Prevalence of topoisomerase I genetic mutations and UGT1A1 polymorphisms associated with irinotecan in individuals of Asian descent

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Abstract. Topoisomerase I (TOP-I) mutations have been shown to be correlated to irinotecan resistance *in vitro*. However, the prevalence of TOP-I germline mutations has yet to be systematically elucidated. On the other hand, polymorphisms of *UGT1A1* have been shown to be associated with CPT-11 toxicity in clinical situations. The primary aim of this study was to investigate the prevalence of mutations in the TOP-I exons associated with CPT-11 resistance, including untreated cancer tissue. A secondary aim was to confirm the less frequent *UGT1A1**28 and more frequent *UGT1A1**6 in individuals of Asian descent compared to Caucasians and individuals of African descent. The prevalence of 5 reported TOP-I mutations in exons was investigated in volunteers (n=236) using DNA sequencing of the PCR products. The prevalence of TOP-I mutations in untreated lung cancer tissues (n=16) was also investigated. Additionally, 3 *UGT1A1* polymorphisms, *UGT1A1**6, *27 and *28, were investigated in volunteers (n=126). There were no mutations of TOP-I in any of the 236 subjects or in the untreated lung tissues. Among 128 subjects, the distribution of homozygous polymorphisms of *UGT1A1* was: *UGT1A1**28 in 3 (2.4%) and *UGT1A1**6 in 4 (3.2%) subjects, and co-occurrence of heterozygous polymorphisms for both *UGT1A1**6 and *UGT1A1**28 in 4 (3.2%) subjects, and for *UGT1A1**27 and *UGT1A1**28 in 1 subject (0.8%). The Hardy-Weinberg deviation test showed there was no significant deviation from the equilibrium, and the association analysis indicated no significant linkage between *UGT1A1**6 and *UGT1A1**28. In conclusion, TOP-I genetic mutations correlated to CPT-11 resistance were not detected in any of the subjects and untreated lung cancer tissues. Less frequent *UGT1A1**28 and more frequent *UGT1A1**6 were confirmed in East Asian individuals compared to Caucasians and individuals of African descent. Linkage disequilibrium was not detected between *UGT1A1**6 and *UGT1A1**28.

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Introduction

Irinotecan hydrochloride (CPT-11), a water-soluble semi-synthetic derivative of camptothecin, has been shown to exert marked antitumor activity (1). It is an inactive prodrug, and its major metabolite SN-38 is a potent topoisomerase I (TOP-I) inhibitor. SN-38 stabilizes covalent TOP-I-DNA complexes, causing DNA strand breaks. Several point mutations of TOP-I were identified as being associated with resistance to CPT-11 (2-4), and structural models were introduced to explain how the mutations of TOP-I hinder the docking of camptothecin derivatives in the ternary complex of TOP-I-DNA (5-7).

The cultured cells with the TOP-I mutation showed no obvious or only minor defects in cell function and proliferation (2-4). Consequently, such mutations may be innocent or not fatal to the cells. Therefore, it is reasonable to consider that the mutations may even occur in germlines.

CPT-11 exhibits inter-individual variations in terms of both pharmacokinetic and pharmacodynamic behavior (8). CPT-11 is hydrolyzed to yield active SN-38 (9) and detoxified via glucuronidation of SN-38 by uridine diphosphate glucuronosyltransferase (UGTs) to yield its β-glucuronides, SN-38G (10). *UGT1A1* is the main isoform of UGTs involved in the formation of SN-38G. Genetic polymorphisms of the *UGT1A1* gene were revealed to explain the variability of CPT-11-related toxicity among patients, particularly *UGT1A1**28 [$(TA)_7TAA$], the existence of which is known to be predictive of CPT-11-induced neutropenia (11). In addition, the *UGT1A1**6 and *27 alleles, two variants in exon 1 of the *UGT1A1* gene, are found mainly among individuals of Asian descent, and have also been indicated to affect enzyme function (12,13). The associated phenotype of *UGT1A1**28, *UGT1A1**6, or *UGT1A1**27 is the Gilbert syndrome (14), and a strong association between

