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Comparative Analysis of *UGT1A9* Genetic Polymorphisms between Chinese Han and Tibetan Populations

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Abstract: Although various polymorphisms have been identified in the *UGT1A9* gene in the main sorts of ethnic groups in the world, no investigations have been focused on the Chinese Tibetan populations and the comparison between Chinese Tibetan and Han populations to date. This study was designed to systematically compare genetic differences between the two populations. We investigated the functional regions of *UGT1A9* in 200 unrelated healthy Chinese volunteers, comprising 100 Tibetan and 100 Han individuals from Qinghai and Shaanxi, respectively, by using direct sequencing. A total of 21 different genetic variants, including 7 novel variants were identified. According to the results of comparative analysis, the allele frequencies of three common variants (-1888T>G, 95246T>C, 96292C>T) were significantly different between Tibetan and Han populations ($p \leq 0.05$). However, there were no differences of linkage disequilibrium patterns, haplotype structures and htSNPs between the two populations. *UGT1A9**1b was the prevalent defective alleles in Chinese population. In addition, -1888T>G, -1819T>C, -441C>T affected the binding of transcriptional factors and four of the missense mutations (P361L, N397H, P448L and Y483D) were highly conserved among the three different species (Homo sapiens, Rattus norvegicus and Danio rerio). In short, there are significant differences of genetic information of *UGT1A9* between Chinese Tibetan and Han populations. The determined genetic information of *UGT1A9* in Chinese Tibetan and Han populations might serve as a baseline for larger studies on pharmacogenomics and also provide important data for the advance of personalized medicine in Chinese Han and Tibetan populations.

Key words: Tibetan, haplotype, LD, polymorphism, *UGT1A9*

INTRODUCTION

The human UDP-Glucuronosyl Transferases (UGTs) is one of phase II drug-metabolizing enzymes that catalyze the glucuronidation of a variety of endogenous and other exogenous compounds, including drugs, carcinogens and other xenobiotics (Fujita *et al*., 2006). According to the primary amino acid sequence homology of UGT, they were categorized into two major families, UGT1 and UGT2 (Chouinard *et al*., 2006). The entire UGT1 family is derived from a single gene locus (*UGT1A*), located on chromosome 2 (2q37), spanning about 210 kb, coding for nine functional proteins (*UGT1A1*, *UGT1A3*-*UGT1A1*0) and three pseudogenes (Gong *et al*., 2001). The UGT2 family can be further divided into two subfamilies, UGT2A and UGT2B, which are encoded by different genes clustered on chromosome 4q13-4q21.1 (Turgeon *et al*., 2000). The *UGT1A* isoforms share the identical exon 1, while differing in exons 2-5, both of which are responsible for the specific expression of *UGT1A* isoforms (Araki *et al*., 2005). The regulation and expression of the *UGT1A* gene are tissue-specific, eg. *UGT1A1*, *UGT1A3*, *UGT1A4*, *UGT1A6* and *UGT1A9* (Strassburg *et al*., 1997; 1998; 1999) are hepatic tissue-specific, whereas *UGT1A7*, *UGT1A8* and *UGT1A10* are exclusively expressed in extrahepatic tissues such as mouth, esophagus, intestine, pancreas and colon (Ockenga *et al*., 2003; Strassburg *et al*., 1997; 1999; Vogel *et al*., 2002).

The *UGT1A9* gene, a member of the UGT1 family, is both involved in the metabolism of endogenous estrogens and thyroid hormones, the exogenous chemicals and drugs such as phenol, acetaminophen,

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propofol, propranolol, (Albert *et al*., 1999). The *UGT1A9* gene is expressed specifically in liver, kidney, colon and esophagus (Albert *et al*., 1999; Tukey and Strassburg, 2000). To date, various polymorphisms have been identified in the *UGT1A9* gene, resulting in decrease of *UGT1A9* activities toward a variety of exogenous substances and affecting efficiencies of drug metabolisms. -118$(dT)_{9>10}$ (*UGT1A9*1b*), for example, is one of the most important polymorphisms in the promoter region of the *UGT1A9* gene reported to be associated with the enhanced transcriptional activity of this gene (Yamanaka *et al*., 2004). This variant was reported (Carlini *et al*., 2005) to cause higher incidence of toxicity and poor tumor response. *UGT1A9* polymorphisms influence SN-38G formation in liver microsomes (Han *et al*., 2006) and *UGT1A9*1b* genotypes might be important for SN-38 glucuronidation. In this regard, polymorphisms of *UGT1A9* gene may exert a significant impact on the pharmacokinetics and toxicity of drugs.

