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

TOMOYA FUKUI¹, HISASHI MITSUFUJI¹, MASARU KUBOTA¹, HIDENORI INAOKA³, MINORU HIROSE³, KEIICHI IWABUCHI², NORIYUKI MASUDA¹ and HIROSUKE KOBAYASHI³

Departments of ¹Respiratory Medicine and ²Pathology, School of Medicine; ³Department of Medical Engineering and Technology, School of Allied Health Sciences, Kitasato University, Kanagawa 252-0373, Japan

Received January 27, 2011; Accepted June 17, 2011

DOI: 10.3892/ol.2011.346

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.

Introduction

Irinotecan hydrochloride (CPT-11), a water-soluble semisynthetic 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-11induced 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

Correspondence to: Dr Hirosuke Kobayashi, Department of Medical Engineering and Technology, School of Allied Health Sciences, 1-15-1 Kitasato, Sagamihara, Minami-ku, Kanagawa 252-0373, Japan E-mail: hiro@kitasato-u.ac.jp

Key words: topoisomerase I, mutation, UGT1A1, polymorphism, CPT-11

A Protein sequence

MSG DHLHND SQIEADFRLNDSHKHK DKHKD REHRH KEHKKE KDREKSKHSNSEH KDSEKKHK EKEK TKHKD GSSEKHK DKHK DRD KEKR KEEK V RA SGD AKIK KEKENG FSSPPQ IKDEPEDD GYF VPPK EDIKPLKR PR DED DADY KPK KIKT ED TKKEK KRK LEEEED GKLKKPKNKD KDKK VPEPDN KKK KPK KEEEQK WKW WEEERY PEG IKW KFLEH KGP VFAPPYEPLPENVKF YYD GKVMKL SPK AEEVAT FFAKMLD HEYTTKE IFR KNFF KDWRKEMT NEEKNIITNL SKCDFT QMSQ YFK AQTEAR KQMSKEEKLK IKEEN EKLLKEY GFCIMD NHKERIA NFK IEPPG L**FRGRGNHPKM²⁻¹** GMLKRRIMP EDI IIN CSKDAKV PSPPPGHKW KEVRHDNK VTW LVSW TENI QGSIKYIMLNPSSRIKGEKD WQKY ETARRLKKC VDK IRNQYREDW KSKEMKVR QRAVALYFIDKL ALRA GNEKEE GETAD TV G⁴ CCSL RVEHINLHPELD GQEYVVEFDFLG KDS²⁻² IRYY NKVP VEKRVF KNLQLFMEN KQPEDDLFDRLNT GILNKHLQ DLMEGLTAKVFRTYNA SIT LQQQLK ELTA PDEN IPAKILS YNRANRAVAILCNH QRAP PKTFE KSMMNLQ TKIDAKKEQLA DARRD LKSAKADAK VMKDAK TKKVV ESK KKAVQRLEEQL MKLEVQ ATDREENKQI AL GTSKLN ¹Y LDPRIT³ VAWCKKW GVPIEKI YNKT QREKFAWAIDMADE 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 μ l whole blood using the QIAamp[®] DNA blood mini kit (Qiagen, Valencia, CA, USA). Paraffinembedded 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 OIAamp[®] 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

