Investigation of the major cytochrome *P450 1A2* genetic variant in a healthy Tibetan population in China

YONGCHAO REN[1-3*], FANG LIU[4*], XUGANG SHI[5,6], TINGTING GENG[3,7], DONGYA YUAN[5,6], LI WANG[5,6], LONGLI KANG[5,6], TIANBO JIN[2,3,5,6] and CHAO CHEN[2,3]

[1]Qiannan Institute for Food and Drug Control, Duyun, Guizhou 558000; [2]School of Life Sciences, Northwest University; [3]National Engineering Research Center for Miniaturized Detection Systems; [4]The Reproductive Centre, Tangdu Hospital, the Fourth Military Medical University, Xi'an, Shaanxi 710069; [5]Key Laboratory of High Altitude Environment and Genes Related to Diseases of the Tibet Autonomous Region, School of Medicine, Xizang Minzu University; [6]Key Laboratory for Basic Life Science Research of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang, Shaanxi 712082; [7]First Affiliated Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, P.R. China

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Abstract. The cytochrome *P450 (CYP) 1A2* gene is involved in the metabolism of several carcinogens and clinically important drugs, generating a high potential for pharmacokinetic interactions. Since no data are available for Tibetan aborigines, the present study aimed to investigate the distribution of variant *CYP1A2* alleles in a population living in Tibetan region of China. Genotyping analyses of *CYP1A2* were conducted in 96 unrelated, healthy volunteers of Tibetan ancestry using direct sequencing assays. A total of 14 different *CYP1A2* polymorphisms, including two novel variants (1690G>A and 2896C>T) in the intron region and a novel non-synonymous one (795G>C, Gln265His) were detected. *CYP1A2*1A* (6.77%), *CYP1A2*1B* (58.33%) and *CYP1A2*1F* (14.58%) were the most frequent defective alleles identified in the sample. The frequencies of the prevalent genotypes *CYP1A2*1A/*1B*, **1B/*1B*, **1B/*1F* were 13.54%, 16.67% and 29.17%, respectively. In addition, the novel non-synonymous variant 795G>C (Gln265His) was predicted to be benign by PolyPhen-2 and SIFT tools. The present study provides useful information on the pattern of *CYP1A2* polymorphisms in Chinese Tibetan population. The current results may have potential benefits for the development of personalized medicine in the Tibetan population.

Correspondence to: Dr Tianbo Jin or Dr Chao Chen, School of Life Sciences, Northwest University, 229 North Taibai Road, Xi'an, Shaanxi 710069, P.R. China
E-mail: tianbojinprofessor@163.com
E-mail: chaochen898@126.com

*Contributed equally

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Introduction

Interethnic differences in drug-metabolizing enzyme activity have been associated with inter-individual differences in the efficacy and toxicity of many medications (1). Among drug-metabolizing enzymes, the cytochrome *P450 (CYP)*, a supergene family involved in the phase I reactions of the metabolism of several drugs and endogenous compounds, has increasingly been recognized to have clinically significant consequences (2). Cytochrome *P450 1A2 (CYP1A2)*, one of the *CYP* enzyme isoforms, is of particular interest because it exhibits a genetic polymorphism.

CYP1A2, mapped to the positive strand of the long arm of chromosome 15 at 15q24.1, is predominantly expressed in the human liver and at lower levels in intestine, pancreas, lung and brain (3). The human *CYP1A2* enzyme has been demonstrated to be responsible for many commonly used drugs, including caffeine, imipramine, paracetamol, clozapine, theophylline, tacrine, phenacetin and some neurotoxins (4). In addition, *CYP1A2* is known to gain further importance in the metabolic activation of numerous carcinogens (5). Therefore, any alteration to *CYP1A2* activity has been suggested to be a susceptibility factor for drug metabolism and the etiology of developing cancers and other diseases (6).

Like other drug metabolizing enzymes, numerous factors have been presented to elucidate the mechanisms underlying the inter-individual differences in *CYP1A2* activity, such as race, gender, environmental exposure to inducers or inhibitors and genetic factors (7). With respect to genetic factors, several alleles and additional haplotype variants have been identified in coding and non-coding regions of the *CYP1A2* gene, in particular in the *CYP1A2* upstream sequence and the intron 1 region (*CYP* allele nomenclature website at http://www.cypalleles.ki.se/). The frequencies of these polymorphisms display interethnic variability particularly between those of European and East Asian ancestry (8).

