Journal of Biological Research-Thessaloniki **18**: 280 – 290, 2012
J. Biol. Res.-Thessalon. is available online at http://www.jbr.gr
Indexed in: WoS (Web of Science, ISI Thomson), SCOPUS, CAS (Chemical Abstracts Service) and DOAJ (Directory of Open Access Journals)

Variants, haplotypes and htSNPs of UDP-glucuronosyltransferase 1A9, 1A7 and 1A1 genes in Chinese Tibetan Population

Yuewen WANG[1#], YUAN YANG[2#], Wei YAN[1], Xiaoqing ZHANG[1], Fangfang YANG[1], Erfei CHEN[1], Min WANG[1], Jin YANG[1*]

[1] *National Engineering Research Center for Miniaturized Detection System, College of Life Sciences, Northwest University, Xi'an, Shaanxi 710069, China*

[2] *Department of Pathology, Shenyang Northern Hospital, Shenyang, Liaoning 110840, China*

Received: 29 November 2011 Accepted after revision: 30 March 2012

No data about the genetic information of *UGT1A9*, *1A7*, *1A1* loci in Tibetan population were available up to now. In the present study, we systematically studied the polymorphisms of promoters, exon 1 of the *UGT1A9*, *1A7*, *1A1* gene and common exons 2-5 in 100 unrelated healthy Chinese Tibetan subjects, using direct sequencing. A total of 36 variants, including eight novel ones were detected in our study. Four, five and six alleles and seven, eight and nine genotypes were detected in *UGT1A9*, *1A7* and *1A1*, respectively. Two LD blocks were calculated; eight common haplotypes were identified for block 1 (account for 98.1% of *UGT1A7* haplotype diversity) and three common haplotypes (account for 99.5% of exons 2-5 haplotype diversity) for block 2. The htSNPs were selected based on haplotype measure. The present knowledge of UGT haplotypes and htSNPs can improve understanding of genetic risk factors and increase the safety and efficiency of individualization of drug dosage while avoiding intoxication by studying drug response and developing the targeted drug therapy for individual patients in Chinese Tibetan.

Key words: UGT, genetic, polymorphism, Tibetan.

INTRODUCTION

Glucuronidation is a critical and elimination process in the detoxification of many different exogenous and endogenous compounds (Radominska-Pandya *et al*., 1999; Tukey & Strassburg, 2001). Glucuronides account for ~35% of all phase II drug metabolites (Evans & Relling, 1999), including therapeutic drugs such as SN-38, which is the active antitumor metabolite of the prodrug irinotecan, as well as endobiotics such as bilirubin and steroid hormones. Glucuronidation is catalyzed by the UDP-glucuronosyltransferases (UGTs) which exist as a superfamily of independently regulated enzymes (Mackenzie *et al*., 1997). Four UGT families have been identified: UGT1, UGT2, UGT3 and UGT8 (Mackenzie *et al*., 2005). The human *UGT1A* gene consists of 13 different isoforms (*UGT1A1-UGT1A13*), all of which are derived from a single gene locus; it spans approximately 200 kb on chromosome 2q37 and consists nine active and four inactive exon 1 segments (in the following segment order: *UGT1A12P*, *1A11P*, *1A8*, *1A10*, *1A13P*, *1A9*, *1A7*, *1A6*, *1A5*, *1A4*, *1A3*, *1A2P* and *1A1*) and common exons 2-5. One of the nine active exon 1 (i.e. 1A1) can be used in conjunction with the same four downstream exons (Gong *et al*., 2001; Tukey & Strassburg, 2001). A number of genetic polymorphisms including single nucleotide polymorphisms (SNPs) in *UGT1As* have been identified and published on the UDP-glucuronosyltransferase homepage (http://www.flinders.edu.au/medicine/sites/clinical-pharmacology/ugt-homepage.cfm). Some of these polymorphisms are known to affect glucuronidation rates (Guillemette *et al*., 2000a; Gagné *et al*., 2002; Huang

* Corresponding author: tel.: 0086 29 88303446851, fax: 0086 29 88303551, e-mail: yangjin@nwu.edu.cn
# Equal scientific contribution

et al., 2002; Elahi *et al*., 2003; Guillemette, 2003; Jinno *et al*., 2003a, b; Villeneuve *et al*., 2003; Ehmer *et al*., 2004; Nagar *et al*., 2004). *UGT1A1* is the principal isoform involved in the glucuronidation of bilirubin (Tukey & Strassburg, 2000; Verlaan *et al*., 2004). The variant of the TATA box [-52(TA) 7, *28 allele] in promoter of *UGT1A1* is implicated in Gilbert syndrome in Caucasians (Bosma *et al*., 1995; Beutler *et al*., 1998) and also is associated with an increased risk of breast cancer (Guillemette *et al*., 2000a) and irinotecan toxicity, a commonly used anticancer agent (Ando *et al*., 2000; Iyer *et al*., 2002; Innocenti *et al*., 2004). In addition, it has been reported that the *UGT1A7* alleles (Guillemette *et al*., 2000b; Gagné *et al*., 2002), *2 [387T>G (Asn129Lys), 391C>A (Arg131Arg) and 392G>A (Arg131Gln)], *3 [(Asn129Lys), (Arg131Arg), (Arg131Gln) and 622T>C (Trp208Arg)] and *4 (Trp208Arg), and UGT1A9 alleles (Villeneuve *et al.*, 2003) show reduced activities towards benzo(a)pyrene metabolites (for SN-38 glucuronidation).

Recent pharmacogenomic studies have suggested that combinations of SNPs (haplotypes) on a chromosome, have the advantage of providing more useful information than individual SNPs to investigate the phenotype-genotype links (Judson *et al*., 2000). Haplotype-tagging SNPs (htSNPs) retain most of the information in high-density marker maps, while reducing genotyping requirements. The htSNPs are being evaluated for their usefulness in pharmacogenetic studies (Ahmadi *et al*., 2005). Thus, it could lead to a cooperative alteration in glucuronidation activity if co-occurrence of the SNPs or segmental haplotypes with functional changes in the UGT1A complex. Kohle *et al.* (2003) reported that *UGT1A1**28 occurs frequently with *UGT1A6**2 (T181A/R184S) as well as *UGT1A7**3 in whites and Egyptians. In Americans, *UGT1A7**3, was completely associated with the *UGT1A9*1*b (delT-118) allele. It is likely that the haplotype structure of the *UGT1A* gene varies with ethnicity and any conclusions of previous studies may be limited by ethnicity. China has 56 ethnic groups and Tibet is one of the ethnic groups in China. However, there is no systematic polymorphism screening and study of patterns of haplotypes and htSNPs of the *UGT1A9*, *1A7* and *1A1* gene in Chinese Tibetan population. Therefore, in the present study, we systematically screened the upstream putative promoter regions exon 1 of *UGT1A9*, *1A7* and *1A1* gene and common exons 2-5 in 100 Chinese Tibetans from Qinghai province. These polymorphism data can be used to explore haplotype structures in *UGT1A9*, *1A7* and *1A1* locus and to identify htSNPs in Tibetan populations. The present knowledge of UGT haplotypes and htSNPs can improve understanding of genetic risk factors and increase the safety and efficiency of individualization of drug dosage while avoiding intoxication by studying drug response and developing the targeted drug therapy for individual patients in Chinese Tibetan population.