В

cDNA sequence

cDNA se	equence					
1		cttaggetgt	tacacaactg	ctggggtctg	ttatcgcage	ccgcccggca
61	gtcaggcagc	dredecadeed	tggtagcagc	ctcagccgtt	tctggagtct	coggeccaca
121			cctcctcgag			
181	gacgqtagcc	qtctqcqtct	cccccacgcc	gcctcgcctg	ccqcqcqct	cqtccctccq
241			acctœacaa			
301			aagataaaca			
361			aaaagtccaa			
421			agaagaccaa			
481			acaaggaaaa			
541	-		agaaggaaaa			
601			atttgttcc			
661	acctcgagat	gaggatgatg	ctgattataa	acctaagaaa	attaaaacag	aagataccaa
721	gaaggagaag	aaaagaaaac	tagaagaaga	agaggatggt	aaattgaaaa	aacccaagaa
781	taaagataaa	gataaæaaag	ttcctgagcc	agataacaag	aaæagaægc	cgaagaaaga
841	agaggaæag	ægtggaaat	ggtgggaaga	agagegetat	cctgaaggca	tcaagtggaa
901	attcctagaa	cataaaggtc	cagtatttgc	cccaccatat	gagectette	cagagaatgt
961	caagttttat	tatgatggta	aagtcatgaa	getgageeœ	aaagcagagg	aagtagetae
1021	-		accatgaata			
1081	ctttaaagac	tggagaaagg	aaatgactaa	tgaagagaag	aatattatca	ccaacctaag
1141	caaatgtgat	tttaccaga	tgagccagta	tttcaaagcc	cagacggaag	ctcggaaaca
1201	gatgagcaag	gaagagaaac	tgaaaatcaa	agaggagaat	gaaaaattac	tgaaagaata
1261	tggattetgt	attatggata	accacaaaga	gaggattgct	aacttcaaga	tagageetee
1321	tggactt ttc	aqtqqacqcq	qcaaccaccc	caaqatqggc	atgetgaaga	gacgaatcat
	Re	gion 2-1				
1381			actgtagcaa		-	
1441			ggcatgataa			
1501			aatacatcat			
1561			agactgctcg			
1621			ggaagtccaa			
1681	-		ttgctctgag			
1741			gctcacttog	tgtggagcæ	atcaatctac	acccagagtt
1801		gion 4 gaatatgtgg	tagagtttga			tcagatacta
1961	taaaata		~~~~		ion 2-2	+
1861			aacgagtttt	taagaaccta	caætattta	
1921	gcagcccgag	gatgatettt	ttgatagact	taagaaccta caatactggt	caactattta attctgaata	agcatettea
1921 1981	gcagcccgag ggatctcatg	gatgatettt gagggettga	ttgatagact cagccaaggt	taagaaccta caatactggt attocgtaca	caactattta attetgaata tacaatgeet	agcatettea ceateaeget
1921 1981 2041	gcagccogag ggatctcatg acagcagcag	gatgatettt gagggettga etaaaagaae	ttgatagact cagccaaggt tgacagcccc	taagaaccta caatactggt attœgtaca ggatgagaac	caactattta attctgaata tacaatgcct atcccagcga	agcatettea ceateaeget agateettte
1921 1981 2041 2101	gcagcccgag ggatctcatg acagcagcag ttataaccgt	gatgatettt gagggettga etaaaagaac gecaategag	ttgatagact cagccaaggt tgacagcccc ctgttgcaat	taagaaccta caatactggt attœgtaœ ggatgagaæ tctttgtaæ	caactattta attetgaata tacaatgeet ateecagega cateagaggg	agcatettea ceateaeget agateette caecaecaaa
1921 1981 2041 2101 2161	gcagcccgag ggatctcatg acagcagcag ttataaccgt aacttttgag	gatgatettt gagggettga etaaaagaac gecaategag aagtetatga	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca	taagaaccta caatactggt attœgtaœ ggatgagaæ tctttgtaæ aactaagatt	caactattta attetgaata tacaatgeet ateecagega cateagaggg gatgeecaaga	agcatettea ceateaeget agateette caccaccaaa aggaacaget
1921 1981 2041 2101 2161 2221	gcagcccgag ggatctcatg acagcagcag ttataaccgt aacttttgag agcagatgcc	gatgatettt gagggettga etaaaagaac gecaategag agtetatga oggagagaec	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca tgaaagtgc	taagaaccta caatactggt attœgtaœ ggatgagaœ tctttgtaœ aactaagatt taaggctgat	caactattta attetgaata taœatgœt atœeagoga cateagaggg gatgeeaaga geœaggtea	agatettea cateaget agateette caceacaaa aggaacaget tgaaggatge
1921 1981 2041 2101 2161 2221 2281	gcagcccgag ggatctcatg acagcagcag ttataaccgt aacttttgag agcagatgcc aaagacgaag	gatgatettt gagggettga etaaaagaac gecaategag agtetatga eggagagaec aaggtagtag	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca tgaaagtgc agtcaaagaa	taagaaccta caatactggt attœgtaœ ggatgagaœ tctttgtaœ aactaagatt taaggctgat gaaggctgtt	caactattta attetgaata taœatgœt atœeagœga cateagaggg gatgeeaaga geœaggtea cagagaetgg	agatettea ecateaget agateette caceaccaaa aggaacaget tgaaggatge aggaacagtt
1921 1981 2041 2101 2161 2221 2281 2341	gcagcccgag ggatctcatg acagcagcag ttataaccgt aacttttgag agcagatgcc aaagacgaag gatgaagctg	gatgatettt gagggettga etaaaagaac gecaategag agtetatga ggaggagaec aggtagtag gaagteaag gaagteaag	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca tgaaagtgc agtcaaagaa ccacagaccg	taagaaccta caatactggt attœgtaœ ggatgagaæ tctttgtaæ aactaagatt taaggctgat gaaggctgtt agaggaaaat	caactattta attetgaata tacaatgoot atoccagoga catcagaggg gatgoccaga gcoaaggtoa cagagactgg aaacagattg	agcatettea ceateaeget agateettte caccaceaa aggaaeaget tgaaggatge aggaaeagtt ceetg ggaae
1921 1981 2041 2161 2221 2281 2341 2401	gcagcccgag ggatctcatg acagcagcag ttataaccgt aacttttgag agcagatgcc aagaacgaag gatgaagctg <u>ctocaaactc</u> Region 1	gatgatettt gagggdtga dtaaaagaac gccaategag agtctatga ggaggagacc aggtagtag gaagttcaag <u>aagttc</u> atgg <u>aagttc</u> ag <u>aagttc</u> ag	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca tgaaagtgc agtcaaagaa ccacagaccg accctaggat Reg	taagaaccta caatactggt attccgtaca ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggctgat agaggaaaat caagtggctgit ion 3	caactattta attotgaata tacaatgoct atoccagogg gatgccaaga gccaaggtca cagagactgg aacagattg tggtgcaaga	agcatettea coateaoget agateette coecacoaaa aggaacaget aggaacaget coetg ggaac agtggggtgt
1921 1981 2041 2101 2221 2281 2341 2401 2461	gcagcccgag ggatctcatg acagcagcag ttataaccgt agcagatgcc aacagcagag gatgaagctg ctccaactc Region 1 cccaattgag	gatgatottt gagggdtga dtaaaagaac gcaatggag agtctatga gaggagacc aaggtagtag gaagttcaag attt ctgg * agatttacag	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaaagtgc agtcaaagaa accctaggat accctaggat accaaaaccca	taagaaccta caatactggt attcgtaca ggatgagaac tctttgtaac aactaagatt taaggctgat ggaggctgtt agaggaaaat cacqgtggct ion 3 gcgggagaag	caactattta attotgaata tacaatgoot atoccagoga gatgccaaga gocaaggtoa cagagactgg aacagattg tggtgcaaga tttgcctggg	agcatettea coateaget agateette coecacoaaa aggaacaget tgaaggatge aggaacagtt coetg ggaac agtggggtgt