Tibet, as a part of China, contains a large number of high altitude populations that have a distinctive suite of

physiological traits that enable them to tolerate environmental hypoxia. Because few data are available on the investigation of the *CYP1A2* genotype in the Tibetan population, the aim of the present study was to determine the *CYP1A2* genotype profile of a random Tibet population by screening for the main allelic variants and compare to the allelic frequencies of those previously reported from other ethnic groups. It is hoped that the results will prospectively offer a preliminary basis for more rational usage of drugs that are substrates for *CYP1A2*.

Materials and methods

Subjects and DNA extraction. A total of 96 unrelated Chinese healthy volunteers (48 males and 48 females) of Tibetan origin, mostly students or employees at Xizang Minzu University (Xi'an, China), were enrolled in the study. All of the individuals lived in the same region at the time of the study and were of Tibetan ancestry without any known ancestry from other ethnicities. The study protocol was approved by The Human Research Committee of Xizang Minzu University (Xi'an, China), and each volunteer gave written informed consent to participate in the study. Peripheral blood samples were collected and stored after centrifugation at -70˚C until analysis, and genomic DNA was isolated and purified using a commercial blood Genomic DNA extraction kit (Xi'an GoldMag Nanobiotech Co., Ltd., Xi'an, China) according to the manufacturer's recommendations.

Polymerase chain reaction (PCR) and DNA sequencing. The primer pairs designed to amplify the 5' flanking regions, all exons and all introns of the *CYP1A2* gene are listed in Table I. The PCR was conducted in a total volume of 10 μl consisting of 1 μl genomic DNA (20 ng/μl), 0.5 μl each primer pair (5 μM), 5 μl HotStart TaqMasterMix (Qiagen China Co., Ltd., Shanghai, China), and 3 μl deionized water. PCR amplification consisted of an initial denaturation step at 95˚C for 15 min followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 55-64˚C for 30 sec, extension at 72˚C for 1 min. The final extension step was performed at 72˚C for 3 min. The PCR products were purified and sequenced on an ABI Prism 3100 sequencer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA) using a BigDye Terminator Cycle Sequencing kit (version, 3.1; Applied Biosystems; Thermo Fisher Scientific, Inc.).

Statistical analysis. The sequences were edited and assembled using Sequencher software (version, 4.10.1; Gene Codes Corporation, Ann Arbor, MI, USA). Allele nomenclature was assigned according to the Human Cytochrome *P450 (CYP)* Allele Nomenclature Committee (http://www.cypalleles.ki.se/). Differences in allele frequencies between Tibet and other ethnic populations were measured by Fisher exact test. P<0.05 was considered to indicate a statistically significant difference. The observed genotype frequencies of *CYP1A2* were also estimated by the Hardy Weinberg law for the predicted frequencies. The linkage equilibrium (LD) coefficient (D') between each genetic variant was analyzed by Haploview software (version, 4.1; Daley Lab at the Broad Institute, Cambridge, MA, USA).

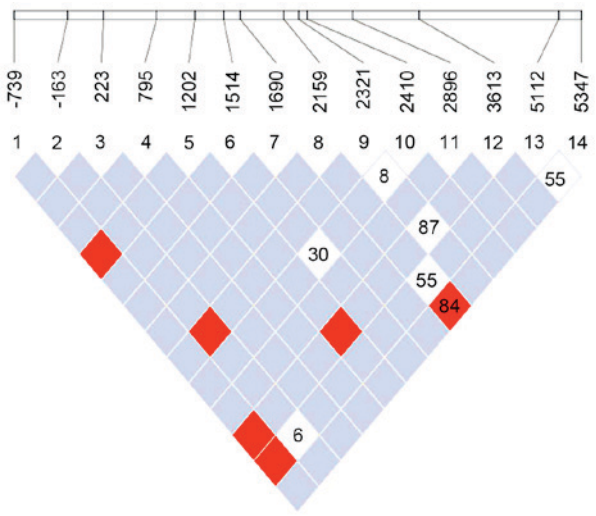

Figure 1. Linkage disequilibrium analysis of CYP1A2. LD is displayed by standard color schemes, with bright red for very strong LD (LOD >2, D'=1), pink red (LOD >2, D'<1) and blue (LOD <2, D'=1) for intermediate LD, and white (LOD <2, D'<1) for no LD. LD linkage equilibrium; LOD, logarithm of odds score; D', coefficient of linkage disequilibrium.