China consists of 56 ethnic groups, among which Han accounts for 90.56% of the total Chinese population, yet Tibetan is a major minority. Up to now, no systematic investigations have been focused on polymorphisms, Linkage Disequilibrium (LD) pattern and haplotype structures of *UGT1A9* in Tibetan population. Similarly, the genetic information comparison between Tibetan and Han is also rare. The genetic information may be different due to ethnic and spatial differences (Mehlotra *et al*., 2007). Polymorphisms, genotypes and haplotypes may collectively provide more effective aids for individualized treatment. In this study, in order to study and compare the identified genotypes, allele frequencies, LD pattern, haplotype structures and haplotype tagSNPs (htSNPs) of *UGT1A9* in the Tibetan and Han groups, a comprehensive study on the genetic information in the Tibetan and Han populations was conducted, The determined genetic information of *UGT1A9* in Chinese Tibetan and Han populations might serve as a baseline for larger studies on pharmacogenomics and also provide important data for the advance of personalized medicine in Chinese Tibetan and Han populations.

MATERIALS AND METHODS

Study populations: Two hundred healthy unrelated Chinese people from two different regions of the Chinese mainland were recruited in the study. Two hundred Chinese can be divided into two groups, one hundred Han volunteers from Yulin, Shaanxi province and one hundred Tibetan volunteers from Qinghai province. Each group includes 50 males and 50 females aging from 18-40 years. All participants provided their detailed information, so we can guarantee that the people in each group were of the same origin. All volunteers provided written consent for the use of their peripheral blood samples for experimental purposes and the present study was reviewed and approved by the ethics committee of Northwest University.

Polymerase Chain Reaction (PCR) and DNA sequencing: Systematic polymorphism screening was performed using PCR and direct sequencing. A 2ml sample of venous blood was collected from each subject. Genomic DNA was extracted from peripheral blood leukocytes of 200 subjects by the standard procedure (Fujita *et al*., 2006). The extracted DNA was dissolved in sterile distilled water and stored at-80°C until PCR analysis. The promoter regions, all exons and 3'UTR of the *UGT1A9* gene were amplified and directly sequenced using an ABI PRISM 3700 DNA analyzer (Applied Biosystems, Foster City, California, USA) using eight sets of primers. The obtained sequences were examined for the presence of variants using Sequencher software (version 4.10.1, Gene Codes Corporation, Ann Arbor, Michigan, USA). The A in the ATG translation start codon is denoted nucleotide +1. The sequence of the complete *UGT1A9* gene described in GeneBank (Gene ID: 54600) was used as a reference.

Statistics analysis: Allele and genotype frequencies were calculated by the counting method. The χ^2 test or Fisher's exact test were used to compare allele, genotype frequencies between the Tibetan and Han populations. Statistical significance was set at $p<0.05$. All the statistical works were implemented on the SPSS 16.0 platform. Haploview, based on the expectation-maximization method, was used to measure LD between each of two loci and to estimate the Lewontin's coefficients D' (Lewontin, 1988) and correlation coefficient r^2 (Hill and Robertson, 1968). r^2 of 0.8 was selected as a threshold for all analysis. The block structures and their haplotype frequencies were also estimated using Haploview version 3.2 (Stephens *et al*., 2001). htSNPs were selected using the Haploview version of the Tagger program.