1921 1981 2041 2101 2221 2281 2341 2401 2461 2521	gcagcccgag ggatctcatg acagcagcag ttataaccgt aacttttgag gacgagatgcc aaagacgaag gatgaacctg ctccaaactc Region 1 cccaattgag ggctgatgaa	gatgatottt gagggdtga dtaaaagaac gcaatggag agtcatga gaggtcatga gaggtcatga gaggtcaag attt ctgg * aggatttaca gagtttacag gagtttacag	ttgatagact cagocaaggt tgacagocoo ctgttgcaat tgaaagtgo agtcaaagaa ccacagacog accotaggat Reg acaaaacocca tttagocagt	taagaaccta caatactggt attccgtaa ggatgagaac tecttgtaac aactaagatt taaggetgat gaaggaaaat cacagtggct jon 3 gegggagaag ctcaagagg	caactattta attotgaata tacaatgact atcocagoga gatgccaaga gccaaggtca cagagactgg aacagattg tggtgcaaga ttggtgcaaga	agcatettea coateaget agateette caceacoaaa aggaacaget tgaaggatge aggaacagtt coetg ggac agtggggtgt coattgacat gaagaggaca
1921 1981 2041 2161 2221 2281 2341 2401 2461 2521 2581	gcagcccgag ggatctatg acagcagcag ttataacogt aacttttgag agcagatgc aagaagatgc aagaagatg gatgaactg ctcaactc Region 1 cccaattgag ggctgatgaa agtgtggttt	gatgatottt gagggdtga daaaagaac gocaatogag agtotatga ggaggagacc aggtagtag gaagttoaag atta ctag atta taca gactatgagt gggaagatg	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaaatgca agtcaaagaa cacaagaccg accctaggat Reg accaaaccca tttagccagt gataaactga	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggctgtt agaggaaaat caagtggct iion 3 gcgggagag gcctcaactgg	caactattta attotgaata tacaatgact atoccagoga gatgccaaga gcaaggtca cagagactgg aacagattg tggtgcaaga ttggcctggg agagttctgt coccgggc	agatettea coateaget agateette caecacoaaa aggaacaget tgaaggatge aggaacagtt coetg ggac agtggggtgt coattgacat gaagaggac tgggggagag
1921 1981 2041 2161 2221 2281 2341 2401 2461 2521 2581 2641	gcagcccgag ggatctcatg acagcagcag ttataaccgt aacttttgag agcagatgcc aaagacgaag gatgaacgtg ctccaaactc Region 1 cccaattgag ggctgatgaa agtgtggttt aggcagcaag	gatgatottt gagggdtga daaaagaac gcaatogag ggagaca aggtagtag gaagttcaag attat ctgg * aagatttaca ggatgagt ggatgagt ggatgagt ggaagatg tottaacaaa	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaaagtgc agtcaaagaa ccacagaccg accatagat Reg acaaaaccca gataaaccaa gataaactga ccacagcatt	taagaaccta caatactggt attcgtaa ggatgagaac totttgtaac aactaagatt taaggetgat gaaggetgtt agaggaaaat cacaytggc googggagag ctcaagagc gcetcacttg tgcgaaaag	caactattta attotgaata tacaatgact ataccagga gatgccaaga ggcaaggtca cagagactgg aaacagattg tggtgcaaga tttgcctggg agagttctgt ccccgtgc taaacctgga	agcatettea coateaoget agateettee coateaoaa aggaacaget tgaaggatge aggaacagtt coetg ggac agtggggtgt coattgacat gaagaggaac tgggggagag gatattataa
1921 1981 2041 2101 2161 2221 2281 2341 2401 2461 2521 2581 2581 2581 2581	gcagcccgag ggatctcatg acagcagcag tataaccgt aacttttgag agcagatgcc aaagacgaag gatgaagstgc ctrcaaatt gag ggctgatgaa agtgtggttt aggcagcag gggaggacga	gatgatottt gagggdtga daaaagaac gcaatogag gggaggacc aggtagtag gaagttcaag attat ctgg * aagatttaca gactatgagt gggaagatg tcttazaaa agccagtgt	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacattgca tgaacatgca agtcaaagaa ccacagaccog accataggat Reg acaaaaccca tttagocagt gataaactga ccaacatctt cctatggaca	taagaaccta caatactggt attogtaa ggatgagaac totttgtaac aactaagatt taaggetgat agaggaaaat cacagtggct ion 3 gogggagag ctcagaggc goctoactg tgogaaaag actatttaa	caactattta attotgaata tacaatgact atacaagagg gatgccaaga gcaaggtca cagagactgg aaacagattg tggtgcaaga tttgcctggg agagttctgt ccctcgtoc taaactgga aaatatttca	agcatettea coateaoget agateettee coecacoaaa aggaacaget tgaaggatge aggaacaget coetg ggaac agtggggtgt coattgacat gaagaggaac tgggggagag gatattataa gatateaaaa
1921 1981 2041 2101 2161 2221 2281 2341 2401 2461 2581 2581 2641 2701 2761	gcagcccgag ggatctcatg acagcagcag acagcagcag agcagatgcc aacattttgag gatgaagctg ctocaactc Region 1 cccaattgag ggctgatgaa agtgtggttt aggcagcag gggagagctg ttctagcgt	gatgatottt gagggdtga dtaaagaac gcaatgag agtctatga gaggtgagacc aggtgtcagg gagttcaag agttatcag gagttcaag x agatttaca gactatgag ggaagatg tottaacaaa agcagtgt atgattgtt	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaactgca tgaaagtgc agtcaaagac accctaggat Reg accaagaccca tttagccagt gataaactga ccaacatctt cctatggaca ttgaatttg	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggaaaat cacagtggct ion 3 gcgggagaag ctcaagagc gcctcacttg tgcgaaaag acttatttaa	caactattta attotgaata tacaatgact atoccagoga gatgccaaga gccaaggtoa cagagactog aacagattg tggtgcaaga tttgcctggg agagttctgt cctcgtgoc taaactgga caagaggga	agcatettea coateaoget agateette caecaccaaa aggaacaget tgaaggatge aggaggggtgt coattgacat gaagaggaac tgggggagag gatattataa gatateaaa agtggatgg
1921 1981 2041 2101 2161 2221 2281 2341 2401 2461 2521 2581 2641 2701 2761 2821	gcagcccgag ggatctcatg acagcagcag acagcagcag agcagatgcc aacattttgag gatgaagctg ctocaactc Region 1 cccaattgag ggctgatgaa agtgtggttg aggcagcag gggaagactg ttctagctgt aatttgtoag	gatgatottt gagggdtga dtaaagaac gcaatgag agtctatga gaggtgagacc aggtgtgagacc aggtgtcagg agttc agg agttc agg agttc agg gagttcaag ggaagatg ggaagatg tcttaacaa agccagtgt atgattgtt ggtctacag	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca tgaaagtgc agtcaaagaa ccacagaccg accctaggat Reg acaaaaccca tttagccagt gataactga ccacatctt cctatggaca ttgaatttg ggcaaattca	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggaaaat cacagtggct ion 3 gcgggagaag ctcaagagg gcctcacttg tgcgaaaag acttatttaa tttttattt	caactattta attotgaata tacaatgoct atoccagoga gatgccaaga gccaaggtoa cagagactog aacagattg tggtgcaaga tttgcctggg agagttctgt cctcgtgoc taaactgga aaatatttoa cagaggoa gaaatgtttg	agcatettea coateaoget agateette coccaccaaa aggaacaget tgaaggatge aggaggggtgt coattgacat gaggaggagg gatattataa gatateaaa agtgggatgg gatettataa