Protein prediction of novel mutations. PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/) and SIFT (http://blocks.fhcrc.org/sift/SIFT.html) software were performed to predict the effect of missense variants on the protein function. Based on the SIFT score, SIFT scores ≤0.05 were predicted by the algorithm to be evolutionary conservation and intolerance to substitution, whereas scores >0.05 were considered tolerant (not likely to affect protein function) (9). The PolyPhen-2 score ranges from 0 to 1, and PolyPhen-2 scores >0.85, between 0.85 and 0.15, and <0.15 were coded as 'probably damaging', 'possibly damaging' and 'benign', respectively (10).

Results

Single nucleotide polymorphism (SNP) discovery. In the current study, the authors used direct sequencing to analyze sequence variation within the *CYP1A2* gene among 96 healthy Tibetans. The analyses covered the proximal promoter region, all exons as well as surrounding intronic regions and variable lengths of the flanking regions. Table II presented all the *CYP1A2* mutation variations in this population. The most frequent polymorphism was the C-163A change in intron 1 which had 88.54% frequency, followed by G2321C change in intron 4 which had 37.5% frequency and T-739G change in intron 1 which had 20.83% frequency in the healthy group. Both 2159G>A and 5347C>T had similar results (13.5%), correspondingly. Additionally, among a total of 14 nucleotide variants detected, the authors detected three novel *CYP1A2* variants (795G>C, 1690G>A and 2896C>T) in exon 2 and intron 5 region with minor allele frequency of 1.04%, of which one variant (795G>C) resulted in an amino acid change from glutamine to histidine at position 265.

Allele & genotype frequencies. A total of eight different *CYP1A2* alleles and genotypes were determined based on the polymorphisms identified in the current study (Table III). Hardy-Weinberg equilibriums were assessed and all *CYP1A2*

Table I. Primers used for human CYP1A2 gene amplification.

Region	Primer sequence (5'-3')	Fragment size (bp)
CYP1A2_1_F	AATCGATATGGCAATCAAATGCAAA	740
CYP1A2_1_R	CCCGTCTTTCTGTCCCCACT	
CYP1A2_2_F	TAGGCTCCCTACCCTGAACC	919
CYP1A2_2_R	AACATGAACGCTGGCTCTCT	
CYP1A2_3_F	GTCACTGGGTAGGGGGAACT	896
CYP1A2_3_R	AAGGTGTTGAGGGCATTCTG	
CYP1A2_4_F	CTGGCACTGTCAAGGATGAG	909
CYP1A2_4_R	ATTGCAGGACTCTGCTAGGG	
CYP1A2_5_F	CAGGACTTTGACAAGGTGAGC	912
CYP1A2_5_R	CATAGCCCAGGCTCAAACC	
CYP1A2_6_F	CCTGTTCAAGCACAGCAAGA	903
CYP1A2_6_R	AACACAGAGGACAAGCAGAGC	
CYP1A2_7_F	CCTGTTATGTGCCTGCTGTG	899
CYP1A2_7_R	GGGGATTCAGGCCTCTTACT	
CYP1A2_8_F	TCCCAGTGCCCTCTGTGCCA	848
CYP1A2_8_R	GCCTTCCTGACTGCTGAACCTGC	
CYP1A2_9_F	AACAGCCAAGTGCGCAGCCA	881
CYP1A2_9_R	TCGCCTGAGGTACCCCACCT	
CYP1A2_10_F	AGGTGGGGTACCTCAGGCGA	930
CYP1A2_10_R	GAGGTGCCTGGGGGAGGGAG	
CYP1A2_11_F	TTTGGTTCCTTCCCACCTACCCTT	511
CYP1A2_11_R	GAAGAGAAACAAGGGCTGAGTCCCC	
CYP1A2_12_F	TGCTGTTTGGCATGGGCAAG	926
CYP1A2_12_R	TCTGGTGATGGTTGCACAATTC	
CYP1A2_13_F	AGAATTGTGCAACCATCACCAGAA	921
CYP1A2_13_R	CCAGTCTCAGGACTCAAGCACCA	