A Protein sequence

MSGDHLHNDSQIEADFRLNDSHKHKDKHKDREHRHKEHKKEKDREKSKHSNSEHKDSEKKHK
EKEKTKHKDGSSEKHKDKHKDRDKEKRKEEKVRASGDAKIKKEKENGFSSPPQIKDEPEDDGYF
VPPKEDIKPLKRPRDEDDADYKPKKIKTEDTKKEKKRKLEEEEDGKLKKPKNKDKDKKVPEPDN
KKKKPKKEEEQKWKWWEEERYPEGIKWKFLEHKGPVFAPPYEPLPENVKFYYDGKVMKLSPK
AEEVATFFAKMLDHEYTTKEIFRKNFFKDWRKEMTNEEKNIITNLSKCDFTQMSQYFKAQTEAR
KQMSKEEKLKIKEENEKLLKEYGFCIMDNHKERIANFKIEPPGL<u>ERGRGNHPKM</u>[2-1]GMLKRRIMP
EDIIINCSKDAKVPSPPPGHKWKEVRHDNKVTWLVSWTENIQGSIKYIMLNPSSRIKGEKDWQKY
ETARRLKKCVDKIRNQYREDWKSKEMKVRQRAVALYFIDKLALRAGNEKEEGETADTVG[4]CCSL
RVEHINLHPELDGQEYVVEFDFLG<u>KDS</u>[2-2]IRYYNKVPVEKRVFKNLQLFMENKQPEDDLFDRLNT
GILNKHLQDLMEGLTAKVFRTYNASITLQQQLKELTAPDENIPAKILSYNRANRAVAILCNHQRAP
PKTFEKSMMNLQTKIDAKKEQLADARRDLKSAKADAKVMKDAKTKKVVESKKKAVQRLEEQL
MKLEVQATDREENKQIAL<u>GTSKLN</u>[1]YLDPRI<u>T</u>[3]VAWCKKWGVPIEKIYNKTQREKFAWAIDMADE
DYEF

Figure 1. TOP I mutation corrrelated to CPT-11 resistance. NCBI reference sequence: NM_003286.1. (A) Protein sequence; Y, active catalytic tyrosine site; shaded location with underline: mutation site related to TOP I resistance; 1, region 1; 2-1 and 2-1, region 2; 3, region 3; 4, region 4 [see Redinbo *et al* (6) for the numbering of each region].

5 single nucleotide polymorphisms (SNPs; 4 introns, and 1 exon: *UGT1A1**6) within the *UGT1A1* gene and serum total bilirubin levels was noted in Japanese subjects (15).

Although the TOP-I mutation-related CPT-11 resistance has been elucidated, the prevalence of TOP-I germline mutation has yet to be investigated. The prevalence of TOP-I germline mutation requires investigation to elucidate the group of TOP-I-resistant patients prior to cancer chemotherapy. On the other hand, polymorphisms of *UGT1A1* have been revealed to be associated with CPT-11 toxicity in clinical situations. Additionally, less frequent (~50%) *UGT1A1*28* and frequent *UGT1A1*6* have been reported in individuals of Asian descent compared to Caucasians and individuals of African descent (16-21). The prevalence of *UGT1A1* polymorphisms in Asian individuals requires further confirmation studies to avoid toxicity-related death in cancer patients.

The primary aim of this study was to investigate the prevalence of mutation in the TOP-I exons associated with CPT-11 resistance, including untreated cancer tissue. A secondary aim was to confirm the frequency of polymorphisms in the *UGT1A1* genes related to CPT-11 toxicity in a Japanese population.

Materials and methods

Materials. The study protocol was approved by the Ethics Committee of Kitasato University School of Medicine, Japan (B03-07 for healthy volunteers, B03-28 and G03-04 for patients with lung cancer). Blood samples of healthy volunteers (n=236 for TOP-I mutations and n=126 for *UGT1A1* polymorphism) as well as lung cancer tissue specimens from untreated patients (n=16) were examined. The lung cancer tissue samples comprising 6 resected tissues and 10 biopsy specimens constituted of 8 cases of small cell carcinoma, 7 of adenocarcinoma and 1 of squamous cell carcinoma. All of the subjects provided written informed consent prior to their being enrolled in this study.

DNA extraction and polymerase chain reaction. DNA was extracted from 200 μl whole blood using the QIAamp® DNA blood mini kit (Qiagen, Valencia, CA, USA). Paraffin-embedded lung cancer tissues obtained from lung cancer patients were stained with hematoxylin and eosin, and the DNA was extracted from LASER-captured microdissected tumor tissue (AS LMD, Leica, Tokyo, Japan) using the QIAamp® DNA Micro kit (Qiagen).