Functional predictions: Polymorphisms in the promoter region may have an influence on Transcriptional Factors (TFs) binding to the specific sites, including sorts and amounts. Web-based TFSEARCH software was used to analyze the transcriptional factor binding to the promoter region. The normal and variant sequences were analyzed by the software, respectively. According to the analysis, the influence of the polymorphisms towards to TFs was speculated. As for non-synonymous variants, conservative assessment was performed on the web-

based Protein BLAST software (http://blast.ncbi.nlm.nih.gov/) in three species which were Homo sapiens (h), Rattus norvegicus (r) and Danio rerio (d).

RESULTS

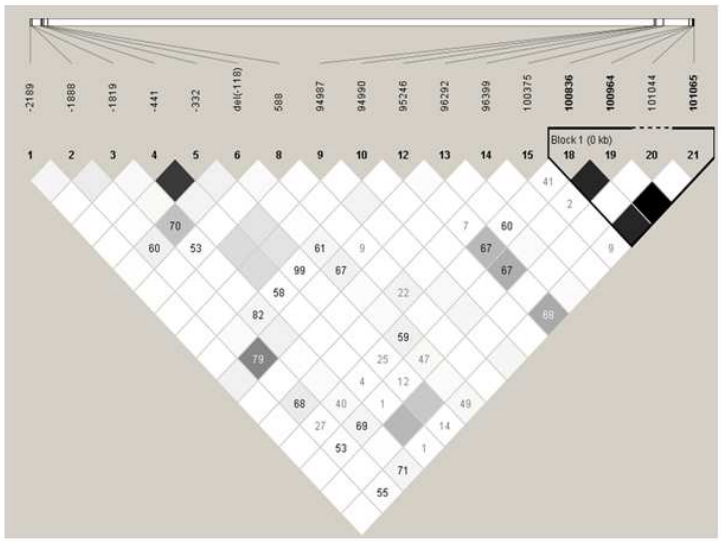
Identification of genetic variants in *UGT1A9* in Chinese Tibetan and Han populations: In the Chinese Tibetan and Han populations, a total of 21 different genetic variants, including 7 novel variants, were detected in the promoter region, five exons, surrounding introns and 3'UTR of *UGT1A9* (Table 1). -2189T>C, -1888T>G, -1819T>C, -441C>T, -332T>C, -118delT and -40C>G in the promoter region were detected, among them -2189T>C and -40C>G were novel yet -40C>G was specific to Han population with minor allele frequency 0.5%, -2189T>C with minor allele frequencies 0.5%, 1.5%, respectively, in Tibetan and Han populations. In the coding region, 9 variants were detected, including one novel nonsynonymous variant (96399A>C, 397 N>H) in one Tibetan subject in exon 4, two novel synonymous variant (588G>T, 95186G>A). Among these variants, two were synonymous variants and four were nonsynonymous variants. Besides, 94987T>C, 94990A>C and 95246T>C in the introns were also identified and 94990A>C was novel. In the 3'UTR, 100813T>G, 100836T>C, 100964G>C, 101044T>C and 101065G>C were detected, including one novel (100813T>G) in one Han subject. Among the 21 variants, nine ones -1888T>G, -1819T>C, -332T>C, -118delT, 95246T>C, 96292C>T (P361L), 100836T>C, 100964G>C and 101065G>C were common SNPs (minor allele frequency ≥ 0.05). Common polymorphic frequencies in the two ethnic groups were compared by statistical analysis. According to the statistical comparison, some of the polymorphic frequencies demonstrated significant differences between the two ethnic groups, such as -1888T>G, p=0.015; 95246T>C, p=0.05; 96292C>T, p = 0.008.

Allele and genotype frequencies: The frequency of the different *UGT1A9* alleles was listed in Table 2. Four different *UGT1A9* alleles were identified based on discoverable polymorphisms in this study which were *UGT1A9**1a, *1b, *1d and *1f. In Tibetan population, the frequent alleles were the wild-type allele *1a (55.05%) and allele*1b (41.41%). Alleles *1d and *1f were detected with frequencies of 1.52%, 1.02%, respectively. In the Han population, the frequent alleles were also the wild-type allele *1a (58.5%) and allele*1b (39.5%). Alleles *1d and *1f were detected with frequencies of 1.50%, 0.50%, respectively. Of these alleles, there were no significant differences between the two ethnic groups.