CYP, cytochrome P450.

allele and genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. The wild-type allele, *CYP1A2*1A*, with a frequency of 6.77%, was classified as normal enzyme activity. Besides the wild-type allele, *CYP1A2*1B* (58.33%) and *CYP1A2*1F* (14.58%) were the best-characterized defect alleles in the Chinese Tibetan population, of which *CYP1A2*1F* alleles were putatively linked to higher inducibility of the enzyme. *CYP1A2*1G*, *CYP1A2*1J*, *CYP1A2*1M*, *CYP1A2*13* and *CYP1A2*14* alleles have been included in the table, as these were the most scarce alleles in the study population. They occurred at a frequency of 1.56-5.21% in the current study population.

In relation to genotypes, the most frequent genotypes were **1A/*1B* (13.54%), **1B/*1B* (16.67%) and **1B/*1F* (29.17%) (Table III). All five other genotypes presented frequencies of <10.5% in the study. In addition, individuals with the **1B/*1B* genotype have been associated with a higher activity of the enzyme.

Interethnic variability. In order to better understand the occurrence and distributional patterns of the common mutation allele amongst different ethnic groups, the data were compared with those from previous investigations in different countries and ethnic groups in Caucasians, Africans, Arabs and Asians (Table IV). C-163A (88.54%) was most frequent among the Tibetan population, when compared with T-739 G (20.83%) and C5347T (13.54%). The allele frequency of C-163A and T-739G was significantly higher than that in Caucasians, Africans, Arabs and Asians, but allelic distributions of C-163A were relatively equal to that in Malays (78%), and T-739G was relatively similar to Tunisia (13.5%), Southern Chinese (9.3%) and Indians (10%). For C5347T, Tibetans demonstrated a relatively lower frequency of mutation compared with Caucasians (48-64.4%), but was similar to that in Africans (20.9%) and Asians (12.0-20.4%) with the only exception of South Asians (35%), which was significantly higher than Tibetans.

LD analysis. To identify relationships between the SNPs identified in the polymorphism screening, linkage disequilibrium (LD) analysis was evaluated in Haploview (http://www.broad.mit.edu/mpg/haploview/) using coefficient of linkage disequilibrium D' values (Fig. 1). Even though no distinct LD blocks or extended haplotypes could be detected in the sequenced data, some SNPs were identified (-739T>G and 1202C>T, -163C>A and 2321G>C, 1202C>T and 3613T>C, -739T>G and 3613T>C, -739T>G and 5112C>T) seemed to be linked with high D'.

Table II. CYP1A2 polymorphisms and their frequencies in a Chinese Tibetan population.

Polymorphism	Location	Flanking sequence	Minor allele	CYP nomenclature	Reference dbSNP	Amino acid translation	Predicted effect on protein structure/function using PolyPhen	Frequency (%)
-739T>G	Intron 1	GGTGTAGGGG K CCTGAGTTCC	G	CYP1A2*1E/*1G/*1J	rs2069526	/		20.83
-163C>A	Intron 1	CTCTGTGGGC M CAGGACGCAT	A	CYP1A2*1F/*1J/*1K	rs762551	/		88.54
223G>A	Exon 2	CTACGGGGAC R TCCTGCAGAT	A		rs150164960	Val75Ile	Benign	1.04
795G>C	Exon 2	GGTTCCTGCA S AAAACAGTCC	C	Novel	Novel	Gln265His	Benign	1.04
1202C>T	Intron 2	TTCACACTAA Y CTTTTCCTTC	T		rs4646425	/		9.38
1514G>A	Exon 3	TAGAGCCAGC R GCAACCTCAT	A	CYP1A2*13	rs35796837	Gly299Ser	Benign	3.13
1690G>A	Intron 3	ACAACATACT R AGATCTGGCT	A	Novel	Novel	/		1.04
2159G>A	Intron 4	GAAGCCTTGA R ACCCAGGTTG	A	CYP1A2*1M/*1Q/*17	rs2472304	/		13.54
2321G>C	Intron 4	TGGGGTATAA S AGGGGATAAT	C		rs3743484	/		37.50
2410G>A	Exon 5	AGGGAGCGGC R GCCCCGGCTC	A		rs55918015	Arg356Gln	Benign	4.17
2896C>T	Intron 5	AATGCCGACA Y GAGCTTCCTC	T	Novel	Novel	/		1.04
3613T>C	Intron 6	GAACTGTTTA Y ATAATGAAAG	C		rs4646427	/		9.38
5112C>T	Exon 7	GCCGATGGCA Y TGCCATTAAC	T	CYP1A2*14	rs45486893	Thr438Ile	Possibly damaging	9.38
5347C>T	Exon 7	TCTCCATCAA Y TGAAGAAGAC	T	CYP1A2*1B/*1G/*1H	rs2470890	Asn516=		13.54