1921 1981 2041 2101 2221 2281 2341 2461 2521 2581 2641 2761 2761 2821 2881	gcagcccgag ggatctatg acqcaccag acctttgag acctttgag agcagatgc aagaagatgc aagaacgag gatgaacgtg <u>ctcaactag</u> ggctgatgaa agtgtggttt agcagacag gggagactg ttctagctg aatttgtcag gctactgta	gatgatottt gagggdtga daaaagaac gocaatogag gagtatag ggaggagco aggtagtag gagttaag gagttaag gagttaag ggaagtotag ggaaagtg tottaacaaa gocagttgt atgattgtt ggtcaacaa gocagttgt atgattgtt	ttgatagact cagccaaggt tgaactgca ctgttgcaat tgaaagtgc agtcaagaa ccacagaccg accctaggat Reg acaaaaccca tttagccagt gataaactga ccaacatctt cctatggaca ttgaatttgg agtaatttgg acatttggaca	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggctgtt agaggaaaat cacagtggct gccggagag gcctcacttg tgcgaaaag acttattta tttttatttt ctgtttcact	caactattta attotgaata tacaatgacta catcagaga gatgccaaga gcaaggtca cagagactgg aacagattg tggtgcaaga ttggccaga ttggcctggc agagttctgt ccctcgtgc taacctgga aaatatttca caagaggga gaatgtttg cagtaggt	agatcttca catcagct agatctttc caccacaaa aggaacagct tgaggatgc aggaacagtt cactg ggac agtggggtgt cattgacat gagagaggac tgggggagag gatattaaa agtggatgg gatctctta tttgcaata
1921 1981 2041 2101 2221 2281 2341 2401 2461 2521 2581 2641 2701 2761 2821 2881 2891	gcagcccgag ggatctatg acagcagcag ttataacqt accttttgag acagtagtgc aagaagtgc aagaagtgc gatgaagtg cccaattgag ggctgatgaa agtgtggttt aggcagcag ggagagctg ttctagctgt ttctagctgt cttctatatt	gatgatottt gagggdtga daaaagaac gcaatogag ggagtatag ggaggtatag gagttacag adtt ctgg * agattaca ggctatagt ggaaagatg tottaacaaa agccagttgt atgattgtt gttotaccag tottacaga gcaagtcg tottaacaaa agcadtgat gttotaccag tottacaaa gcaagtcg tottaacaaa gcaagtcg tottaacaaa gtattagat	ttgatagact cagccaaggt tgaacgcccc ctgttgcaat tgaacgtgc agtcaagaa ccacagacg accataggat acaaaaccca tttagccagt gataactga ccaacatctt cctatggaca ttggcaattca ggcaaattca ttagtttg ggcaaattca	taagaaccta caatactggt attcgtaa ggatggagac tctttgtaac aactagatt taaggctgat gaaggctgtt agaggaaat caagtggct dion 3 gcgggagag gcctcacttg tgcgaaaga acttattta tttttatttt tgcgtttcact tgcgtttcact	caactattta attotgaata tacaatgact atoccagoga gatgccaaga gcaaggactgg aaacagattg tggtgcaaga tttgoctggg aagattotgt coctogtgoc taaacctgga aadatttta caagaggoa gaatgtttg cagtagggt cagtagggt	agatettea cateaget agateette caecacaaa aggaacaget tgaaggatge aggaacagtt ceetg ggac agtggggtgt ceetg ggac agtgggggag gatattataa gatateaaa agtggatgg gateetta tttgeaata atteagaa
1921 1981 2041 2101 2221 2281 2341 2401 2401 2521 2581 2581 2641 2701 2761 2881 2881 2881 2941 3001	gcagcccgag ggatctcatg acagcagcag ttataaccgt aacttttgag agcagatgcc aagaagag gatgaagstg chocaactc Region 1 cccaattgag ggctgatgaa agtgtggttt aggcagcaga gggagagstg ttctagctgt aatttgtcag gctactgtat tttaaaatta	gatgatottt gagggdtga daaaagaac gcaatogag agtctatga ggaggagacc aggtgtatag gaagttcaag attat ctgg * aggatttaca gactatgagt ggaagatg tottaacaaa agccagtgt atgattgtt gttatcacaa gaaagtcog ttaatagaaa gatagacaa	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaaagtgc agtcaaagaa ccacagacog accctaggat Reg acaaaaccca tttagccagt gataaactga ccaacatctt cctatggaca ttgagcaattca aataattgg taaattca aaaaccaa	taagaaccta caatactggt attcgtaa ggatgagaac tctttgtaac aactagatt taaggctgat gaaggctgtt agaggaaat caca gtggct joon 3 gogggagaag ctcaagagc goctcactty tgcgaaaag acttatttaa tttttatttt ctgtttcact gcgtttta aactocttc gcactgtta	caactattta attotgaata tacaatgact atacaagagg gatgccaaga gagactag aacagactag agagtcag agagtcog agagtcog cagaggca tttgcctggg aacagatcog caccogga aacagttog caccogga gaatgttta gaatgttta gaatgttta gaatgttagggt cagttagggt cagtcaggac	agcatettea coateaoget agateettee coaceoaaa aggaacaget tgaaggatge aggaacaget coetg ggaac agtggggtgt coattgacat gaagaggaac tgggggagag gatattataa gatateaaaa agtggatgg gatettetta tttgeaataa tttgeagaa tetetattg
1921 1981 2041 2101 2221 2281 2341 2401 2401 2521 2581 2581 2581 2581 2581 2581 258	gcagcccgag ggatctcatg acagcagcag acattttgag agcagatgcc aagacgag gatgaagetg ctocaactgag ggctgatgaa agtgtggttt aggcagcag gggaggetg ttctagetg ttctagetg ctdctattt ttttaaatta catggggatc	gatgatottt gagggdtga daaaagaac gcaatogag agtotatga ggaggagac aagtagtag gaagttcaag attat ctgg aattat ctgg ggaagattg ggaagatg tottazaaa agccagttgt agacttgtt gtaatgattgtt gtaatagaa agtagacaa aatagacag aatagacaa aatagacaa attutcatt	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaaagtgc agtcaaagaa ccacagacog accctaggat Reg acaaaaccca tttagccagt ccaaatctt cctatggaca ttgaattctg ggcaaattca attattgg taaatcca aaaacccag aaaaccga aaaactgaag	taagaaccta caatactggt attogtaa ggatgagaac totttgtaac aactaagatt taaggctgat gaaggctgtt agaggaaaat cacagtggct ion 3 gogggagag ctcagaggc goctoactg tgcgaaaag acttattta tttttatttt ctgtttcact tgcgtttta aactocctic gcacctgtta cagtgggc	caactattta attotgaata tacaatgact atacaagagg gatgccaaga gcaaggtca cagagactgg aaacagattg tggtgcaaga tttgcctggg agagttctgt ccctcgtoc taacctgga aaatatttoa caagaggga gaatgtttg cagtagggt cctctctacc gagtcgtac tttggcagtg	agcatettea coateaoget agateettee coecacoaaa aggaacaget tgaaggatge aggaacaget coetg ggaac agtggggtgt coetggggagag gatattataa gatateaaaa agtggatgg gatteetta tttgeaataa atteeagaa teetetteg
1921 1981 2041 2101 2221 2281 2341 2401 2401 2521 2581 2581 2641 2701 2761 2881 2881 2881 2941 3001	gcagcccgag ggatctcatg acagcagcag acattttgag agcagatgcc aagacgag gatgaagctg ctrcaaacte Region 1 cccaattgag ggctgatga agtgtggttt aggcagcag ggtagagctg ttctagctg aatttgtcag gctagctgt aatttgtcag gctcaattgtcag gctagctgt aatttgtcag gcagccgatg acatcgtat cttctaatt tttaaacta	gatgatottt gagggdtga dtaaagaac gcaatogag agtctatga aggtgagacc aggtggagacc aggtagtag gaagttcaag attat ctgg gaagttcaag tctaagagt gggaagatg tcttaacaa agccagttgt agattgtt ggtactaca gcaagtcg tuatagaaa agtagaaca agtagaacaa attuttcatt cacagaaaa	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca tgaaagtgc agtcaaagaa ccacagaccg accataggat Reg acaaaaccca tttagccagt ccaaatcta cctatggaca ttgaattctg ggcaaattca attatttgg taaattccta aaaaccagc aaacttgaag gcatgatggg	taagaaccta caatactggt attccgtaa ggatgagaac tccttgtaac aactaagatt taaggctgat gaaggaaaat cacagtggct ion 3 gcgcgaaag ctcaagagc gcctcactg tgcgaaag actattta ttttattt ctgtttcac gcactgtta gcaccgtgac aaaatattte	caactattta attotgaata tacaatgact ataccagga gatgccaaga gcaaggtca cagagactgg aacagattg tggtgccaaga ttggtgccaga ttggtgccaga acagagtctgt cactcggg agagttctgt cactcggg agaatgtttg cagtaggga gaatgtttg cagtaggg cagtcgtcac tttggccagtg cagtcgtcac	agcatettea coateaoget agateettee coecacoaaa aggaacaget tgaaggatge aggaacaget coetggggac agtggggtgt coattgacat gagaggaag gatattataa ggtateaaa agtggatgg gatteetta tttgcaataa attecagaa tteettgt ttttggttea