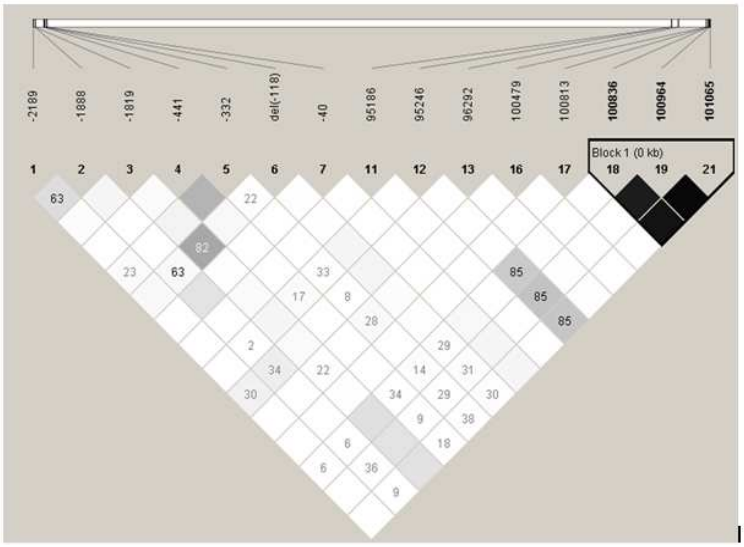
Table 1: Frequencies of *UGT1A9* polymorphisms in two different ethnic groups of China

				Minor allele frequency (%)			
Marker	Region	dbSNP (NCBI)	Effect	Tibetan (n = 100)	Han (n = s100)	P -value	Flanking sequence
-2189T>C	promoter	novel		0.5	1.5	0.315	tataatggcgt/cgatctcagct
-1888T>G	promoter	rs6731242		11.0	4.5	0.015**	actagaagcct/gtaccaataac
-1819T>C	promoter	rs13418420		36.0	41.5	0.259	tgtattatcat/caatgaagtca
-441C>T	promoter	rs2741045		3.5	2.0	0.359	ttgcttagagc/tatgagttgcc
-332T>C	promoter	rs2741046		4.5	6.5	0.380	caaatttactt/cttactttatc
-118delT	promoter	rs3832043		44.9	41.5	0.545	actgatttttttt/-atgaaa
-40C>G	promoter	novel		0.0	0.5	0.317	atcataggagc/gtt AGATTCCC
588G>T	Exon1	novel	196 G/G	2.0	0.0	0.123	TTCTCTTAGGG/TTTCTCAGATG
94987T>C	Intron1	rs6708136		0.5	0.0	0.317	atctcaaacac/tgcatgccttt
94990A>C	Intron1	novel		0.5	0.0	0.317	tcaaacacgca/ctgcctttaat
95186G>A	Exon2	novel	314 K/K	0.0	1.0	0.156	TTCCAGAGAAG/AAAAGCTATGG
95246T>C	Intron2	rs4148327		9.5	4.5	0.05**	gaagattctat/caccatggcct
96292C>T	Exon4	rs34946978	361 P/L	8.5	2.5	0.008**	tcag GTCACCC/TGATGACCCGT
96399A>C	Exon4	novel	397 N/H	1.0	0.0	0.156	TCAGATGGACA/CATGCAAAGCG
100375C>T	Exon5	rs114982090	448 P/L	2.0	0.0	0.440	AAGGACCGCCC/TGGTGGAGCCG
100479T>G	Exon5	rs34993780	483 Y/D	0.0	1.5	0.079	CTGGTACCAGT/GACCATTCCTT
100813T>G	3'UTR	novel		0.0	0.5	0.312	GTAAAGATATT/GTGAATATGTA
100836T>C	3'UTR	rs10929303		15.0	14.5	0.888	GTGCCCCCTCT/CGGTGTCTTTG
100964G>C	3'UTR	rs1042640		12.1	13.0	0.791	GTGGTCCCACG/CGCTGCCCCTA
101044T>C	3'UTR	rs34942353		1.0	0.0	0.156	TAACCAATAAT/CGGTCAGTCCT
101065G>C	3'UTR	rs8330		13.0	13.5	0.883	CATCTCTGTCG/CTGCTTCATAG