CYP, cytochrome P450; dbSNP, The Single Nucleotide Polymorphism Database.

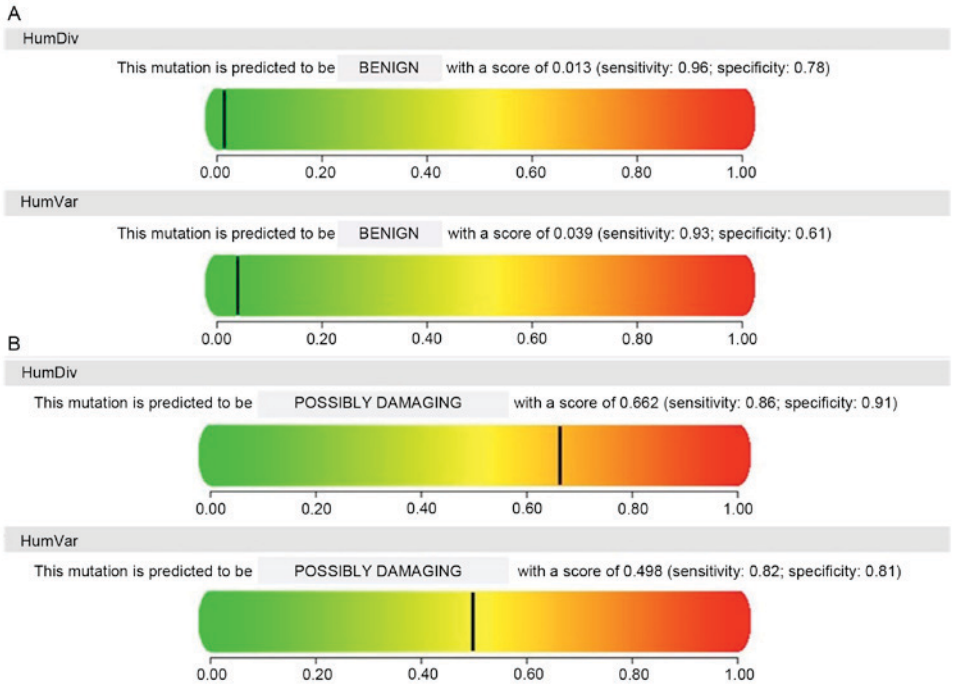

Figure 2. Protein prediction of the variants 795G>C (novel mutation) and 5112C>T using the PolyPhen-2 tool. (A) Prediction of the novel mutation 795G>C (B) Prediction of the variant 5112C>T.

Table III. Allele and genotype frequencies of CYP1A2 variants in Chinese Tibetan subjects.