Polymerase chain reaction (PCR) was performed to amplify the targeted regions of TOP-I (or *UGT1A1*) with a thermal cycler (Takara Thermal Cycler SP, Takara, Shiga, Japan; or PTC-200 DNA Engine, MJ Research, Watertown, MA, USA) using each primer (Table I), HotStarTaq DNA polymerase and the Q-Solution Kit (Qiagen) in accordance with the manufacturer's protocol, with the exception of the case of *UGT1A1**28 (see below). The PCR products were identified by gel electrophoresis. After excising the DNA fragment band from the agarose gel, high final concentrations of DNA were extracted using a MinElute® gel extraction kit (Qiagen).

Genetic analyses of TOP-I. Five TOP-I mutations correlated to CPT-11 resistance were previously reported (2-7), as follows [see Redinbo *et al* (6) for the numbering of each region]: region 1, exon 20 (541-558); region 2-1, exon 12 (501-530); region 2-2, exon 15 (186-194); region 3, exon 20 (577-579); and region 4, exon 15 (99-101) (Fig. 1A and B). The 5 mutation-hotspot regions of TOP-I were amplified using the relevant primer pairs (Table I). Direct sequencing of the PCR products was performed using ABI PRISM 3100 Genetic Analyzer (Life Technologies Corporation, Carlsbad, CA, USA). Sequencing reactions were performed in both the forward and reverse directions.

Genetic analyses of UGT1A1. The following variant sequences were investigated: a 2-extra-nucleotide insertion (TA) within the TATA box in the promoter, resulting in the sequence