*The A of the ATG of the initiator Met codon is denoted nucleotide +1, as recommended by the Nomenclature Working Group (Hum Mut 1998; 11:1-3). The nucleotide sequence (Gene ID: 54600) was used as a reference sequence. 3'UTR, 3' Untranslated region. &The *P* value is for the comparison of the minor allele frequencies among the two different ethnic groups of China. **Significantly different between the Chinese Tibetan and Han population in the present study with p≤0.05



(A)



(B)

Fig. 1: Pairwise linkage disequilibrium associations between the SNPs in the *UGT1A9* gene in the two ethnic groups of China (A) Tibetan (B) Han. A correlation coefficient r2 color scheme is used to display LD by a scale of color intensity, black for very strong LD (r2=1), white for no LD (r2=0) and shades of gray for intermediate LD (0<r2<1)

Table 2: Frequencies of *UGT1A9* allele in two different ethnic groups of China

	Allele frequencies (%)		
UGT1A9 alleles	Tibetan (n =100)	Han (n = 100)	*P*-value
*1a(wild)	55.05	58.5	0.544
*1b	41.41	39.5	0.759
*1d	1.52	1.5	1.000
*1f	2.02	0.5	0.214

#The P value is for the comparison of the allele frequencies among the two different ethnic groups of China.

With regard to genotypes, the frequencies of genotypes were listed in Table 3. Seven genotypes were found in this study. In Tibetan population, 55.56% of the subjects carried one mutated allele which were *UGT1A9* *1a/*1b (50.51%), *1a/*1d (2.02%), *1a/*1f (3.03%) and 17.17% carried two defective alleles being homozygous for *1b/*1b (15.15%), heterozygote for *1b/*1f and *1b/*1d with the same frequency of 1.01%. In Han population, 47.00% of the subjects

carried one mutated allele which were *1a/*1b (45.00%), *1a/*1d (2.00%) and 14.14% carried two defective alleles being homozygous for *UGT1A9* *1b/*1b (16.00%). *UGT1A9* *1a/*1f was only detected in Han populations. Of these genotypes, there were no significant differences between the two ethnics.

LD/Haplotyping analysis: LD in pairwise SNPs was calculated for the two ethnic groups through Haploview analysis by calculating D' and correlation of alleles at two loci (r^2). The results were shown in Fig. 1. The results indicated that the degrees of linkage disequilibrium seem to be no different in the two populations.

The haplotype frequencies and htSNPs determination in the two ethnic groups analyzed by Haploview were summarized in Table 4.

Table 3: Frequencies of *UGT1A9* genotypes in two different ethnic groups of China

	Frequencies (%)		
UGT1A7 genotype	Tibetan (n = 100)	Han (n = 100)	**P*-value
*1a/*1a	a27.27	35	0.284
*1a/*1b	50.51	45	0.479
*1a/*1d	2.02	2	1.000
*1a/*1f	3.03	0	0.121
*1b/*1b	15.15	16	1.000
*1b/*1d	1.01	1	1.000
*1b/*1f	1.01	1	1.000
*1d/*1d	0.00	0	1.000
*1d/*1f	0.00	0	1.000
*1f/*1f	0.00	0	1.000

The *P* value is for the comparison of the minor allele frequencies among the two different ethnic groups of China