Allele	Total (n=192)	Phenotype	Frequency (%)
*1A	13	Normal	6.771
*1B	112	/	58.333
*1F	28	Higher inducibility	14.583
*1G	10	/	5.208
*1J	10	/	5.208
*1M	7	/	3.646
*13	3	/	1.563
*14	9	/	4.688
Genotype	**Total (n=96)**	**Phenotype**	**Frequency (%)**
*1A/*1B	13	/	13.542
*1B/*1B	16	Higher activity	16.667
*1B/*1F	28	/	29.167
*1B/*1G	10	/	10.417
*1B/*1J	10	/	10.417
*1B/*1M	7	/	7.292
*1B/*13	3	/	3.125
*1B/*14	9	/	9.375

Protein function prediction of non-synonymous mutation. The SIFT scores for the amino acid substitutions Val75Ile (223G>A), Gln265His (novel variant 795G>C), Gly299Ser (1514G>A) and Arg356Gln (2410G>A), ranged between 0.07 and 0.72 and were predicted as being tolerated. In contrast, the Thr438Ile (5112C>T) mutations gave SIFT scores of 0.00, predicting they were highly likely to affect protein function. To validate the prediction of SIFT scores, the PolyPhen-2 algorithm was used to predict variations Val75Ile, Gln265His, Gly299Ser and Arg356Gln as benign with scores of 0.415, 0.039, 0.045 and 0.002, respectively, and Thr438Ile as possibly damaging, with a score of 0.281. Four substitutions (Gln265His, Gly299Ser, Arg356Gln and Thr438Ile) were consistently computationally predicted using both PolyPhen-2 and SIFT, while Val75Ile was not consistent. The protein function prediction of variants 5112C>T and 795G>C (novel variant) is presented Fig. 2 (PolyPhen-2).

Discussion

CYP1A2, one of the major P450 isoforms, accounts for ~5-20% of the total hepatic *CYP* content and contributes to the metabolism of 10% of clinically relevant drugs, including clozapine and caffeine (3). It has been demonstrated that *CYP1A2* activity has been influenced by the presence of polymorphic variants, which displays wide interindividual and interethnic variability. In the present study, the *CYP1A2* gene polymorphisms were systematically screened in 96 healthy Chinese Tibetan subjects. To the best of the authors' knowledge, these efforts are the first to investigate allelic variants of *CYP1A2* among the Tibetan population to date.

A total of 14 SNPs were detected in the current study. There were eight SNPs detected in the intron region. The -163 C>A (*1F/*1J/*1K/*1M allele) in intron 1 is the most common *CYP1A2* polymorphism in various population studies (Table IV). In Tibetans, -163C>A is the most frequently observed SNP, with an overall frequency of 88.54%, which is significantly higher than that in Caucasians, Africans, Arabs and Asians (except Malays). Possible explanations for these differences include: Genetic background, cultural variants and other factors, such

Table IV. Distribution of mutant allele frequencies of CYP1A2 -739T>G , -163C>A and 5347C>T in different ethnicities.