B

cDNA sequence

```
1     caaatgcgaa cttaggctgt tacacaactg ctgggtctg ttctcgccgc ccgcccggca
61    gtcaggcagc gtcgccgccg tggtagcagc ctcagccgtt tctggagtct cgggcccaca
121   gtcaccgccg cttacctgcg cctcctcgag cctccggagt ccccgtcccg cccgcacaag
181   gccggtcgcc gtctgcgtct cccccacgcc gcctcgcctg ccgccgcgct cgtccctccg
241   ggccgacatg agtggggacc acctccacaa cgattcccag atcgaagcgg atttccgatt
301   gaatgattct cataaacaca aagataaaca caaagatcga gaacaccggc acaaagaaca
361   caagaaggag aaggaccggg aaaagtccaa gcatagcaac agtgaacata aagattctga
421   aaagaaacac aaagagaagg agaagaccaa acacaaagat ggaagctcag aaaagcataa
481   agacaaacat aaagacagag acaaggaaaa acgaaaagag gaaaaggttc gagcctctgg
541   ggatgcaaaa ataaagaagg agaaggaaaa tggcttctct agtccaccac aaattaaaga
601   tgaacctgaa gatgatggct attttgttcc tcctaaagag gatataaagc cattaaagag
661   acctcgagat gaggatgatg ctgattataa acctaagaaa attaaaacag aagataccaa
721   gaaggagaag aaaagaaaac tagaagaaga agaggatggt aaattgaaaa aacccaagaa
781   taaagataaa gataaaaaag ttcctgagcc agataacaag aaaaagaagc cgaagaaaga
841   agaggaacag aagtggaaat ggtgggaaga agagcgctat cctgaaggca tcaagtggaa
901   attcctagaa cataaaggtc cagtatttgc cccaccatat gagcctcttc cagagaatgt
961   caagttttat tatgatggta aagtcatgaa gctgagcccc aaagcagagg aagtagctac
1021  gttctttgca aaaatgctcg accatgaata tactaccaag gaaatattta ggaaaaattt
1081  ctttaaagac tggagaaagg aaatgactaa tgaagagaag aatattatca ccaacctaag
1141  caaatgtgat tttacccaga tgagccagta tttcaaagcc cagacggaag ctcggaaaca
1201  gatgagcaag gaagagaaac tgaaaatcaa agaggagaat gaaaaattac tgaaagaata
1261  tggattctgt attatggata accacaaaga gaggattgct aacttcaaga tagagcctcc
1321  tggacttttc cgtggccgcg gcaaccaccc caagatggc atgctgaaga gacgaatcat
                Region 2-1
1381  gcccgaggat ataatcatca actgtagcaa agatgccaag gttccttctc ctcctccagg
1441  acataagtgg aaagaagtcc ggcatgataa caaggttact tggctggttt cctggacaga
1501  gaacatccaa ggttccatta aatacatcat gcttaaccct agttcacgaa tcaagggtga
1561  gaaggactgg cagaaatacg agactgctcg gcggctgaaa aaatgtgtgg acaagatccg
1621  gaaccagtat cgagaagact ggaagtccaa agagatgaaa gtccggcaga gagctgtagc
1681  cctgtacttc atcgacaagc ttgctctgag agcaggcaat gaaaaggagg aaggagaaac
1741  agcggacact gtgggctgct gctcacttcg tgtggagcac atcaatctac acccagagtt
              Region 4
1801  ggatggtcag gaatatgtgg tagagtttga cttcctcggg aaggactcca tcagatacta
                                                  Region 2-2
1861  taacaaggtc cctgttgaga aacgagtttt taagaaccta caactattta tggagaacaa
1921  gcagcccgag gatgatcttt ttgatagact caatactggt attctgaata agcatcttca
1981  ggatctcatg gagggcttga cagccaaggt attccgtaca tacaatgcct ccatcacgct
2041  acagcagcag ctaaaagaac tgacagcccc ggatgagaac atcccagcga agatcctttc
2101  ttataaccgt gccaatcgag ctgttgcaat tctttgtaac catcagaggg caccaccaaa
2161  aacttttgag aagtctatga tgaacttgca aactaagatt gatgccaaga aggaacagct
2221  agcagatgcc cggagagacc tgaaaagtgc taaggctgat gccaaggtca tgaaggatgc
2281  aaagacgaag aaggtagtag agtcaaagaa gaaggctgtt cagagactgg aggaacagtt
2341  gatgaagctg gaagttcaag ccacagaccg agaggaaaat aaacagattg ccctgggaac
2401  ctccaaactc aattatctgg accctaggat cacagtggct tggtgcaaga agtgggtgt
      Region 1      *          Region 3
2461  cccaattgag aagatttaca acaaaaccca gcgggagaag tttgcctggg ccattgacat
2521  ggctgatgaa gactatgagt tttagccagt ctcaagaggc agagttctgt gaagaggaac
2581  agtgtggttt gggaaagatg gataaactga gcctcacttg ccctcgtgcc tgggggagag
2641  aggcagcaag tcttaacaaa ccaacatctt tgcgaaaaga taaacctgga gatattataa
2701  gggagagctg agccagttgt cctatggaca acttatttaa aaatatttca gatatcaaaa
2761  ttctagctgt atgatttgtt ttgaattttg tttttatttt caagagggca agtggatggg
2821  aatttgtcag cgttctacca ggcaaattca ctgtttcact gaaatgtttg gattctctta
2881  gctactgtat gcaaagtccg attatattgg tgcgttttta cagttagggt tttgcaataa
2941  cttctatatt ttaatagaaa taaattccta aactccctic cctctctccc atttcaggaa
3001  tttaaaatta agtagaacaa aaaacccagc gcacctgtta gagtcgtcac tctctattgt
3061  catggggatc aattttcatt aaacttgaag cagtcgtggc tttggcagtg ttttggttca
3121  gacacctgtt cacagaaaaa gcatgatggg aaaatatttc ctgacttgag tgttcctttt
3181  taaatgtgaa ttttatttct ttttaattat tttaaaatat ttaaaccttt ttcttgatct
3241  taaagatcgt gtagattggg gttggggagg gatgaagggc gagtgaatct aaggataatg
3301  aaataatcag tgactgaaac cattttccca tcatccttig ttctgagcat tcgctgtacc
3361  ctttaagata tccatctttt tctttttaac cctaatcttt cacttgaaag attttattgt
3421  ataaaaagtt tcacaggtca ataaacttag aggaaaatga gtatttggtc caaaaaaagg
3481  aaaaataatc aagatttiag ggcttttatt ttttctttig taattgtgta aaaatggaa
3541  aaaaacataa aaagcagaat tttaatgtga agacattttt tgctataatc attagtttta
3601  gaggcattgt tagtttagtg tgtgtgcaga gtccatttcc cacatctttc ctcaagtatc
3661  ttctatttit atcatgaatt ccctttiaat caactgtagg ttatttaaaa ataaattcca
3721  tcaacttaat cnaaactaa
```

Figure 1. (B) cDNA sequence; *tat, active catalytic tyrosine site. Shaded location with bold character(s) and underline: mutation site correlated to TOP I resistance.