MATERIALS AND METHODS

Subjects and human genomic DNA

A total of 100 unrelated Tibetan subjects residing in Qinghai province for *UGT1A9, 1A7* and *1A1* were included in the genotyping study. The genomic DNA was extracted directly from peripheral blood leukocyte (Gabriel *et al*., 2002) using DNA blood mini kit (Qiagen, Hilden, Germany). There were 50 males and 50 females (their age ranged from 18 to 40 years old). All participants were in good health according to their medical history and after a physical examination. All study subjects provided their written consent as regards their participation approved by the ethics committee of Northwest University, Shaanxi.

Polymerase chain reaction (PCR) and DNA sequencing

The promoters and exon 1 of *UGT1A9*, *1A7* and *1A1*, as well as common exons 2-5 were amplified from genomic DNA using the primers designed with the aid of the Web-based primer3 software (Table 1). The amplified DNA was purified using a Product Pre-Sequencing Kit (USB Co., Cleveland, OH, USA) and directly sequenced using an ABI BigDye Terminator Cycle Sequencing Kit (Applied Biosystems, Foster City, CA, USA). The results were analyzed on an ABI Prism3700 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). The sequence data were analyzed using the Sequencher4.9 software (Gene Codes Corp., Ann Arbor, MI, USA; www.genecodes.com) and followed by visual inspection. The A of the ATG of the initiator Met codon is denoted nucleotide +1 for *UGT1A9*, *1A7* and *1A1* and common exons 2-5 according to *UGT1A1*. Genbank accession numbers of reference sequences are NM021027.2, NM019077.2 and NM000463.2 for *UGT1A9*, *1A7* and *1A1* gene, respectively.

Linkage Disequilibrium (LD) Block Determination and Haplotype Analysis

Genotype and allelic frequencies were determined for all the variants of UGT1A9, 1A7, and 1A1 for

TABLE 1. Primers used for amplifying *UGT1A1, 1A7, 1A9* and exons 2-5 and the size of PCR products

Gene		Primer name	Primer	Product size
UGT1A9	1	P1F	AAAGCTCTTTCTATAATAACAGTGG	481
		P1R	TGTAAATGCAGTCAAGTTTACAGT	
	2	P2F	CTTACATTCTGGTGCTTGGT	501
		P2R	AGATTCATGAAGACATTGCTG	
	3	P3F	TTTTGCAATGGTCCTTATCT	502
		P3R	ATTTCGTGAGGGATTCAAC	
	4	P4F	AGCTCACCCATTAAAATCTG	516
		P4R	ATTGCAGAGACACAGGTGAG	
	5	P5F	ATTGCAGAGACACAGGTGAG	519
		P5R	CGACATTGAAAGATAAAAAGG	
	6	P6F	GCATTGCTTAATAATTTTGTTT	291
		P6R	AGAGAACTTCAGCCCAGAG	
	7	E1(1)F	CCAAGGCAAAGACCATAAGCTA	619
		E1(1)R	CAAACTCCTGCAATTTGAAAAA	
	8	E1(2)F	CATATACCCTGGAGGATCTGGA	623
		E1(2)R	ACTTACCATAGGCAACGGCTTT	
UGT1A7	9	P1F	ATTGCAGAGACACAGGTGAG	519
		P1R	CGACATTGAAAGATAAAAAGG	
	10	P2F	AACTCATATTGCAGCACAGG	266
		P2R	AGAGAACTTCAGCCCAGAG	
	11	E1(1)F	AAACTCATATTGCAGCACAGG	624
		E1(1)R	AAGTCAAAAATACCATTGGATGAA	
	12	E1(2)F	CATTGCGAAGTGCATTTTCT	610
		E1(2)R	TCTTTTAATTTCCAAAGCCAGA	
UGT1A1	13	P1F	TACAGGACTGGCTCTTTCAG	500
		P1R	AGAGGAAGAAGGACGACTATG	
	14	P2F	TCTTCCTCTCTGGTAACACTTG	302
		P2R	GTTCGCCCTCTCCTACTTAT	
	15	E1(1)F	ACTCCCTGCTACCTTTGTGG	667
		E1(1)R	GCAGTGCATGCAAGAAGAAT	
	16	E1(2)F	TGTCTGGCTGTTCCCACTTA	667
		E1(2)R	CCAGAAGATGATGCCAAAGA	
E2-E5	17	E2F	TGGATTTTGCATCTCAAGGA	481
		E2R	GCAGGGAAAAGCCAAATCT	
	18	E3-E4F	TTCAGAGGACCCCTGTTTTC	687
		E3-E4R	CCTTTTGTCATTGATGACTGC	
	19	E5(1)F	TTCTTAAGCAGCCATGAGCA	588
		E5(1)R	GGGGGCACGATACATATTCA	
	20	E5(2)F	ATTAATCAGCCCCAGAGTGC	655

Chinese Tibetan population by SNPAlyze8 software. Hardy-Weinberg equilibrium analysis by χ^2 test and LD analysis were performed using Haploview4.2 software for Tibetan population, and pairwise two-dimensional maps between SNPs were obtained for the |D'| and r^2 values. LD block was assigned according to Gabriel's definition (Gabriel *et al*., 2002). SNPs with a Hardy-Weinberg equilibrium *p* value smaller than the cutoff value of 0.05 were included in LD analysis. The haplotypes structures in each LD block were obtained by an expectation-maximization algorithm with the Haploview4.2 program for the whole region of UGT1A9, 1A7 and 1A1 locus. The htSNPs, a subset of SNPs sufficient to quantify genetic variability of haplotypes, were selected using Haploview4.2 software. A group of haplotypes without amino acid changes was defined as *1 allele, and the groups with amino acid changes were numbered according to the

UGT Alleles Nomenclature home page (http://galien.pha.ulaval.ca/labocg/alleles/alleles.html).