Ethnic group	Study population no.	-163C>A (*1F/*1J/*1K)	-739T>G (*1E/*1G/*1J)	5347C>T (*1B/*1H/*1G)	Reference
Tibetan	96	88.54	20.83	13.54	Present study
Caucasian					
British	65	66.2[b]	0.77[b]	ND	PMID: 12534642
Bulgarian	138	72.0[b]	ND	ND	PMID: 18021343
Caucasian	495	68.2[b]	1.6[b]	ND	PMID: 16307269
Caucasian	194	73.7[b]	4.1[b]	64.4[b]	PMID: 18231117
Caucasian	236	68.0[b]	ND	ND	PMID: 10233211
Costa Rican	932	60.0[b]	ND	ND	PMID: 15466009
European	166	69.0[b]	5.0[b]	48.0[b]	PMID: 22948892
German	150	68.0[b]	ND	ND	PMID: 21918647
Hawaiian	194	71.4[b]	ND	ND	PMID: 12925300
Hungarian	396	68.6[b]	ND	ND	PMID: 25461540
Italian	95	66.8[b]	ND	ND	PMID: 16188490
Roman	404	56.9[b]	ND	ND	PMID: 25461540
Serbian	262-264	61.1[b]	3.4[b]	ND	PMID: 20390257
Swedish	194	71.4[b]	2.3[b]	ND	PMID: 17370067
Swedish	1170	71.0[b]	ND	ND	PMID: 12445029
Spanish	117	2.0[b]	2.0[b]	ND	PMID: 12920202
Swiss	100	68.0[b]	ND	ND	PMID: 12851801
Turkish	101	73.2[b]	1.0[b]	ND	PMID: 20797314
Turkish	110	73.0[b]	1.0[b]	ND	PMID: 18825963
Turkish	146	66.8[b]	4.8[b]	49.7[b]	PMID: 19450128
African					
Ethiopia	173	60.0[b]	10.0[a]	ND	PMID: 12920202
Ethiopia	50-391	51.3[b]	6.6[a]	20.9	PMID: 20881513 a genomic biography of the gene behind the human drug-metabolizing enzyme
Tanzanian	71	49.0[b]	ND	ND	PMID: 15387446
Tunisia	98	44.0[b]	13.5	ND	PMID: 19332078
Tunisia	27	59.3[b]	ND	ND	PMID: 25921178
South African	983	61.0[b]	ND	ND	PMID: 22118051
Ovambo	177	46.0[b]	ND	ND	PMID: 16933202
Zimbabwean	143	57.0[b]	ND	ND	PMID: 15387446
Arab					
Egyptian	212	68.0[b]	3.0[b]	ND	PMID: 12630986
Saudi Arabian	136	10.0[b]	10.0[a]	ND	PMID: 12920202
Jordanian	550-560	67.3[b]	6.0[b]	ND	PMID: 22426036
Asian					
Zhejiang	43	57.0[b]	ND	ND	PMID: 25117321
Chinese					
Chinese	38-42	71.0[a]	4.0[a]	12.0	PMID: 20930417
Chinese	168	67.0[b]	ND	ND	PMID: 11470995
Chinese	79	66.0[b]	ND	ND	PMID: 12445035
Chinese	200	69.3[b]	10.4[a]	15.3	PMID: 18231117
South	27	70.4[a]	9.3	20.4	PMID: 16153396
Chinese					
Taiwan	204-208	35.0[b]	9.7[b]	14.0	PMID: 21121774
Indians	41-42	58.0[b]	10.0	12.0	PMID: 20930417

Table IV. Continued.

Ethnic group	Study population no.	-163C>A (*1F/*1J/*1K)	-739T>G (*1E/*1G/*1J)	5347C>T (*1B/*1H/*1G)	Reference
Malays	38-42	78.0	7.0[a]	18.0	PMID: 20930417
Mongolian	153	21.2[b]	ND	ND	PMID: 16933202
Japanese	160	70.0[b]	1.9[b]	18.7	PMID: 18231117
Japanese	250	62.8[b]	3.2[b]	19.2	PMID: 15770072
Japanese	159	61.3[b]	8.2[b]	ND	PMID: 10551315
Korean	150	62.7[b]	2.7[b]	ND	PMID: 17370067
Korean	1015	62.5[b]	ND	ND	PMID: 19579025
Korean	250	31.6[b]	ND	ND	PMID: 16933202
Korean	160-186	66.1[b]	5.4[b]	18.3	PMID: 18231117
South Asian	166	38.0[b]	6.0[b]	35.0[b]	PMID: 22948892

ND, not determined. [a]P<0.05 vs. the Tibetan population; [b]P<0.01 vs. the Tibetan population.