Fig. 2: (a) Human transcriptional factor targeted site in the promoter sequence of *UGT1A9* gene. Numbers indicate the position of nucleotide sequence. The asterisks indicate the positions at which mutation occurs. [&]transcription factor. [#] The score is predicted by TFSEARCH software. Transcriptional factor targeted sites are underlined. **, no transcription factors (b) Alignment of partial amino acid sequences of *UGT1A9* from three species including Homo sapiens (h), Rattus norvegicus (r) and Danio rerio (d). Numbers indicate the position of the amino acid sequence. The asterisks indicate the positions at which missense mutations occur (P361L, N397H, P448L and Y483D)

Table 4: Frequencies of haplotypes of the *UGT1A9* gene in two different ethnic groups of China

Ethnic groups	SNPs in haplotypes	Haplotypes	Frequencies (%)	Tag SNPs
Tibetan	100836T>C	CCC	85.0	100836T>C; 100964G>C
	100964G>C	TGG	12.5	
	101065G>C	TCC	2.0	
Han	100836T>C; 100964G>C	CCC	85.5	100836T>C;
	101065G>C	TGG	13.0	100964G>C

*Only haplotypes with a frequency greater than 1% were listed. # htSNPs are haplotype tag SNPs

Every ethnic group has only one LD block and the haplotype structures were analyzed by using Haploview (Fig. 1). In the LD block, the two groups had similar haplotype structures. Haplotype CCC was the dominating haplotype with the frequencies of 85.0%, 85.5%, respectively, in Tibetan and Han populations. Haplotype TGG had the frequencies of 12.5%, 13%, respectively, in Tibetan and Han populations.

Functional predictions: In the promoter region, the TFSEARCH software analysis showed that some polymorphisms altered transcriptional factor binding efficiency. For-1888T (binding C/EBP transcriptional factor), if the variant occurred, there was no related transcriptional factor in binding to the site. For -1819T (binding Oct-1 and CdxA transcriptional factors), if the variant occurred, transcriptional factor transformed into Nkx-2. For -441C (binding CP2), if the variant occurred, there was no related transcriptional factor binding to the site (Fig. 2). Towards the four nonsynonymous variants in the coding region, BLAST software showed that the four missense mutations (P361L, N397H, P448L, Y483D) occurred in highly conserved among three different species (Homo sapiens, Rattus norvegicus and Danio rerio) as shown in Fig. 2b.

DISCUSSION

Although many polymorphisms of *UGT1A9* have been identified in multi-ethnic groups, there are no relative investigations among the Chinese Tibetan population and comparison with Han population to date. This study conducted a comprehensive analysis of *UGT1A9* polymorphisms between the two ethnic groups of China. In this study, of all the 21 genetic variants, the allele frequencies of three common variant (-1888T>G, 95246T>C, 96292C>T) were significantly different between Tibetan and Han population ($p \leq 0.05$). Moreover, we also detected some ethnic-specific variants. For instance, 588G>T, 94987T>C, 94990A>C, 96399A>C (N397H), 100375C>T (P448L), 101044T>C were detected only in Tibetan population, while -40C>G, 95186G>A, 100479T>G (Y483D), 100813T>G only in Han population. Despite that three nonsynonymous variants [8G>A (C3Y), 98T>C (M33T) and 766G>A (D256N)] and a variant at 726T>G resulting in a premature termination codon TAG (Y242X) have been reported in exon1 in other ethnic groups (http://www.pharmacogenomics.pha.ulaval.ca/sgc/ugt_alleles), we have not detected these variants in this study. Taken together, there are ethnic differences in polymorphic and prevailing mutations of *UGT1A9.*

*UGT1A9**1b, a functional and prevalent defective allele identified in our study, occured at the frequencies of 41.41%, 39.50% in Tibetan and Han populations, respectively. *UGT1A9*2* (C3Y)、*UGT1A9*3* (M33T)、*UGT1A9*4* (Y242X) and *UGT1A9*5* (D256N) ,which were identified in other ethnic groups (Ehmer *et al*., 2004; Villeneuve *et al*., 2003; Jinno *et al*., 2003; Saeki *et al*., 2003) were not detected in the present study. These results showed that different genetic polymorphisms of *UGT1A9* existed in inter-ethnics.

