 3100-Avant Genetic Analyzer (Applied Biosystems).

TaqMan assays of PCR products were performed according to the manufacturer's protocol. Specific forward/reverse PCR primers and TaqMan probes for *UGT1A1**93 were custom-synthesized by Applied Biosystems. Primers and probes for *UGT1A1**6, *UGT1A1**27, *UGT1A1**60, *UGT1A7* (-57) were purchased from Applied Biosystems (TaqMan SNP Genotyping Assays). Reaction mixtures were loaded into 384 well plates and placed in the ABI Prism 7900HT Sequence Detection System (Applied Biosystems). PCR amplifications were performed as follows: initial denaturation at 95 ℃ for 10 min, followed by 40 cycles of PCR with a denaturation at 95 ℃ for 15 s, and one step annealing/extension for 1 min at 60 ℃.

Statistical analysis and power calculation

Proportions of wild-type, hetero-type and homo-type were calculated with 95% Agresti-Coull confidence intervals (95% CI)[24]. Fisher's exact test with a two-sided significance level of 0.05 was used for comparing the areas. For a two-sided 95% CI for a binomial proportion whose true value is varied from 0.5 to 0.1, a sample size of 50 yields a half-width of, at most, 14% in any situations of the true value.

RESULTS

Tables 3 and 4 list the polymorphisms of *UGT1A1* allele *28, *6, *60, *27 and *93 (-3156), *UGT1A7 *3* (N129K, W208R, -57) and *UGT1A9**22*. The incidence of wild-type *UGT1A1**28* in the Akita, Kochi and Yamaguchi cohorts was 82% (95% CI: 69 to 90), 74% (95% CI: 60 to 84) and 74% (95% CI: 60 to 84), respectively (*P*-value = 0.663). The incidence of homozygous *UGT1A1**28* across the three districts was only 1.3% (95% CI: 0.0 to 5.0).

The only statistical difference in allele polymorphisms examined among the three groups was in *UGT1A1**6*. The incidence of wild-type *UGT1A1**6* across the Akita, Kochi and Yamaguchi populations was 58% (95% CI: 44 to 71), 72% (95% CI: 58 to 83) and 76% (95% CI: 62 to 86), respectively, while the incidence of heterozygous-type *UGT1A1**6* was 40%, 28% and 18%, respectively. Volunteers from Akita showed the most heterozygosity in *UGT1A1**6*, although the *P*-value was 0.0496.

DISCUSSION

The participants in this study were mostly nurses and other medical staff from hospitals in the three Japanese prefectures. Around 95% of the nurses in Japan are women; thus the predominance of female subjects in this study.

There are several reports about the distribution of *UGT1A1* polymorphisms worldwide. However, these studies were limited to the promoter region, *UGT1A1**28*[8,25-27], and demonstrated that *UGT1A1**28* homozygosity is frequent in Europe (5.0%-14.8%), Africa (5.9%-17.9%) and the Indian subcontinent (19.2%-24.0%), compared to East Asia, which comprises mainly of the Chinese (1.2%-5.0%)[25,26]. Hall *et al*[25] showed that sub-Saharan Africa, especially Cameroon, was 33% homozygous for

Figure 1 The location of the three prefectures. Akita represents the northern part of Japan, while the Kochi prefecture on Shikoku Island was obstructed from communication with other prefectures by the Shikoku mountain (dotted line) range in ancient times. Yamaguchi is one of the nearest prefectures to the Korean Peninsula in Japan.

*UGT1A1*28*, which is a fairly high frequency even compared to Caucasians and Indians.

The incidence of homozygous *UGT1A1*28* across the three districts of our data in Japan was only 1.3%, which is comparable to the 1.0% reported by Hall *et al*[25]. Premawardhena *et al*[26] also reported a wider diversity of repeat numbers among individuals from North and Central America with varying degrees of African ancestry. Our data demonstrated that the repeat number of (TA) was 6/6, 6/7 and 7/7, which is the same as those reported for Europeans and other Asians. Hitherto, no studies have investigated the regional diversity in *UGT1A1*-family polymorphism within one country, although our study now indicates that there is no diversity of *UGT1A1*28* polymorphism in Japan.

In this study, we selected the Akita, Kochi and Yamaguchi prefectures (Figure 1). Akita represents the northern part of Japan, while the Kochi prefecture on Shikoku Island was obstructed from communication with other prefectures by the Shikoku mountain range in ancient times. Thus, both prefectures have developed a unique dialect and less communication with each other historically. On the other hand, Yamaguchi is one of the nearest prefectures to the Korean Peninsula in Japan. All the prefectures chosen have also developed a unique culture.