RESULTS

UGT1A9, 1A7, and 1A1 polymorphisms detected in Chinese Tibetan population

All promoter regions and exon 1 of *UGT1A9, 1A7* and *1A1*, as well as the common exons 2-5, were systematically and comprehensively screened and a total of 36 variants were detected in the DNA from 100 Chinese Tibetan subjects, of which 14 variants were sequenced in the promoter regions, 12 in exon 1, two in intron 1, one in intron 2, two in exon 4, one in the exon 5 and four in 3′-UTR (Table 2). Of the 36 polymorphisms, seven [UGT1A9: -2189T>C, 588G>T (Gly196Gly); UGT1A7: -561A>C, -103G>C, 128-129insC (Frameshift), UGT1A1: 181G>A (Ala61Thr), 596C>G (Ser199Cys), 6637A>C] were novel polymorphisms. The information of the polymorphisms was reported in Table 2. Using the χ^2 test for deviation from Hardy-Weinberg equilibrium for the group, only one SNP (211G>A in *UGT1A1*) did not fit with the expected allele frequencies.

Seven polymorphisms were found in the *UGT1A9* (Table 2). Of the seven polymorphisms, six polymorphisms, [i.e. -2189T>C, rs6731242 (-1888T>G), rs13418420 (-1819T>C), rs2741045 (-441C>T), rs2741046 (-332T>C), rs67695772 (delT-118, *1b allele)] were detected in promoter of *UGT1A9* gene (rs number corresponds to the SNP name). The polymorphism -2189T>C was a novel one and therefore no rs number was found in GenBank. The six polymorphisms occurred at frequencies of 0.005, 0.110, 0.360, 0.035, 0.045 and 0.449, respectively in Tibetan population. In exon 1 of *UGT1A9* gene, a novel synonymous polymorphism 588G>T (Gly196Gly) was found and the minor allele frequency was 0.020 in our studied subjects.

For *UGT1A7* gene, thirteen variants were screened. In promoter region of *UGT1A7*, five variants [i.e. -561A>C, rs4530361 (-543A>G), rs28946877 (-341C>T), -103G>C and rs7586110 (-57T>G)] were found at the frequencies of 0.055, 0.245, 0.126, 0.005 and 0.298, respectively (variants -561A>C and -103G>C were novel). In the exon 1 region, eight variants were found; four were synonymous [rs7577677 (33C>A, Pro11Pro), rs17863778 (391C>A, Arg131Arg), rs45462096 (660C>T, Cys220Cys) and rs17864686 (756G>A, Leu252Leu) and occurred at frequencies of 0.291, 0.449, 0.005 and 0.136, respectively], three were nonsynonymous variants [rs17868323 (387T>G, Asn129Lys), rs17868324 (392G>A, Arg131Gln) and rs11692021 (622T>C, Trp208Arg) and presented frequencies of 0.444, 0.444 and 0.298, respectively] and one (128-129insC) was a novel frameshift variant and occurred at 0.005 frequency in Tibetan population.

Six *UGT1A1* polymorphisms were detected; three were in promoter region and three were in exon 1 region. In the promoter region, the variants rs887829 (-364C>T *80 allele), rs873478 (-64G>C *81 allele) and rs3064744 (-52$(TA)_{6>7}$ *28 allele) were observed at frequencies of 0.135, 0.082 and 0.013, respectively. The variants 181G>A (Ala61Thr), rs4148323 (211G>A Gly71Arg *6 allele) and 596C>G (Ser199Cys) appeared at frequencies of 0.030, 0.390 and 0.010, respectively, in exon 1 region; 181G>A (Ala61Thr) and 596C>G (Ser199Cys) were novel non-synonymous.

We detected ten variants in common exons 2-5 from the Tibetan subjects. A known variant rs6708136 (6634C>T) and a novel variant 6637A>C were detected in intron 1 (minor allele frequencies were 0.005 and 0.005, respectively) and another known variant rs4148327 (6893T>C) was detected in intron 2 (minor allele frequency was 0.095). Three nonsynonymous variants [rs34946978 (7939C>T, Pro364Leu, *63allele, exon 4), rs111033540 (8046A>C, Asn400His, exon 4) and rs114982090 (12022C>T, Pro451Leu, exon 5)] were found at frequencies of 0.085, 0.010 and 0.020, respectively. In 3′-UTR, four variants were detected [i.e. rs10929303 (12483T>C), rs1042640 (12611G>C), rs34942353 (12691T>C) and rs8330 (12712G>C)] and the observed frequencies were 0.150, 0.121, 0.010 and 0.130, respectively.

The allele and genotype frequencies of UGT1A9,1A7 and 1A1 in Chinese Tibetan population

In present study, four alleles [*1a (wild-type), *1b (delT -118), *1d (-441C>T, -332T>C and delT-118), *1f (-1819T>C, -441C>T, -332T>C and delT -118)] and seven genotypes were detected in *UGT1A9* gene. As to *UGT1A7* gene, five alleles [*1 (wild-type), *2 (387T>G Asn129Lys, 391C>A Arg131Arg, and 392G>A Arg131Gln), *3 (387T>G Asn129Lys, 391C>A Arg131Arg, 392G>A Arg131Gln and 622T>C Trp208Arg), *4 (622T>C Trp208Arg) and *11 (392G>A Arg131Gln)] and eight genotypes were detected. For *UGT1A1* gene, six alleles [*1 (wild-type), *6 (211G>A Gly71Arg), *28 (-52$(TA)_{6>7}$), *63 (7939C>T Pro364Leu), *80 (-364C>T), *81 (-64G>C)] and nine genotypes were found (the sequence of

TABLE 2. Summary of *UGT1A1, 1A7, 1A9* polymorphisms detected in Chinese Tibetan population