1921 1981 2041 2101 2221 2281 2341 2461 2521 2581 2581 2641 2701 2761 2881 2941 3001 3061 3121 3181	gcagcccgag ggatctatg acqcacag acttttgag agcagatgc aagacgag gatgaagctg ctocaactc Region 1 cccaattgag ggctgatgaa agtgtggttt aggcagcag gggagactg ttctagctgt aatttgtcag gctactgtat cttctattt tttaaaatta	gatgatottt gagggdtga dtaaaagaac gocaatogag agtotatga ggaggagac aggtagtag gagttoaag attataca gcaatgat gggaagatg tottaacaag gggaagatg tottaacaag agccagtgt dtgattgtt gttotacca gcaagtcog taatagacaa agtagacaa agttoataa attttoatt	ttgatagact cagccaaggt tgaactgca ctgttgcaat tgaaagtgc agtcaaagaa ccacagaccg accctaggat Reg acaaaacca tttagccagt gataaactga ccaaatctt cctatggaca attatattgg taaatccta aaattcta aaaaccag gcaagtggg tttaattat	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggaaaat caaggtggct cion 3 gcgggagaag ctcaagaggc gcctcactg tgcgaaaga actatttaa tttttattt ctgtttcact tgcgtttta aactoccttc gcactgtggc aaaatatttc	caactattta attotgaata tacaatgocta catoagaga gatgccaaga gcoaaggtoa cagagactog aaacagattg tggtgccaaga ttggtgccaaga ttggtgccaaga tttgcctggg agagttctgt coctogtoc tacacctgga gaatgtttg cagaggog gaatgtttg cagtagggc gagtogtcac tttggccagg ctgacttgag ttggccagtg ttggccagtg	agatettea cateaget agatette caecacoaa aggaacaget tgaggatge aggaacaget coetg ggac agtggggtgt coetg ggac tggggggagag gatattataa gatateaaa agtgatggg gatetetta tttgeaata tetteatgt ttttggttea
1921 1981 2041 2101 2221 2281 2341 2401 2521 2581 2641 2701 2761 2881 2881 2941 3001 3061 3121 3181 3241	gcagcccgag ggatctatg acqcaccag acctttgag acqcaccag acctttgag gatgaagtgc aagaagatgc aagaagag ggtgaactg ggctgatgaa agtgtggttt aggcagcag gggagactg ttctagctg ttctagctg aatttgtoag gctactgtat cttctatatt tttaaaatta catggggtc acccgttt taaagtgtga ttaaggagtt	gatgatottt gagggdtga daaaagaac gocaatogag gagtatag gaggtagag gagttaag gagttaag gagttaag gagttaag gacatagat gygaagatg tottaacaa gocagtgt atgattgtt gytotacaa gocagtgt atgattgtt gytotacaa gaagtcog ttaatagaa attttoatt gagatagaaaa tttattct gtagatggg	ttgatagact cagcaaggt tgaacagcccc ctgttgcaat tgaaagtgc agtcaagaa ccacagaccg accctaggat accacagaccg gataaactga ccaacatctt cctatggaca attatttgg gaaattca attatttg gaaattca ggcaagtca aaactgaag gcatgatggg tttaattat gtgggggggggg	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggctgtt aagggaaaat cacagtggct gccggagag gcctcacttg tgcgaaaag actattta tttttatttt tgcgtttta aactocctc gcacctgtggc aaatattcc tttaaatt gatgaaggg	caactattta attotgaata tacaatgacta atoccagoga gatgccaaga gcoaggtca cagagactgg aacagattg ttggcgcaaga ttggcgcaaga tttgcctggc aagagttctg caccogtgoc taacctgga aaatatttca cagagggoa gaatgtttg cagttagggt ctgcttctccc ttggcagtag ttggcgcagtac tttggcagtag ttaaccttgg	agatettea cateaget aggatette caecacaaa aggaacaget tgaggatge aggaacagtt coetg ggac agtggggtgt coettgacat gagagagaac tgggggagag gatattaaa gatateaaa agtggatgg gatetetta tttgcaataa atteagaa tetteatga ttteggtet tttggtet tuttggtet aggatagg
1921 1981 2041 2101 2221 2281 2341 2401 2401 2521 2581 2641 2701 2761 2881 2881 2941 3001 3061 3121 3181 3241 3301	gcagcccgag ggatctatg acagcagcag ttataacqt aacttttgag acagagtgc aagaagtgc aagaagtgc gatgaagtg ggctgatgaa agtgtggttt aggcagcaga ggagagctg ttctagctgt ttctagctgt tttaactgta cttctatatt tttaaaatta cagggatga gaacctgtat catagtgga taaagtgtga	gatgatottt gagggdtga daaaagaac gcaatogag gagtatag ggaggacc aggtagtag gagttacag gaagttcaag ggatataca gctatagat gggaagatg tottaacaaa agcctagatg tottaacaaa agcagttgt atgatttgt gttotaccag taatagaaa agtagaaca agtagaaca attttcatt cacagaaaa ttttattct gtactagaaaa	ttgatagact cagccaaggt tgaacgcccc ctgttgcaat tgaaagtgc agtcaagaa ccacagacog accctaggat gacaaaccca tttagccagt ggtaaactga ccacactctt cctatggaca tttagccagt ggcaaattca aatatattgg taaattcg ggcaattca aatatcca ggcatgatggg gttatattsg tttaatttg taaattca aaacccagc aacttgaagg gcatgatggg gttttaatta	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactagatt taaggctgat gaaggctgtt agaggaaaat cacagtggct ctcagaggc gcctcacttg tgcgaaaga acttattta ttttatttt tgcgtttta aactocttc gcactgtga caatatttc gaatatttc tttaaatat gatgaaggc tcacttg tgcgaaaga cttattta	caactattta attotgaata tacaatgact ataccagoga gatgccaaga gcaaggactag aacagactgg aacagattg ttggtgcaaga tttgcctggg agagttctgt ccctogtgoc taacctgga gaatgtttg cagtagggca gaatgtttg cagtagggca cagtagggca tttggcagtg ctgcttcac tttggcagtg ctgacttgg gtggaatt ttaaccttt gagtggaatt ttcggagtg	agatettea cateaget agateette caecacaaa aggaacaget tgaaggatge aggaacagtt coetg ggac agtgggtgt coettgacat gaagaggac tggggggaga gatattaaa gatateaaa agtggatgg gatettet tttgeataa ttteagtaa tgeteette tttggteette tgtteettt ttettgateet aggataatg togetgacat tgetgacat
1921 1981 2041 2101 2221 2281 2341 2401 2401 2521 2581 2641 2701 2761 2881 2881 2881 2941 3001 3061 3121 3181 3241 3301 3361	gcagcccgag ggatctatg aacacagcag ttataacogt aacttttgag agcagatgc aagaagatgc aagaacgaag gatgaagstg chccaactc Region 1 cccaattgag ggctgatgaa agtgtggtt aggcagcaga gggagagctg ttctagctgt ttaaagtgg ctactgtat cttctatatt tttaaaatta catggggatc gaaccagtt taaatgtgaa taaagatogt aaattatggaa taaagatogt caactagta	gatgatottt gagggdtga daaaagaac gcaatogag ggaggagac aagttatga ggaggtagag attat ctag ggaggtagag attat ctg ggaagttaa ggatataga ggaagttaa ggaagttga tottaacaaa agccagttgt atgattgat gttatacaa gcaagtcg ttataacaa agcagttgt atgattgaa ttatattatt cagagaaaa attttatt gtagatggg ttattattet gtagatggg ttattattet	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaaagtgc agtcaaagaa ccacagacog accctaggat gataaactga ccaaaactga ggataaactga ccaacatctt cctatggaca tttagccagt gggcaaattca aatatattgg taaattca aaaaccgc aacctgaagg gctagatggg ttttaattat