$(TA)_7TAA$ (-39 to -53, *UGT1A1**28: rs8175347); transition (+295 from the initial site of transcription, G to A) at codon 71 in exon 1 that is associated with a substitution of glycine to arginine (G71R, *UGT1A1**6: rs4148323); and a transversion (+770, C to A) at codon 229 in exon 1 that alters proline to glutamine (P229Q, *UGT1A1**27: rs35350960). The variant longer sequence of *UGT1A1**28 was distinguished from the wild-type sequence using poly-acrylamide gel electrophoresis (PAGE) with a DNA-sequencer (Long-Read Tower™, Visible Genetics, Suwanee, GA, USA) following amplification of the targeted sequence using PCR with Pfu DNA Polymerase (native) (Fermentas Life Sciences, Crt Burlington, Ontario, Canada). The primers (Table II) were designed to amplify a 216-bp segment of *UGT1A1**28 variant-type sequence as compared

Table I. Primers for topoisomerase I and *UGT1A1*.

	Forward primer 5'-3'	Reverse primer 5'-3'
TOP-I		
exon20	TAGGGTAGTAGAGTCAAAGAAGAA	GCCAGAAGTTTCCCCAGAGG
exon12	GACTTTCCTCTACCTTGACTTA	GACGCCCTCCACCCCCTTTTT
exon15	TTCCATTCATGCTCATCTTTTCTT	TGTGCCTGTTGCCTGTCTCA
UGT1A1		
*UGT1A1**6	AAGTAGGAGAGGGCGAACC	GTGGGCAGAGACAGGTACT
*UGT1A1**27	AGTACCTGTCTCTGCCCAC	GTCCCACTCCAATACACAC
*UGT1A1**28	TATAGTCACGTGACACAGTC	CCACTGGGATCAACAGTATCT

TOP-I, topoisomerase I; *UGT1A1*, UDP-glucuronyltransferase 1A1; *UGT1A1**6: G→A on exon 1 (protein, G71R); *UGT1A1**27, 770C→A on exon 1 (protein, P229Q); *UGT1A1**28, $(TA)_7TAA$ on the promoter (protein, reduced expression of *UGT1A1*).

Table II. Co-occurence of *UGT1A1**28, *UGT1A1**6 and *UGT1A1**27 polymorphisms in healthy volunteers (n=126).

Number of cases among the 126 subjects		*UGT1A1**28 Wild	Hetero	Homo	Total in *UGT1A1**6 or *27
*UGT1A1**6	Wild	71	18	3	92
	Hetero	26	4	0	30
	Homo	4	0	0	4
*UGT1A1**27	Wild	101	21	3	125
	Hetero	0	1	0	1
	Homo	0	0	0	0
Total in *UGT1A1**28		101	22	3	126

UGT1A1, UDP-glucuronyltransferase 1A1 gene; wild, wild-type; hetero, heterozygous; homo, homozygous.

with the 214-bp segment of the wild-type sequence. The separation ability was previously verified using guaranteed wild-type and variant-type genome DNA obtained from Daiichi Pure Chemicals (now Sekisui Medical Co. Ltd., Japan). For analysis of *UGT1A1**6 and *UGT1A1**27, direct sequencing of the PCR amplification product obtained using specific primers (Table I) was performed using the ABI PRISM 3100 genetic analyzer.

Results

Mutations of TOP-I related to CPT-11 resistance. Since no TOP-I mutations were observed in any of the 126 subjects, an additional 110 healthy volunteers, as well as untreated lung cancer tissue specimens in patients (n=16), were investigated. The results revealed that no genetic mutations correlated to CPT11-resistance in exons 12, 15, 16 or 20 of TOP-I.

UGT1A1 genetic polymorphism associated with CPT-11 toxicity. Homozygous polymorphisms of *UGT1A1* were detected in 126 subjects, and were distributed as follows: *UGT1A1**28 in 3 (2.4%) subjects and *UGT1A1**6 in 4 (3.2%) subjects (Table II), and co-occurrence of heterozygous polymorphisms for both *UGT1A1**6 and *UGT1A1**28 in 4 subjects (3.2%), and for both *UGT1A1**27 and *UGT1A1**28 in 1 (0.8%) subject (Table III). The frequency of the *UGT1A1**28 variant allele was found to be 19.8%. In general, either homozygous or heterozygous polymorphisms of *UGT1A1**6 were detected in 30 (23.8%) subjects, and either homozygous or heterozygous polymorphisms of *UGT1A1**6, *27 or *28 were detected in 55 (43.7%) subjects.