Gene	Variant*	LD&	dbSNP SNP ID (NCBI)	Location	Position	Amino acid change	Minor allele frequencies	Flanking sequences (5′-3′)
UGT1A9	-2189T>C		novel	promoter	chr2:234578392		0.005	TATAATGGCGT/CGATCTCAGCT
	-1888T>G		rs6731242	promoter	chr2:234578693		0.110	ACTAGAAGCCT/GTACCAATAAC
	-1819T>C		rs13418420	promoter	chr2:234578762		0.360	TGTATTATCAT/CAATGAAGTCA
	-441C>T		rs2741045	promoter	chr2:234580140		0.035	TTGCTTAGAGC/TATGAGTTGCC
	-332T>C		rs2741046	promoter	chr2:234580249		0.045	CAAATTTACTT/CTTACTTTATC
	delT -118		rs67695772	promoter	chr2:234580463		0.449	CAGTGACTGATTTTTTTTTT/-ATGAAAGGAT
	588G>T		novel	exon 1	chr2:234581168	Gly196Gly	0.020	TTCTCTTAGGG/TTTCTCAGATG
UGT1A7	-561A>C		novel	promoter	chr2:234590023		0.055	CCACTAAAATA/CCAAAAAGTTA
	-543A>G		rs4530361	promoter	chr2:234590041		0.245	TTAGCTGGGCA/GTGGCAGCGTG
	-341C>T		rs28946877	promoter	chr2:234590243		0.126	TTATTAGCTTC/TGTTCAAATTT
	-103G>C		novel	promoter	chr2:234590475		0.005	GAATGAATAAG/CTACACGCCTT
	-57T>G	c	rs7586110	promoter	chr2:234590527		0.298	CTTCTTCCACT/GTACTATATTA
	33C>A	c	rs7577677	exon 1	chr2:234590616	Pro11Pro	0.291	GCCTCCTTCCC/ACTATATGTGT
	128-129insC		novel	exon 1	chr2:234590712	Frameshift	0.005	CCATGCAGTC-/CGGTGGTGGAG
	387T>G	a	rs17868323	exon 1	chr2:234590970	Asn129Lys	0.444	GTTTGTTTAAT/GGACCGAAAAT
	391C>A	a	rs17863778	exon 1	chr2:234590974	Arg131Arg	0.449	GTTTAATGACC/AGAAAATTAGT
	392G>A	a	rs17868324	exon 1	chr2:234590975	Arg131Gln	0.444	TTTAATGACCG/AAAAATTAGTA
	622T>C	c	rs11692021	exon 1	chr2:234591205	Trp208Arg	0.298	GGAGAGAGTAT/CGGAACCACAT
	660C>T		rs45462096	exon 1	chr2:234591243	Cys220Cys	0.005	ATTTATTTTGC/TCCCTATTTTT
	756G>A		rs17864686	exon 1	chr2:234591339	Leu252Leu	0.136	CAATTTGGTTG/ATTGCGAACTG
UGT1A1	-364C>T	d	rs887829	promoter	chr2:234668570		0.135	TGTTTAATTTC/TTGGAAAAGAA
	-64G>C		rs873478	promoter	chr2:234668870		0.082	TGTATCGATTG/CGTTTTTGCCA
	-52(TA)7	d	rs3064744	promoter	chr2:234668880		0.130	GTTTTTGCCATATATATATATA−/TATAAGTAGGAG
	181G>A		novel	exon 1	chr2:234669114	Ala61Thr	0.030	AGTTGTCCTAG/ACACCTGACGC
	211G>A		rs4148323	exon 1	chr2:234669144	Gly71Arg	0.390	CATCAGAGACG/AGAGCATTTTA
	596C>G		novel	exon 1	chr2:234669529	Ser199Cys	0.010	CCTCTCTCCTC/GTCATTCAGAT
Exons 2-5	6634C>T		rs6708136	intron 1	chr2:234675567		0.005	ATCTCAAACAC/TGCATGCCTTT
	6637A>C		novel	intron 1	chr2:234675570		0.005	TCAAACACGCA/CTGCCTTTAAT
	6893T>C		rs4148327	intron 2	chr2:234675826		0.095	GAAGATTCTAT/CACCATGGCCT
	7939C>T		rs34946978	exon 4	chr2:234676872	Pro364Leu	0.085	TCAGGTCACCC/TGATGACCCGT
	8046A>C		rs111033540	exon 4	chr2:234676979	Asn400His	0.010	TCAGATGGACA/CATGCAAAGCG
	12022C>T		rs114982090	exon 5	chr2:234680955	Pro451Leu	0.020	AAGGACCGCCC/TGGTGGAGCCG
	12483T>C	b	rs10929303	3'UTR	chr2:234681416		0.150	GTGCCCCCTCT/CGGTGTCTTTG
	12611G>C	b	rs1042640	3'UTR	chr2:234681544		0.121	GTGGTCCCACG/CGCTGCCCCTA
	12691T>C		rs34942353	3'UTR	chr2:234681624		0.010	TAACCAATAAT/CGGTCAGTCCT
	12712G>C	b	rs8330	3'UTR	chr2:234681645		0.130	CATCTCTGTCG/CTGCTTCATAG

* The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group. The NM021027.2, NM019077.2 and NM000463.2 (GenBank accession numbers) were used as UGT1A9, 1A7, 1A1 reference sequence.

& The apparent linkage disequilibrium (LD), defined by r^2 more than 0.8, was indicated by a-d for Tibetan population in the LD column. The nucleotide positions of polymorphisms in the exons 2-5 are numbered as in UGT1A1. UTR: untranslated region.

TABLE 3. Allelic frequencies of *UGT1A1, 1A7, 1A9* recorded in Chinese Tibetan population

Gene	*UGT1A1*						*UGT1A7*				*UGT1A9*			
Location		exon 1	promoter	exon 4	promoter	promoter	exon 1	exon 1	exon 1	exon 1	promoter	promoter	promoter	
variant	wild	211	-53TA6>7	7939	-364	-64	wild	387(T>G) 391(C>A) 392(G>A)	387(T>G) 391(C>A) 392(G>A) 622(T>C)	622(T>C)	392(G>A)	wild	-118(dT)$_{10>9}$	-441(T>C) -332(C>T) -118(dT)$_{10>9}$	-1818(T>C) -441(T>C) -332(C>T) -118(dT)$_{10>9}$
Amino acid change	none	Gly71Arg		Pro364Leu			none	Asn129Lys Arg131Lys	Asn129Lys Arg131Lys Trp208Arg	Trp208Arg	Arg131Gln	none			
Allele[#]	1	6	28	63	80	81	1	2	3	4	11	1a	1b	1d	1f
Frequency	0.295	0.390	0.125	0.080	0.095	0.015	0.546	0.147	0.288	0.010	0.010	0.551	0.414	0.015	0.020

[#]The allele was based on the criteria posted on the UGT alleles Nomenclature home page (http://galien.pha.ulaval.ca/labocg/alleles/alleles.html)

TABLE 4. Genotypic frequencies of *UGT1A1, 1A7, 1A9* in Chinese Tibetan population