gttgggagg cattttccca tcttttccca	taagaaccta caatactggt attcgtaa ggatgagaac totttgtaac aactagatt taaggctgat gaaggctgtt agaggaaat cacaytggc gcogggagag gctcaactg tgcgaaaga acttattta tttttatttt cgcgtttta aactoccttc gcacctgta cagtgggc aaatatttc tttaaatat gatgaaggc tcaatgatta cagtogtgc aaatatttc	caactattta attotgaata tacaatgact atoccagoga gatgccaaga gcaaggactgg aacagactgg aacagattg ttggtgcaaga tttggcctggg agagttctgt cctcgtgoc taacctgga gaatgtttg cagtagggca gaatgtttg cagtaggg ttggcatcac tttggcagtg ttaaccttta gagtagtact ttdggcagtac tttdgcagtg ctactcgga ttaaccttg agatgtatta cagtaggtac ttdggcagtac tttdggcagtac ttdggcagtac ttdggagatct	agatettea cateaget agateette caecacaaa aggaacaget tgaagage aggaacaget coefgggatge aggagggggg gatataaa gataggagg gatattaaa gatacaaaa agtggaggg gatettea tttegaaaa atteaggaa teteatag ttteggtea tggteettet ttteggtea tgeteettet ttettgatee aggataatg togetgtaec aggataatg togetgtaec atteatgt
1921 1981 2041 2101 2221 2281 2341 2401 2401 2521 2581 2641 2701 2761 2881 2881 2941 3001 3061 3121 3181 3241 3301	gcagcccgag ggatctcatg acagcagcag tataacogt aacttttyag agcagatgc aagacgaag gatgaagstg chocaattyag ggctgatgaa agtgtggtt agtcggtt aggcagcaga gggaggctg ttctagdgt tttaacdgt tataagtgat cttctatatt tttaaaatta catggggat aatagggat gcacccgtt taaagatogt aataagatogt aataagatogt aataagatogt aataagatga ataaagatgt	gatgatottt gagggttga daaaagaac gcaatogag agtotatga ggaggagac aaggtagtag gaagttcaag attat ctgg attat ctgg ggaagattaaa gccagttgt agatttaca gcaagttgt agaagtcog ttaatagaaa gtagacaa agtagacaa attttcatt ggaagacaa attttattc gtagatagaaca tocagaaaa ttttattct gtagatgaaca	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca tgaaagtgc agtcaagaa ccacagacog accctaggat Reg acaaaaccca tttagccagt gataaactga ccaacatctt cctatggaca tttagccagt ggacaattca aaaaccagc aaacttgaag gcatatcgg gcatatcgg gcatatcgg gcatatcga gcatatcga gcatatcga gcatatcga gcatatcga tttattttccaa tttatttccaa tttatttccaa tctttttccaa	taagaaccta caatactggt attcrgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggaaaat caaggctgtt agaggaaag ctcagggc gctcactgg tgcgaaaga actattta ttttatttt ctgtttcac tggatttta aactoccttc gcactgtgc caagaggc gcactgtgc cagacggtggc aaaatattc tttaaaatat gatgagagc cccattgg cccattgg cccattgta cagacgtggc aaaatattc	caactattta attotgaata tacaatgact ataccagoga gatgccaaga gccaaggtca cagagactgg aaacagattg tggtgcaaga tttgcctggg agagttctgt cctcgtgoc tacactgga gaatgtttg cagtaggta gagtstaggt tttggcagtg ctgacttgag ttagacagt tttggaattt ttctgagat	agcatettea coateaoget agateettee coaceoaaa aggaacaget tgaaggatge aggaacaget coetg ggaac agtggggtgt coetggggagag gatattataa gatateaaaa agtggatgg gatateaaaa agtggatgg gatateaaaa agtggatgg gateettet tttgggtea teteettet ttttggttea tgtteetttt ttettgatet aaggataatg togetgtace atttattgt coatagaaaagg
1921 1981 2041 2101 2161 2221 2281 2341 2401 2401 2521 2581 2581 2581 2581 2581 2581 258	gcagcccgag ggatctatg acqcacag acttttgag agcagatgc aagacgag gatgaacgtg cocaactc Region 1 cocaattgag ggctgatgaa agtgtggttt aggcagaag gggagactg ttctagdst aatttgtcag gctactstat cttctattt tttaaaatta catggggatc gacacctgtt taaagatog ataaatatcag ctttaagata	gatgatottt gagggdtga dtaaaagaac gcaatogag agtotatga ggaggagac aggtagtag gagttoaag attataca gcaatogag ggaagatc x agattacag ggaagatg tottaacaaa agccagtgt dtatagat gtagaaga agtagacaa attttottt gtagatgaga tuttattot gtagatgaga tuttattot gtagatgaga attttoatt gagatgaga attttatttot gtagatgaga attttatttot gtagatgaga attttatttot gtagatgag agattgagaga attttatttot gtagatgaga attttatttot gtagatgaga attttatttot gtagatgaga attttatttot gtagatgaga attttatttot gtagatgaga agatttaga gtagatgaga attttatttot gtagatgaga attttatttot gtagatgaga attttatttot gtagatgaga gaagttgagac sad attttatttot gtagatgaga sad sattttatttot gtagatgaga sad sattttatttot gtagatgaga sad sad sad sattttatttot gtagatgaga sad sad sad sad sad sad sad sad sad sa	ttgatagact cagccaaggt tgaactgca ctgttgcaat tgaaagtgc agtcaaagaa ccacagaccg accctaggat tttagccagt gataaactga ccaaaacttt cctatggaca attatattgg tgaaattca aaactgaag gcatgatggg ttttaattat gttgggagg cattacat gttgggggg ttttaattat gtgggggg ttttaattat gtgggggg gcatgatggg gtttttaactaa ggcattttcaa atatacttag gcatgatggg ttttaattat	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggaaaat cacagtggct ion 3 gcgggagaag ctcaagaggc gcctcacttg tgcgaaaga actatttaa tttttattt ctgtttcact tgcgtttta gcacctgta gaactattta ttttatttt ctgtttcact tgcgtttta aactaccttu gcacctgtag caatatttc tttaaatat gatgaaggc tctaagggc tctaagggc aaatatttc	caactattta attotgaata tacaatgact ataccagoga gatgccaaga gcaaggactag aaacagattg tggtgcaaga ttggtgcaaga ttggtgcaaga tttgcctggg agagttctgt cctcgtgoc taaacctgga gaatgtttg cagtaggga gaatgtttg cagtaggg ttggcagtag ttggcagtag ttggcagtg ttggcagtg ttggcagtg ttggcagtg ttggcagtg tttggcagtg tttggcagtg tttggcagtg tttggcagtg ttctggagatct ttctggagat ttctggagat ttctggagat aactgtggatat	agatettea cateaget aggacaget tgaggatge aggacaget catgaggatge aggagaggtg agtggggtgt cattgacat gaggagggag gatattataa gatateaaa agtggatggg gatetetta tttgeaataa attegatag tetteatgt ttttggttea tgtgetgtaec aggatagg aaaaagg aaaatggaa