Our study revealed no regional diversity of *UGT1A1*, *UGT1A7* and *UGT1A9* polymorphisms in Japan. Only *UGT1A1*6* showed a statistically significant difference among these three regions in Japan, with more G/A type in the Akita prefecture compared to the other two regions. However, the *p*-value for the *UGT1A1*6* polymorphism was marginal (*P*-value = 0.0496) and the statistical significance is easily changeable due to the selection of the sampling population. The number of *UGT1A1*6* homozygotes was not different among the three districts, with allele frequencies for Akita, Kochi and Yamaguchi of 2.2%, 1.4% and 1.5%, respectively.

Our study is an exploratory research about the diversity of *UGT1A1* in Japan. Before the study, we speculated that Akita may have the same tendency of *UGT1A1* polymorphism as Caucasians, i.e. Akita may have more polymorphism in *UGT1A1*28* and less polymorphism in *UGT1A1*6*. However, our study revealed that *UGT1A1*28* showed no diversity and *UGT1A1*6* did not show less polymorphism, although this was not random sampling and generalizability of our population could not be guaranteed.

As described, heterozygotes of *UGT1A1*28* are extremely rare in the Japanese population compared to Caucasians and the incidence of heterozygotes and homozygotes of *UGT1A1*28* across the three districts combined was 22.0% and 0.013%, respectively.

Our study also demonstrated that the *UGT1A1*6* polymorphisms, G/A and A/A, occurred at a rate of 28.7% and 2.7%, respectively, in Japan. Kaniwa *et al*[28] examined the variants of *UGT1A1*6* in Caucasian and African-American populations. Caucasians showed only two heterozygotes among 150 blood samples, while none were found among the African-Americans. Our study confirmed the Japanese standard data for *UGT1A1* polymorphism frequencies, which shows more variants for *UGT1A1*6* compared to Caucasian and African-American samples.

Jinno *et al*[29] examined the glucuronidation of SN-38, a potent inhibitor of topoisomerase 1, by human UGT1A1 variants in Cos-1 cells. The variant 211G<A (G71R) (*UGT1A1*6*) reduced the glucuronidation activity more than 686C>A (P229Q) (*UGT1A1*27*). Moreover, hyperbilirubinemia observed in Japanese and Taiwanese patients with the P229Q variant is mainly attributable to the TA7 variation. Thus, *UGT1A1*6* plays an important role during chemotherapy with irinotecan in East Asian populations[28,30].

Finally, the variant sequences in exon 1, *UGT1A1*6* and *UGT1A1*27*, have been identified only in the Japanese. Thus, Japanese studies could focus more on these two genotypes, which might be more closely associated with drug sensitivity in Japanese patients than in Caucasians[31-33].

Our ongoing studies will compare *UGT1A* gene polymorphism worldwide, starting in Asian populations and gradually spreading to Europeans. Such investigations may also clarify the movement of people throughout history.

COMMENTS

Background

Irinotecan with fluoropyrimidine is approved worldwide as a first-line chemotherapeutic agent for metastatic colorectal cancer. Although prolonged survival has been reported with the use of this drug, severe diarrhea and neutropenia have also been reported as dose-limiting toxicities in 20%-35% of patients treated by the agent. Recent studies revealed that the risk of such severe toxicities might be associated with genetic variation in irinotecan metabolism, indicating a possible predictive factor.

Research frontiers

This study aimed to clarify the regional differences in *UGT* enzyme polymorphisms among three different districts in Japan that are widely distant, both geographically and culturally.

Innovations and breakthroughs

The authors enrolled 50 healthy volunteers from each of the Yamaguchi (west-

ern part of Japan), Kochi (southern part of Japan), and Akita (northern part of Japan) prefectures. Blood samples were collected from each participant and stored in EDTA for subsequent genotyping by fragment size analysis, direct sequencing, and TaqMan assay of *UGT1A1**28, *UGT1A7**3/*UGT1A9**22, and *UGT1A1**93/*UGT1A1**6/*UGT1A1**27/*UGT1A1**60/*UGT1A7* (-57), respectively.

Applications

The authors found that the only statistically significant differences in allele polymorphisms among the group examined were for *UGT1A1*6*. The Akita population showed more *UGT1A1*6* heterozygosity. This study revealed no regional diversity among *UGT1A1*, *UGT1A7* or *UGT1A9* polymorphisms in Japan.

Peer review

Kobayashi *et al* aimed to clarify the regional differences in UGT enzyme polymorphisms among three different districts in Japan that are widely distant, both geographically and culturally. The study seems interesting, but the sample size is somewhat small.

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