Gene	Genotype	Frequency	Gene	Genotype	Frequency	Gene	Genotype	Frequency
UGT1A1	*1/*1	0.050	*UGT1A7*	*1/*1	0.263	*UGT1A9*	*1a/a	0.273
	*1/*6	0.480		*1/*2	0.182		*1a/b	0.505
	*1/*28	0.010		*1/*3	0.364		*1a/d	0.020
	*1/*63	0.000		*1/*4	0.020		*1a/f	0.030
	*1/*80	0.000		*1/*11	0.000		*1b/b	0.152
	*1/*81	0.000		*2/*2	0.020		*1b/d	0.010
	*6/*6	0.030		*2/*3	0.070		*1b/f	0.010
	*6/*28	0.070		*2/*4	0.000		*1d/d	0.000
	*6/*63	0.140		*2/*11	0.000		*1d/f	0.000
	*6/*80	0.000		*3/*3	0.070		*1f/f	0.000
	*6/*81	0.030		*3/*4	0.000			
	*28/*28	0.000		*3/*11	0.000			
	*28/*63	0.000		*4/*4	0.000			
	*28/*80	0.170		*4/*11	0.000			
	*28/*81	0.000		*11/*11	0.010			
	*63/*63	0.000						
	*63/*80	0.020						
	*63/*81	0.000						
	*80/*80	0.000						
	*80/*81	0.000						
	*81/*81	0.000						

UGT1A1 was used as reference sequence for common exons 2-5). The most frequent alleles and genotypes were *1a (0.551) and *1a/b (0.505), *1 (0.546) and *1/*3 (0.364) and *6 (0.390) and *1/*6 (0.480) in *UGT1A9*, *1A7* and *1A1* for Tibetan population, respectively. All the results are shown in Table 3 and Table 4.

Linkage Disequilibrium and Haplotype Analysis

Pairwise LD analysis for all detected polymorphisms (included the rare polymorphisms) was performed by calculating $|D'|$ and r^2 using Haploview4.2. The region from *UGT1A9* to *1A1* was decomposed into two discrete LD blocks: Block 1 (1A7) and Block 2 (common exons 2-5) (Fig. 1). The data from the $|D'|$ values supported this block partitioning (Fig. 1); a pattern of LD is present also between some UGT1A9 and UGT1A7 variants and also between UGT1A1 and UGT1A9 variants. In addition, a few exceptional strong linkages ($r^2 > 0.8$) beyond the LD blocks were also observed (Table 2 and Fig. 1). More specifically, the *UGT1A7* 391C>A (Arg131Arg) was strongly linked with *UGT1A7* 387T>G (Asn129Lys) ($r^2 = 0.98$) and 392G>A (Arg131Gln) ($r^2 = 0.899$). The *UGT1A7* -57T>G was linked with *UGT1A7* 33C>A (Pro11Pro) ($r^2 = 0.808$) and 622T>C (Trp208Arg) ($r^2 = 0.927$), while the *UGT1A1* -52$(TA)_{6>7}$ was strong linked with *UGT1A* -364C>T ($r^2 = 0.873$). The 3′-UTR 12712G>C was strongly linked with 12611G>C ($r^2 = 0.954$) and 12483T>C ($r^2 = 0.847$).

Subsequent analysis of LD blocks of Tibetan population revealed eight common haplotypes for block 1 (> 1% frequency accounted for 98.1% of *UGT1A7* haplotype diversity) and three common haplotypes for block 2 (accounted for 99.5% of exons 2-5 haplotype diversity). The haplotypes of LD block 1 was made by the polymorphisms -543A>G, -341C>T, -57T>G, 33C>T, 387T>G and 391C>A of *UGT1A7* and the haplotype tag SNPs (htSNPs) were -543A>G, -341C>T, -57T>G, 33C>T and 387T>G. The LD block 2 consisted of 12483T>C, 12611G>C and 12712G>C and the haplotype tag SNPs (htSNPs) were 12483T>C and 2712G>C (Table 5).

DISCUSSION

This study directly sequenced and provided a comprehensive analysis of the genetic variations of the *UGT1A9*, *1A7* and *1A1* loci using genomic DNA from

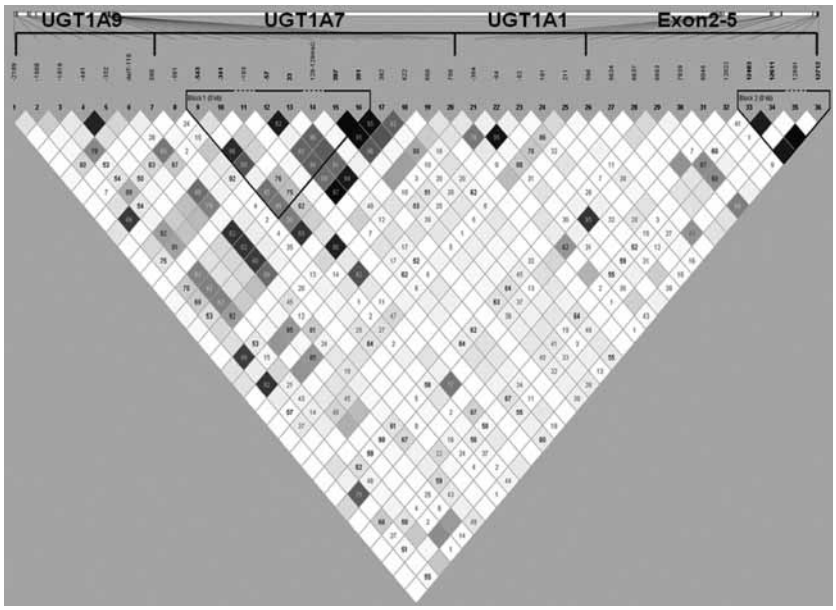

FIG. 1. Linkage disequilibrium (LD) analysis for *UGT1A1*, *1A7*, *1A9* single nucleotide polymorphisms (SNPs); LD for all *UGT1A1*, *1A7*, *1A9* SNPs identified in Tibetan population. The pairwise LD is expressed as r^2 for the polymorphisms in *UGT1A1, 1A7, 1A9*, and the common exons 2-5, which are located in the same order on the chromosome. A denser color represents a higher linkage. The nucleotide positions of polymorphisms in intron 2 and exon 5 are numbered as in *UGT1A1*. Data in the square indicate the D' value.