as living environment, medication use, body composition and dietary habits (11,12). In addition, much confusion and controversy still arises as to the available data in literature about the functional consequences and allele frequencies of *CYP1A2* variants, mainly because of limitation of sample size and the differing designations of the *CYP1A2*1F* allele (defined as having-163A by The HumanCytochrome P450 Allele Nomenclature Committee). Sachse *et al* (4) first reported that smokers homozygous for the *C*-allele had, on average, 40% lower CYP1A2 activity in comparison with those with the *A/A* genotype. In contrast, some inconsistent studies have reported that *CYP1A2 *1F* mutation was associated with a high inducibility of *CYP1A2* in smokers as well as in nonsmokers (13). It is tempting to speculate the divergence may be the possibility of the -163C>A occurring in linkage disequilibrium with another mutation that is responsible for the increased *CYP1A2* inducibility (14). The present study identified a strong linkage disequilibrium between -163C>A and 2321G>C polymorphisms (Fig. 1), providing researchers in the field with abundant clues, however, more studies are required to shed more light on this idea. Another most prevalent polymorphism in intron 1 region, -739T>G, was first reported in in a Japanese population (5). -739T>G is located on the *CYP1A2*1E, *1G, *1J* or **1K* allele, and previous research demonstrated that this polymorphism has no effect on the enzyme activity (6). -739T>G is the most common variant among Asians and the frequency of 20.83% found in the present study is significantly higher than other Asians (Table IV), Caucasians (0.77-5%) (6,8), Africans (6.6-13.5%) (15,16) and Arabs studied elsewhere (3-10%) (17,18). Interethnic differences in the prevalence of -739T>G may be one of the major factors to consider in large pharmacogenetic studies and clinical applications in populations of Asian ancestry, such as Chinese Tibetans, since the proportion of high expressers due to the presence of -739T>G varies depending on the ethnic background. Among the six SNPs identified in the exons, the synonymous 5347T>G (**1B/*1G/*1H*), was the most common variant among Caucasians and the frequency of 13.54% identified in the present study presented a frequency significantly lower than Caucasians, but it was quite similar to Asians (except South Asians) (Table IV). This may be because these populations are distributed in different geographical regions, which may result in the formation of numerous, small, genetically isolated groups.

In the tested Chinese Tibetan population, *CYP1A2*1A* is referred to as the wild-type allele with a frequency of 6.77%, which is significantly less when compared with Swedes (24.4%), Koreans (21.7%), Japanese (34.8%), Caucasians (33.4%) and Serbs (33.4%) (19-21). The occurrence of the most prevalent defective alleles, *CYP1A2*1B* (5347T>G), evaluated in Chinese Tibetan subjects (58.3%) in the present study is slightly lower compared to the occurrence reported in Caucasians (61.8%), but is higher than other Chinese population (20.4%) (22). However, the genotype frequencies observed for **1B, *1B* in Tibetans (16.67%) was slightly higher than that in Caucasian (6.19%), Japanese (7.5%), Korean (10.75%) and other Chinese population (9%). Currently, only Chen *et al* (22) reported that CYP1A2*1B homozygotes demonstrated marginally higher CYP1A2 activity, when compared with *CYP1A2*1A/*1A* homozygotes (22). Because the **1B, *1B* genotype is relatively common in Chinese Tibetan subjects, this genotype may have a major influence in altered *CYP1A2* activity, of course, this requires further investigation. *CYP1A2* 1F* resulted from a C>A substitution at -163 in intron 1 of the promoter region. The haplotype *1F allele is common with high and comparable frequencies in various studies. However, the frequencies of *CYP1A2*1F* (-163A allele) in Tibetans is 14.58%, which was far less frequent compared with Caucasians (73.7%) (23), Africans (61%) (24), Arabs (68%) (17) and Asians (69.3%) (23). Since *CYP1A2*1F* is reported to be associated with an effect on enzyme inducibility, the estimates of their frequencies in the Tibetan population may be of extreme importance. Compared with the alleles *CYP1A2*1B* and *CYP1A2*1F, *1G, *1J, *1M, *13* and **14* are relatively rare in Tibetans, thus the clinical applicability of this pharmacogenetic testing seems to be limited to a small number of individuals. In addition, the-163C>A variant is present in the CYP1A2*1F allele, but it is also presented in several other CYP1A2 haplotypes, two

of which (*CYP1A2***1J* and **1M*) were identified in the sample population. Therefore, it is informative to take the complete haplotypes into consideration when investigating associations of phenotype rather than focusing on single SNPs.

After systematically screening the polymorphisms of the *CYP1A2* gene in the healthy population of Chinese Tibetan subjects, three novel variants were detected that included one nonsynonymous change at position G795C in exon 2. These variants are rare but not absent, occurring in <1.04% of the population, but the current study is the first to report these variants in Chinese Tibetan subjects. Although the c.795 G>C variation is predicted to not have an affect on protein function by the SIFT or PolyPhen algorithms, further functional studies are still necessary to clarify the role of their clinical significance.

It should be acknowledged that the current research was designed to investigate the unique distribution of the *CYP1A2* alleles in the Tibetan population. The characterization of *CYP1A2* genetic polymorphisms among different races may contribute to the outcome and risks to certain drug therapies.

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