1921 1981 2041 2101 2221 2281 2341 2461 2521 2581 2581 2641 2701 2761 2881 2941 3001 3121 3181 3121 3181 3241 3361 33421 3481	gcagcccgag ggatctatg acqcaccag acqcaccag aacttttgag aacttttgag gatgaagcg recaactc Region 1 cccaattgag ggctgatgaa agtgtggttt aggcagcag gggagactg ttctagctg ttctagctg ttctagctg taattgtcag gctactgtat cttctatatt tttaaaatta catggggatc gacacctgtt taaatgtga taaagatogt aataatacag cttaaagata ataaaagtt aaaaaatta aaaaacataa	gatgatottt gagggdtga dtaaaagaac gcaatogag gagtatag ggaggagac aggtagtag gagttaag gagttaag gagttaag ggaagttaag ggaagatg ttaacagatg tagattgtt gttotacaa gcaagtcg taatagaaa attttattc gtagatggg ggaagaaa tttatttc gtagatggg gaatgaacaa attttatttc gagatggg gaatgaacaa	ttgatagact cagccaaggt tgacagcccc ctgttgcaat tgaacttgca tgaaagtgc agtcaagaa ccacagacog accctaggat Reg acaaaaccca tttagccagt gataaactga ccaacatctt cctatggaca tttagccagt ggacaattca aaaaccagc aaacttgaag gcatatcgg gcatatcgg gcatatcgg gcatatcga gcatatcga gcatatcga gcatatcga gcatatcga tttattttccaa tttatttccaa tttatttccaa tctttttccaa	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactaagatt taaggctgat gaaggctgtt aagggaaag ctaaggtggc gcctaacttg tgcgaaaga acttattta agcccgttgt cagtogtggc aaatatttc ttttaattt gatgaaggg tctaacttg gaacggtggc aaatatttc tttaaatat gatgaaggg tcatctttg agagaaata tttttttttt	caactattta attotgaata tacaatgocta cacaagagg gatgccaaga gcoaaggtca cagagactog aacagattog agagttotgt coctogtgoc taaactgga gaatgtttg cagtagggca gaatgtttg cagtagggc ttggcagg ttggcagg ttggcagg ttggcagg ttggcagg ttggcagtoc ttggcagtac ttggcagtg ttggcagtac ttggcagtg ttggcagtac ttggcagtg ttggcagtac ttggcagtg ttggcagtac tggtgaatot ttggcagtac	agatettea cateaget aggatette caecaocaaa aggaacaget tgaggatge aggaacaget coetg ggac agtggggtgt coetg ggac gatgagggag gatattaaa gatgagggg gatateaaa agtggatgg gatetetta tttgcaataa atteagaa tetteatge ttttggttea taggatagg gatettett ttttggttea aggatagg gatattaaa agtggatgg gatetetta
1921 1981 2041 2161 2221 2281 2341 2461 2521 2581 2641 2761 2761 2821 2881 2941 3001 3061 3121 3181 3241 3301 3361 3421 3481 3541	gcagcccgag ggatctatg acagcagcag ttataacqt aacttttgag acagagtgc aagaagtgc aagaagtgc gatgaagtg ccaattgag ggctgatgaa agtgtggttt aggcagcag ggtagagagtg ttctagctgt ttctagctgt tttaaagtgt gatactgtat cttctatatt tttaaaatta catgggatc gaacctgtt taaatgtga taaagtgtga attagtga taaagtgtga aatatgtga taaagatgt aaatatcag ctttaaagtt aaaaaagtt aaaaaagtt aaaaaagtt aaaaaagtt	gatgatottt gagggdtga daaaagaac gcaatogag gagtatag ggaggacc aggtagtag gagttacag gaagttacag gacatgag ggaagatg tottaacaa gccagtgt atgattgat gytaagaag atgattgat gtotacaa agcagaag ttaatagaa agtagaaca attttoatt cacagaaaa ttttattct gtaattggg tgactgaac taattgag gaagtcag attttatto gaagatgaa attttattto gaagtgaaca attttatto gaagtgaaca tttattto gaagtgaaca attttatto gaagtgaaca tttattto gaagtgaaca tttattto gaagtgaaca tttatto gaagtgaaca tttattott	ttgatagact cagcaaggt tgaactgca ctgttgcaat tgaaatgca agcaagaccg agcaagaa ccacagaccg aacctaggat tttagccagt gataactga ccaacatctt cctatggaca attatattgg taaattcca attatattgg caaactca ggcatgatggg ttttaattat gttgggagg catgtggg catttccca tttagccagt ggcatatcat gttgggagg catttccca ttttattat tttaattattg tttaattatt gttggggagg cattttccca ttttattatt gttgggagg	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactagatt taaggctgat gaaggctgtt agaggaaaat cacagtggct tion 3 gcgggagag gcctcacttg tgcgaaaga acttattta tttttatttt tgcgtttta aactocttc gcactgtgc aaatattcc tttaaatat gatgaaggc tcatcttg catacttg catacttg catacttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttt gggaaatg	caactattta attotgaata tacaatgact ataccagoga gatgccaaga gcaaggactag aacagactgg aacagactgg aacagattg ttggccaaga ttggtgcaaga ttggtgcaaga cagaaggtca caagaggga gaatgtttg ccctctccc gagcagtttg ccgctaggg ttaacctgg ttaaccttg ggtggaatc tttggcagtg gtattcgag ttaaccttgag ttaaccttt aactgaag gtatttggta cagaaggta ctgcttaccc ggtggaatc ttcggagt taacctttgagat tacttgaga tggtattggta	agatettea cateaget agateette caecacaaa aggaacaget tgaaggatge aggaacaget coetg ggac agtggggtgt coetg ggac agtgggggag gatattaaa gatagaggag gatattaaa gatacaaa agtggatgg gatettea tttggataa tetetattgt ttttggttea tgetcettt ttettgatet aagataatg togetgtace attettattgt caasaaaagg aataatga aggataatg togetgtace attettattgt caasaaaagg aataatga agaagaagaa tetetttattgt ttettgatet aagaataatg togetgtace attettattgt
1921 1981 2041 2101 2221 2281 2341 2401 2461 2521 2581 2641 2701 2761 2881 2941 3001 3061 3121 3181 3241 3301 3361 3481 3481 3541 3601	gcagcccgag ggatctatg acagcagcag ttataacqt aacttttgag agagatgc aagacgag gatgaagtgc aagacgag ggtgaagtg cccaattgag ggctgatga agtgtggtt aggcagcag ggagagctg ttctaactgt cttctaactgt cttctaatt tttaaagtgt aattgtgag gaaccggtc gacacctgtt taaatgtgaa taaagtgtg aattagtgaa taaagtgtg aattagtgaa taaagtgta aataatacg gcaacctgtt taaatgtgaa taaagatgt aaataatacg ctttaagatt taaaagtta aaaaatatc aaaaacataa gaggcattgt	gatgatottt gagggdtga daaaagaac gcaatogag gagtatag ggaggacc aggtagtag gagttacag gaagttacag gacatgag ggaagatg tottaacaa gccagtgt atgattgat gytaagaag atgattgat gtotacaa agcagaag ttaatagaa agtagaaca attttoatt cacagaaaa ttttattct gtaattggg tgactgaac taattgag gaagtcag attttatto gaagatgaa attttattto gaagtgaaca attttatto gaagtgaaca tttattto gaagtgaaca attttatto gaagtgaaca tttattto gaagtgaaca tttattto gaagtgaaca tttatto gaagtgaaca tttattott	ttgatagact cagccaaggt tgaacgcccc ctgttgcaat tgaactgca tgaacgccc agtcaagaa cacacagacg accctaggat gacaagaccg acacaactct cctatggaca tttagccagt ggcaactca attatttg ggcaattca aaaccca aacttgagg ggcagatggg ggcagtggg gcatgtggg gttttaattat gttggggag cattttccca tctttttaac ataacttag ggcttttatt tttagcaga ggcttttatt	taagaaccta caatactggt attccgtaa ggatgagaac tctttgtaac aactagatt taaggctgat gaaggctgtt agaggaaaat cacagtggct tion 3 gcgggagag gcctcacttg tgcgaaaga acttattta tttttatttt tgcgtttta aactocttc gcactgtgc aaatattcc tttaaatat gatgaaggc tcatcttg catacttg catacttg catacttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttg catactttt gggaaatg	caactattta attotgaata tacaatgact ataccagoga gatgccaaga gcaaggactag aacagactgg aacagactgg aacagattg ttggccaaga ttggtgcaaga tttgcctggg aagattotgt coctogtgoc taaccctgga gaatgtttg cogtagggta ttggcgtag ttggcgtag gaatgtttg cogtagggt ttaaccttt gagtgaatct ttdggagtg ttaaccttt aattggat gtattggta taattggta	agatettea cateaget agateette caecacaaa aggaacaget tgaaggatge aggaacaget coetg ggac agtggggtgt coetg ggac agtgggggag gatattaaa gatagaggag gatattaaa gatacaaa agtggatgg gatettea tttggataa tetetattgt ttttggttea tgetcettt ttettgatet aagataatg togetgtace attettattgt caasaaaagg aataatga aggataatg togetgtace attettattgt caasaaaagg aataatga agaagaagaa tetetttattgt ttettgatet aagaataatg togetgtace attettattgt