We analyzed the LD pattern of the two ethnic groups separately. Strong LD was observed among 100836T>C, 100964G>C, 101065G>C in the two populations. The results indicated that the degrees of linkage disequilibrium seem to be no different in the two populations.

Owing to the different htSNPs selection, the different haplotypes structures were made and with a different haplotypes distributions in different populations. The combined effects of some decreased-function variants will lead to inactive enzymes. Different polymorphisms and their combinations may generate markedly different results with respect to *UGT1A9* activity. Thus, according to htSNPs detection and haplotype analysis the metabolizer phenotype would be identified easily (Chen *et al*., 2008).

There existed no significant differences in LD pattern, haplotypes and htSNPs between the two populations. Since these parameters could fluctuate owing to small sample sizes, it should increase the number of the study samples to get precise information. It is known that TFs, regulate gene expression by identifying and combining gene promoter cis-regulatory elements. Web-based TFSEARCH (version1.3) was used to analyze the TFs binding. Software analysis showed -1888T>G, -1819T>C and -441C>T affected the transcriptional factor binding, leading to conversions from C/EBP binding site to none, from Oct-1, CdxA to Nkx-2 and from CP2 to none (Fig. 2). Further investigations are needed to confirm the impact

on the gene expression. Apart from the known functional variants *UGT1A9**1b, *1d, *1f, we conducted the conservative assessment of other variants by the web-based Protein BLAST software. Four of the missense mutations (P361L, N397H, P448L and Y483D) occurred in highly conserved among three different species. The result indicates that these mutations may give rise to significant effects on the function of *UGT1A9*. In order to determine the effects, further functional researches should be done.

Glucuronidation, catalyzed by UDP-Glucuronosyl Transferases (UGTs), is one of the critical steps in the detoxification and elimination of various endogenous and exogenous compounds. Some of the polymorphisms of UGTs isoforms are known to affect glucuronidation rates (Radominska *et al*., 1999; Tukey and Strassburg, 2001). Numerous studies of functional characterizations of *UGT1A9* polymorphisms which may be associated with altered metabolism/pharmacokinetics of certain drugs have been conducted. 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 shown that *UGT1A9**1b with impaired enzyme function have the major effect on the SN-38 detoxification (Gagné *et al*., 2002; Cecchin *et al*., 2009). *UGT1A9* enzyme variant M33T, heterologously expressed in HEK293 cells, showed 1.7-fold reduced intrinsic clearance for mycophenolic acid 7-O-glucuronide (Bernard and Guillemette, 2004) and 26-fold reduced intrinsic clearance for SN-38 glucuronide (Villeneuve *et al*., 2003). Another *UGT1A9* enzyme variant D256N, showed 22-fold reduced intrinsic clearance for SN-38 glucuronide (Jinno *et al*., 2003). Besides, -118$(dT)_9$, one of the *UGT1A9* polymorphisms in the promoter region increased tumor response. -118$(dT)_{9/9}$ genotype was significantly associated with efficacious tumor response when compared with all other genotypes and also associated with a lower incidence of toxicity, whereas the -118$(dT)_{10/10}$ genotype predicted for poor tumor response (Carlini *et al*., 2005). Thus, these data have important public health implications. Clinical testing for the *UGT1A9* polymorphisms should be implemented as predictors of toxicity/effectiveness of some drugs. In this regard, systematically studying the polymorphisms of *UGT1A9* might play an important role in the prediction of toxicity and responsiveness to cytotoxic agents, as described for other detoxifying enzymes (Martino *et al*., 2011).

CONCLUSION

In conclusion, our results showed that there are ethnic differences in polymorphic and prevailing mutations of *UGT1A9* in Chinese Tibetan and Han populations. The predicted potential variants 96292C>T (P361L), 96399A>C (N397H), 100375C>T (P448L), 100479T>G (Y483D) may be functional variants. Our data may serve as a baseline for large samples studies on pharmacogenomics and also provide important data for the advance of personalized medicine in Chinese Han and Tibetan populations.

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