TABLE 5. The haplotypes and haplotype tag SNPs (htSNPs) of Chinese Tibetan population (n = 100)

Block	Markers	Haplotype	Population frequencies	Total frequencies	htSNPs
1	-543A>G; 341C>T;	ACTCTC	0.529	0.981	-543A>G;
	-57T>G; 33C>A;	GCGAGA	0.214		-341C>T;
	387T>G; 391C>A	ATTCGA	0.114		-57T>G;
		ACGAGA	0.053		33C>A;
		ACTCGA	0.025		387T>G
		GCGCGA	0.020		
		ACTAGA	0.016		
		ATTCTC	0.010		
2	12483T>C; 12611G>C;	CCC	0.850	0.995	12483T>C;
	12712G>C	TGG	0.125		12712G>C
		TCC	0.020		

Tibetan population. Seven, thirteen and six genetic polymorphisms were detected in the promoter and exon 1 of *UGT1A9, 1A7* and *1A1*, respectively, and ten polymorphisms in common exons 2-5 (Table 2).

Some of the polymorphisms of *UGTs* isoforms are known to affect glucuronidation rates (Radominska-Pandya *et al*., 1999; Tukey & Strassburg, 2001). The SN-38, the active antitumor metabolite of the irinotecan (a main therapeutic drug for the treatment of metastatic colorectal cancer patients), is detoxified by UGT1A isoforms. Previous studies have disclosed that UGT1A1*28, UGT1A7*3 and UGT1A9*1b with impaired enzyme function have the major effect on the SN-38 detoxification (Gagné *et al*., 2002; Cecchin *et al*., 2009). In this regard, systematically studying the polymorphisms of *UGT1A9*, *1A7* and *1A1* might play an important role in the prediction of toxicity and responsiveness to cytotoxic agents, as described for other detoxifying enzymes (Di Martino *et al*., 2011).

Except the mechanism of detoxifying enzymes to regulate the sensitivity of cancer cells to cytotoxic drugs, other mechanisms also exist. One of these me-

chanisms is based on the expression of nucleotide carriers on cancer cells as in the case of gemcitabine and its transporter human equilibrative nucleoside transporter 1 (Santini *et al*., 2011). The polymorphisms of these genes that regulate the pharmacokinetics and pharmacodynamics of cytotoxic agents in the efficacy and safety would affect the prediction of toxicity and responsiveness to cytotoxic agents.

In order to improve understanding of genetic risk factors and increase the safety and efficiency of drug individualization and develop the targeted drug therapy for individual patients, we need innovative technological approaches to effectively and easily identify the new genetic polymorphisms correlated with anticancer drug activity or toxicity on a wide population and on different gene regions. For instance, the emerging technology of DMET microarray platform, would be useful for easy identification of new genetic variants correlated with anticancer drug activity or toxicity for personalized medicine (Di Martino *et al*., 2011).

From the published results, it is known that the luciferase-reporter activity of *UGT1A9* -118 T10 (1A9*1b allele) was 2.6-fold as compared to that of *1A9* -118 T9 (Yamanaka *et al*., 2004). In our study the frequency of delT -118 was 0.449 in Tibetan population. The UGT1A7*3 allele, which was detected at frequency of 0.288 in Tibetan population, has been reported to encode for a 50% reduced catalytic activity enzyme (Strassburg *et al*., 1999; Guillemette *et al*., 2000b) and has effect on SN-38 pharmacokinetics in Asian patients (Han *et al*., 2006; Fujita *et al*., 2007). It is known that the *UGT1A7* -57T>G reduce 70% of the luciferase activity (Lankisch *et al*., 2005) and is linked with either 1A7*3 or *4 in Germans; in our study, -57T>G was also linked with 1A7*3 or *4 in Tibetan population (Fig. 1) and the observed frequency was 0.298 in Tibetan population. The UGT1A1*28, in which a variable number of the thymine-adenine (TA) repeats change the length of the TATA element, the recorded frequency was 0.130 in our study. The majority of Gilbert syndrome (GS) cases are also associated with the polymorphism *UGT1A1**28, which is the binding site for factor IID and important in the transcription mechanism (Monaghan *et al*., 1996). The presence of seven repeats, instead of the wild-type six repeats, has been found to be associated with reduction in the efficiency of transcription of the *UGT1A1* gene, and patients with clinically diagnosed GS were homozygous for the (TA)7 allele (Bosma *et al*., 1995). In our study, UGT1A7 variants are not in complete LD with UGT1A1*28 (Fig. 1), suggesting that the phenotypic effects ascribed to UGT1A7*3 variants might not be fully dependent from those of UGT1A1*28.

Each group differs from the others in its diet, cultural habits, and primary language. The differences in allele frequencies indicate that genetic composition also varies among different geographical populations. Systematic analysis of the polymorphisms of *UGT1A9*, *1A7* and *1A1* gene in Chinese Tibetan populations and a mapping of the polymorphism distribution over this group population will be useful for personalized medicine in the Chinese Tibetan population. Linkages among the SNPs in *UGT1A9*, *1A7* and *1A1* have been reported in Americans (Carlini *et al.*, 2005). Our results demonstrated that genetic polymorphisms of *UGT1A9*, *1A7* and *1A1* locus in Tibetan population significantly differ from American population. The LD analysis of the identified polymorphisms revealed two strong linkages in Tibetan population: LD block 1 within the polymorphisms in *UGT1A7*, and LD block 2 with polymorphisms in exons 2-5. We know that D' values are known to fluctuate upward when a small number of samples or rare alleles are examined. In our study, the study samples were 100 (small size) and all identified polymorphisms (including the low frequency polymorphisms) were used to analyze the LD blocks. It seems that the low allelic frequency of some of the included variants and the small sample size could make it difficult to highlight these patterns. Some strong linkages were found beyond the LD blocks in Tibetan population: strong linkages were found between 391C>A (Arg131Arg) and 387T>G (Asn129Lys) (r^2 = 0.980) or 392G>A (Arg131Gln) (r^2 = 0.899) in *UGT1A7*, between -57T>G and 33C>A (Pro11Pro) (r^2 = 0.808) or 622T>C (Trp208Arg) (r^2 = 0.927) in *UGT1A7*, between -52$(TA)_{6>7}$ and -364C>T (r^2 = 0.873) in *UGT1A1*, between 12712G>C and 12611G>C (r^2 = 0.954) or 12483T>C (r^2 = 0.847) in 3′-UTR. These results suggest the presence of larger LD blocks in *UGT1A9*, *1A7* and *1A1* locus and some recombination events may break the LD blocks to smaller ones.