The Hardy-Weinberg deviation test showed that *UGT1A1**6 (p=0.43), *27 (p=0.96) and *28 (p=0.19) was not significantly deviated from the equilibrium. The linkage analysis revealed that normalized linkage disequilibrium coefficient D' for *UGT1A1**6 and *UGT1A1**28 was 0.05. However, the linkage disequilibrium was not detected (p= 0.64).

Discussion

This is the first study to exmaine the frequency of TOP-I mutation associated with CPT-11 resistance in healthy subjects and in untreated lung cancer tissue specimens. Although *in vitro* reports have demonstrated that CPT-11-resistant cancer cell lines exposed to CPT-11 possessed TOP-I mutations with no obvious or only minor defects in cell function, these changes were undetectable in healthy subjects and in untreated lung cancer tissue as mutation. Therefore, in the initial chemo-

therapy with CPT-11, the resistance-related TOP-I mutation is unlikely to occur.

Tsurutani *et al* (22) examined 16 samples obtained from 8 CPT-11-treated patients with lung cancer, and detected 2 types of TOP-I mutations in exon 21 in 1 tumor specimen. In a human colon cancer cell line (HCT-15) (23), one exonic mutation was detected in a heterozygous state in exon 19. Since the mutations coded on exon 19 and 21 have not been reported in previous *in vitro* CPT-11-resistant cancer cell lines, these regions were not examined in our study. However, the results indicated that the development of some acquired CPT-11 mutations was possible in patients in the course of treatment with CPT-11.

According to the SNP database (http://www.ncbi.nlm.nih.gov/SNP/), missense mutations in exons 4 (1 locus), 9 (1 locus), 15 (3 loci), and 21 (1 locus) are currently registered. These SNPs are not correlated to CPT-11 resistance, although a haplotype-tagging SNP in the intervening sequence region has been found to be associated with toxicity (grade 3/4 neutropenia) in patients treated with CPT-11 (17).

Determination of the *UGT1A1* genotypes is clinically significant for the prediction of CPT-11-related severe toxicity (11). Individuals who have at least 1 variant (heterozygous) allele for the *UGT1A1**28 may exhibit reduced elimination of SN-38 and increased probability of development of dose-limiting neutropenia (11).

The frequency of the *UGT1A1**28 variant allele has been reported to be 30-45% in Caucasian, African and Indian populations, which is approximately twice that of the 10-20% reported in East Asian populations (17-22), as confirmed by the frequency of this study (19.8%). On the other hand, *UGT1A1**6 has been detected in 16-40% of Asian individuals, as confirmed by the frequency of 23.8% found in this study; *UGT1A1**6, however, was extremely rare in the Caucasian and African populations (17). The *UGT1A1**27 allele detected only in Asian individuals was reported to be harboured exclusively by a *UGT1A1**28 haplotype, as findings of this study show. Either homozygous or heterozygous polymorphisms of *UGT1A1**6, *27 or *28 were detected in 55 (43.7%) subjects in this study; the risk of *UGT1A1* polymorphism-related toxicity of CPT-11 is considered to be high in East Asian individuals.

The Food and Drug Administration in the United States has approved an amendment of the label for Camptosar (irinotecan hydrochloride), to which a warning to reduce the starting dose of irinotecan for *UGT1A1**28 homozygous patients has been added. In East Asian individuals, both *UGT1A1**6 and *UGT1A1**28 require examination, since there is no linkage disequilibrium between the two polymorphisms (24), as indicated in the present study.

As a clinical relevance, the risk of TOP-I mutation-related resistance to CPT-11 is unlikely and it is not necessary to test for TOP-I mutation prior to chemotherapy with CPT-11. However, the risk of *UGT1A1* polymorphism-related toxicity of CPT-11 is markedly higher in East Asian individuals, and *UGT1A1* polymorphisms, not only of *UGT1A1**28, but also of *UGT1A1**6, should be tested prior to treatment with CPT-11 to avoid severe adverse effects.

In conclusion, the main findings in this study were twofold. First, TOP-I genetic mutations related to CPT-11 resistance were not detected in any of the subjects or in untreated lung cancer tissues. Second, compared to Caucasians and individuals of African descent, it was observed that *UGT1A1**28 was less frequent and *UGT1A1**6 was more frequent in Japanese subjects. Moreover, linkage disequilibrium was not noted between *UGT1A1**6 and *UGT1A1**28.

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