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 TowerTM, 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 $UGTIAI^*28$ 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	GACGCCCTCCACCCCTTTTT		
exon15	TTCCATTCATGCTCATCTTTTCTT	TGTGCCTGTTGCCTGTCTCA		
UGTIAI				
UGT1A1*6	AAGTAGGAGAGGGGGGAACC	GTGGGCAGAGACAGGTACT		
UGT1A1*27	AGTACCTGTCTCTGCCCAC	GTCCCACTCCAATACACAC		
<i>UGT1A1</i> *28	TATAGTCACGTGACACAGTC	CCACTGGGATCAACAGTATCT		

TOP-I, topoisomerase I; *UGT1A1*, UDP-glucuronyltransferase 1A1; *UGT1A1**6: G \rightarrow A on exon 1 (protein, G71R); *UGT1A1**27, 770C \rightarrow 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		<i>UGT1A1</i> *28 Wild Hetero		Homo	Total in <i>UGT1A1</i> *6 or *27
UGT1A1*6	Wild	71	18	3	92
	Hetero	26	4	0	30
	Homo	4	0	0	4
<i>UGT1A1</i> *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 chemotherapy 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.

Acknowledgements

We thank Yakult Honsha Co., Ltd., and Masumi Tanaka (Department of Respiratory Medicine) for their partial technical support. This study was supported by Grant-in-Aid No. 15590826 and Grant-in-Aid for Development of leading-edge cancer professionals in the South Kanto area, from the Ministry of Education, Culture, Sports, Science and Technology.

References

- 1. Pizzolato JF and Saltz LB: The camptothecins. Lancet 361: 2235-2242, 2003.
- 2. Arakawa Y, Suzuki H, Saito S and Yamada H: Novel missense mutation of the DNA topoisomerase I gene in SN-38-resistant DLD-1 cells. Mol Cancer Ther 5: 502-508, 2006.
- Kubota N, Kanzawa F, Nishio K, Takeda Y, Ohmori T, Fujiwara Y, Terashima Y and Saijo N: Detection of topoisomerase I gene point mutation in CPT-11 resistant lung cancer cell line. Biochem Biophys Res Commun 188: 571-577, 1992.
- 4. Chang JY, Liu JF, Juang SH, Liu TW and Chen LT: Novel mutation of topoisomerase I in rendering cells resistant to camptothecin. Cancer Res 62: 3716-3721, 2002.
- Chrencik JE, Staker BL, Burgin AB, Pourquier P, Pommier Y, Stewart L and Redinbo MR: Mechanisms of camptothecin resistance by human topoisomerase I mutations. J Mol Biol 339: 773-784, 2004.
- Redinbo MR, Stewart L, Kuhn P, Champoux JJ and Hol WGJ: Crystal structures of human topoisomerase I in covalent and noncovalent complexes with DNA. Science 279: 1504-1513, 1998.
- Laco GS, Collins JR, Luke BT, Kroth H, Sayer JM, Jerina DM and Pommier Y: Human topoisomerase I inhibition: docking camptothecin and derivatives into a structure-based active site model. Biochemistry 41: 1428-1435, 2002.
- Mathijssen RH, van Alphen RJ, Verweij J, Loos WJ, Nooter K, Stoter G and Sparreboom A: Clinical pharmacokinetics and metabolism of irinotecan (CPT-11). Clin Cancer Res 7: 2182-2194, 2001.
- Humerickhouse R, Lohrbach K, Li L, Bosron WF and Dolan ME: Characterization of CPT-11 hydrolysis by human liver carboxylesterase isoforms hCE-1 and hCE-2. Cancer Res 60: 1189-1192, 2000.
- Kawato Y, Aonuma M, Hirota Y, Kuga H and Sato K: Intracellular roles of SN-38, a metabolite of the camptothecin derivative CPT-11, in the antitumor effect of CPT-11. Cancer Res 51: 4187-4191, 1991.
- 11. Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K and Hasegawa Y: Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. Cancer Res 60: 6921-6926, 2000.
- Ando Y, Fujita K, Sasaki Y and Hasegawa Y: UGT1A1*6 and UGT1A1*27 for individualized irinotecan chemotherapy. Curr Opin Mol Ther 9: 258-262, 2007.
- 13. Araki K, Fujita K, Ando Y, Nagashima F, Yamamoto W, Endo H, Miya T, Kodama K, Narabayashi M and Sasaki Y: Pharmacogenetic impact of polymorphisms in the coding region of the UGT1A1 gene on SN-38 glucuronidation in Japanese patients with cancer. Cancer Sci 97: 1255-1259, 2006.
- 14. Strassburg CP: Pharmacogenetics of Gilbert's syndrome. Pharmacogenomics. 9: 703-715, 2008.
- Saito A, Kawamoto M and Kamatani N: Association study between single-nucleotide polymorphisms in 199 drug-related genes and commonly measured quantitative traits of 752 healthy Japanese subjects. J Hum Gen 54: 317-323, 2009.

- 16. Kaniwa N, Kurose K, Jinno H, Tanaka-Kagawa T, Saito Y, Saeki M, Sawada K, Tohkin M and Hasegawa R: Racial variability in haplotype frequencies of UGT1A1 and glucuronidation activity of a novel single nucleotide polymorphism 686C> T (P229L) found in an African-American. Drug Metab Dispos 33: 458-465, 2005.
- Hoskins JM, Marcuello E, Altes A, Marsh S, Maxwell T, Van Booven DJ, Paré L, Culverhouse R, McLeod HL and Baiqet M: Irinotecan pharmacogenetics: influence of pharmacodynamic genes. Clin Cancer Res 14: 1788-1796, 2008.
- Chowbay B, Zhou S and Lee EJ: An interethnic comparison of polymorphisms of the genes encoding drug-metabolizing enzymes and drug transporters: experience in Singapore. Drug Metab Rev 37: 327-378, 2005.
- 19. Mercke Odeberg J, Andrade J, Holmberg K, Hoglund P, Malmqvist U and Odeberg J: UGT1A polymorphisms in a Swedish cohort and a human diversity panel, and the relation to bilirubin plasma levels in males and females. Eur J Clin Pharmacol 62: 829-837, 2006.
- 20. Liu JY, Qu K, Sferruzza AD and Bender RA: Distribution of the UGT1A1*28 polymorphism in Caucasian and Asian populations in the US: a genomic analysis of 138 healthy individuals. Anticancer Drugs 18: 693-696, 2007.

- Innocenti F, Grimsley C, Das S, Ramirez J, Cheng C, Kuttab-Boulos H, Ratain MJ and Di Rienzo A: Haplotype structure of the UDP-glucuronosyltransferase 1A1 promoter in different ethnic groups. Pharmacogenetics 12: 725-733, 2002.
- 22. Tsurutani J, Nitta T, Hirashima T, Komiya T, Uejima H, Tada H, Syunichi N, Tohda A, Fukuoka M and Nakagawa K: Point mutations in the topoisomerase I gene in patients with non-small cell lung cancer treated with irinotecan. Lung Cancer 35: 299-304, 2002.
- 23. Moisan F, Longy M, Robert J and Le Morva V: Identification of gene polymorphisms of human DNA topoisomerase I in the National Cancer Institute panel of human tumour cell lines. Br J Cancer 95: 906-913, 2006.
- 24 Saito Y, Maekawa K, Ozawa S and Sawada J. Genetic polymorphisms and haplotypes of major drug metabolizing enzymes in east Asians and their comparison with other ethnic populations. Curr Pharmacogenomics 5: 49-78, 2007.