Analysis of the haplotypes structure of LD blocks and htSNPs across the entire *UGT1A9*, *1A7* and *1A1* for Tibetan population showed that there were eight and three common haplotypes in LD block1 and block 2, respectively. From the result we can see that the difference in the haplotypes structure made a different htSNP selection for the population (Table 5). For the block 1, the htSNPs would be account for 98.1% of 1A7 haplotype diversity, and for the block 2, the htSNPs

would be account for 99.5% of exons 2-4 haplotype diversity. The htSNPs selection would reduce the burden of genotyping all SNPs for genetic association studies.

Since it was reported that the UGT1A9, 1A7 and 1A1 were major components in irinotecan detoxification and elimination, we only comprehensively analyzed the genetic variations of the *UGT1A9, 1A7* and *1A1* locus in Tibetan population in this study, even if a minor role is suggested for UGT1A6, UGT1A8, and UGT1A10 (Gagné *et al*., 2002).

This is the first study of the genetic structure of the *UGT1A9*, *1A7* and *1A1* gene in the Chinese Tibetan population. The combined effects of some decreased-function variants will result in enzyme inactivation. Different polymorphisms and their combinations may produce markedly different results in terms of *UGT1A9*, *1A7* and *1A1* activity, so htSNP detection and haplotype analysis would be helpful to identify the metabolizer phenotype and may aid in the development of effective and targeted drug therapy for individual patients and reduce drug toxicity.

ACKNOWLEDGEMENT

We are grateful to all the participants in this study. This work was supported by a grant from the National High Technology Research and Development Program of China (863 Program) (No.2009AA022710). We would like to express our sincere gratitude to Changfeng Li for his technical support for our study.

REFERENCES

Ahmadi KR, Weale ME, Xue ZY , Soranzo N, Yarnall DP, Briley JD, Maruyama Y, Kobayashi M, Wood NW, Spurr NK, *et al*., 2005. A single-nucleotide polymorphism tagging set for human drug metabolism and transport. *Nature Genetics*, 37: 84-89.

Ando Y, Saka H, Ando M, Sawa T, Muro K, Ueoka H, Yokoyama A, Saitoh S, Shimokata K, Hasegawa Y, 2000. Polymorphisms of UDP-glucuronosyltransferase gene and irinotecan toxicity: a pharmacogenetic analysis. *Cancer Research*, 60: 6921-6926.

Beutler E, Gelbart T, Demina A, 1998. Racial variability in the UDP-glucuronosyltransferase 1 (UGT1A1) promoter: A balanced polymorphism for regulation of bilirubin metabolism? *Proceedings of the National Academy of Sciences of the United States of America*, 95: 8170-8174.

Bosma PJ, Chowdhury JR, Bakker C, Gantla S, de Boer A, Oostra BA, Lindhout D, Tytgat GNJ, Jansen PLM, Oude Elfrink RPJ, Chowdhury NR, 1995. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *New England Journal of Medicine*, 333: 1171-1175.

Carlini LE, Meropol NJ, Bever J, Andria ML, Hill T, Gold P, Rogatko A, Wang H, Blanchard RL, 2005. UGT1A7 and UGT1A9 polymorphisms predict response and toxicity in colorectal cancer patients treated with capecitabine/irinotecan. *Clinical Cancer Research*, 11: 1226-1236.

Cecchin E, Innocenti F, D'Andrea M, Corona G, De Mattia E, Biason P, Buonadonna A, Toffoli G, 2009. Predictive role of the UGT1A1, UGT1A7, and UGT1A9 genetic variants and their haplotypes on the outcome of metastatic colorectal cancer patients treated with fluorouracil, leucovorin, and irinotecan. *Journal of Clinical Oncology*, 27: 2457- 2465.

Di Martino MT, Arbitrio M, Leone E, Guzzi PH, Rotundo MS, Ciliberto D, Tomaino V, Fabiani F, Talarico D, Sperlongano P, *et al.*, 2011. Single nucleotide polymorphisms of ABCC5 and ABCG1 transporter genes correlate to irinotecan-associated gastrointestinal toxicity in colorectal cancer patients: A DMET microarray profiling study. *Cancer Biology & Therapy*, 12: 780-787.

Ehmer U, Vogel A, Schütte JK, Krone B, Manns MP, Strassburg CP, 2004. Variation of hepatic glucuronidation: novel functional polymorphisms of the UDP-glucuronosyltransferase UGT1A4. *Hepatology*, 39: 970-977.

Elahi A, Bendaly J, Zheng Z, Muscat JE, Richie JP Jr, Schantz SP, Lazarus P, 2003. Detection of UGT1A10 polymorphisms and their association with orolaryngeal carcinoma risk. *Cancer*, 98: 872-880.

Evans WE, Relling MV, 1999. Pharmacogenomics: Translating functional genomics into rational therapeutics. *Science*, 286: 487-491.

Fujita K, Ando Y, Nagashima F, Yamamoto W, Eodo H, Araki K, Kodama K, Miya T, Narabayashi M, Sasaki Y, 2007. Genetic linkage of UGT1A7 and UGT1A9 polymorphisms to UGT1A1*6 is associated with reduced activity for SN-38 in Japanese patients with cancer. *Cancer Chemotherapy and Pharmacology*, 60: 515-522.

Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, *et al*., 2002. The structure of haplotype blocks in the human genome. *Science*, 296: 2225-2229.

Gagné JF, Montminy V, Belanger P, Journault K, Gaucher G, Guillemette C, 2002. Common human UGT1A polymorphisms and the altered metabolism of irinotecan active metabolite 7-ethyl-10-hydroxycamptothecin (SN-38). *Molecular Pharmacology*, 62: 608-617.

Gong QH, Cho JW, Huang T, Potter C, Gholami N, Basu NK, Kubota S, Carvalho S, Pennington MW, Owens IS, Popescu NC, 2001. Thirteen UDP-glucuronosyltransferase genes are encoded at the human UGT1 gene complex locus. *Pharmacogenetics and Genomics*, 11: 357-368.

Guillemette C, 2003. Pharmacogenomics of human UDP-glucuronosyltransferase enzymes. *The Pharmacogenomics Journal*, 3: 136-158.

Guillemette C, Millikan RC, Newman B, Housman DE, 2000a. Genetic polymorphisms in uridine diphosphoglucuronosyltransferase 1A1 and association with breast cancer among African Americans. *Cancer Research*, 60: 950-956.

Guillemette C, Ritter JK, Auyeung DJ, Kessler FK, Housman DE, 2000b. Structural heterogeneity at the UDP-glucuronosyltransferase 1 locus: functional consequences of three novel missense mutations in the human UGT1A7 gene. *Pharmacogenetics and Genomics*, 10: 629-644.

Han JY, Lim HS, Shin ES, Yoo YK, Park YH, Lee JE, Jang IJ, Lee DH, Lee JS, 2006. Comprehensive analysis of UGT1A polymorphisms predictive for pharmacokinetics and treatment outcome in patients with non-small-cell lung cancer treated with irinotecan and cisplatin. *Journal of Clinical Oncology*, 24: 2237-2244.

Huang YH, Galijatovic A, Nguyen N, Geske D, Beaton D, Green J, Green M, Peters WH, Tukey RH, 2002. Identification and functional characterization of UDP-glucuronosyltransferases UGT1A8*1, UGT1A8*2 and UGT1A8*3. *Pharmacogenetics and Genomics*, 12: 287-297.

Innocenti F, Undevia SD, Iyer L, Chen PX, Das S, Kocherginsky M, Karrison T, Janisch L, Ramírez J, Rudin CM, *et al.*, 2004. Genetic variants in the UDP-glucuronosyltransferase 1A1 gene predict the risk of severe neutropenia of irinotecan. *Journal of Clinical Oncology*, 22: 1382-1388.

Iyer L, Das S, Janisch L, Wen M, Ramírez J, Karrison T, Fleming GF, Vokes EE, Schilsky RL, Ratain MJ, 2002. UGT1A1*28 polymorphism as a determinant of irinotecan disposition and toxicity. *The Pharmacogenomics Journal*, 2: 43-47.

Jinno H, Saeki M, Saito Y, Tanaka-Kagawa T, Hanioka N, Sai K, Kaniwa N, Ando M, Shirao K, Minami H, *et al.*, 2003a. Functional characterization of human UDP-glucuronosyltransferase 1A9 variant, D256N, found in Japanese cancer patients. *Journal of Pharmacology and Experimental Therapeutics*, 306: 688-693.

Jinno H, Saeki M, Tanaka-Kagawa T, Hanioka N, Saito Y, Ozawa S, Ando M, Shirao K, Minami H, Ohtsu A, *et al.*, 2003b. Functional characterization of wild-type and variant (T202I and M59I) human UDP-glucuronosyltransferase 1A10. *Drug Metabolism and Disposition*, 31: 528-532.

Judson R, Stephens JC, Windemuth A, 2000. The predictive power of haplotypes in clinical response. *Pharmacogenomics*, 1: 15-26.

Köhle C, Möhrle B, Münzel PA, Schwab M, Wernet D, Badary OA, Bock KW, 2003. Frequent co-occurrence of the TATA box mutation associated with Gilbert's syndrome (UGT1A1*28) with other polymorphisms of the UDP-glucuronosyltransferase-1 locus (UGT1A6*2 and UGT1A7*3) in Caucasians and Egyptians. *Biochemical Pharmacology*, 65: 1521-1527.

Lankisch TO, Vogel A, Eilermann S, Fiebeler A, Krone B, Barut A, Manns MP, Strassburg CP, 2005. Identification and characterization of a functional TATA box polymorphism of the UDP glucuronosyltransferase 1A7 gene. *Molecular Pharmacology*, 67: 1732-1739.

Mackenzie PI, Owens IS, Burchell B, Bock KW, Bairoch A, Bélanger A, Fournel-Gigleux S, Green M, Hum DW, Iyanagi T, *et al.*, 1997. The UDP glycosyltransferase gene superfamily: recommended nomenclature update based on evolutionary divergence. *Pharmacogenetics and Genomics*, 7: 255-269.

Mackenzie PI, Bock KW, Burchell B, Guillemette C, Ikushiro S, Iyanagi T, Miners JO, Owens IS, Nebert DW, 2005. Nomenclature update for the mammalian UDP glycosyltransferase (UGT) gene superfamily. *Pharmacogenetics and Genomics*, 15: 677-685.

Monaghan G, Ryan M, Seddon R, Hume R, Burchell B, 1996. Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet*, 347: 578-581.

Nagar S, Zalatoris JJ, Blanchard RL, 2004. Human UGT1A6 pharmacogenetics: identification of a novel SNP, characterization of allele frequencies and functional analysis of recombinant allozymes in human liver tissue and in cultured cells. *Pharmacogenetics and Genomics*, 14: 487-499.

Radominska-Pandya A, Czernik PJ, Little JM, Battaglia E, Mackenzie PI, 1999. Structural and functional studies of UDP-glucuronosyltransferases. *Drug Metabolism Reviews*, 31: 817-899.

Santini D, Schiavon G, Vincenzi B, Cass CE, Vasile E, Manazza AD, Catalano V, Baldi GG, Lai R, Rizzo S, *et al*., 2011. Human equilibrative nucleoside transporter 1 (hENT1) levels predict response to gemcitabine in patients with biliary tract cancer (BTC). *Current Cancer Drug Targets*, 11: 123-129.

Strassburg CP, Strassburg A, Nguyen N, Li Q, Manns MP, Tukey RH, 1999. Regulation and function of family 1 and family 2 UDP-glucuronosyltransferase genes (UGT1A, UGT2B) in human oesophagus. *Biochemical Journal*, 338: 489-498.

Tukey RH, Strassburg CP, 2000. Human UDP-glucuronosyltransferases: metabolism, expression, and disease. *Annual Review of Pharmacology and Toxicology*, 40: 581-616.

Tukey RH, Strassburg CP, 2001. Genetic multiplicity of the human UDP-glucuronosyltransferases and regulation in the gastrointestinal tract. *Molecular Pharmacology*, 59: 405-414.

Verlaan M, te Morsche RHM, Pap A, Laheij RJ, Jansen JBMJ, Peters WHM, Drenth JPH, 2004. Functional polymorphisms of UDP-glucuronosyltransferases 1A1, 1A6 and 1A8 are not involved in chronic pancreatitis. *Pharmacogenetics and Genomics*, 14: 351-357.

Villeneuve L, Girard H, Fortier LC, Gagné JF, Guillemette C, 2003. Novel functional polymorphisms in the UGT1A7 and UGT1A9 glucuronidating enzymes in Caucasian and African-American subjects and their impact on the metabolism of 7-ethyl-10-hydroxycamptothecin and flavopiridol anticancer drugs. *Journal of Pharmacology and Experimental Therapeutics*, 307: 117-128.

Yamanaka H, Nakajima M, Katoh M, Hara Y, Tachibana O, Yamashita J, McLeod HL, Yokoi T, 2004. A novel polymorphism in the promoter region of human UGT1A9 gene (UGT1A9*22) and its effects on the transcriptional activity. *Pharmacogenetics and Genomics*, 14: 329-332.