Int J Clin Exp Pathol 2016;9(3):3717-3725
www.ijcep.com /ISSN:1936-2625/IJCEP0020587

Original Article

Genetic analysis of drug-metabolizing enzyme CYP3A5 polymorphisms in Tibetans in China

Tianbo Jin[1,2,3,4*], Pengfei Zhang[5*], Xugang Shi[3], Yongri Ouyang[3], Na He[1,2], Xue He[1,2], Li Wang[1,2], Dongya Yuan[1,2], Longli Kang[1,2]

[1]Key Laboratory of High Altitude Environment and Genes Related to Diseases of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang, China; [2]Key Laboratory for Basic Life Science Research of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang, Shaanxi, China; [3]National Engineering Research Center for Miniaturized Detection Systems, Xi'an, China; [4]School of Life Sciences, Northwest University, Xi'an, Shaanxi, China; [5]Department of Otorhinolaryngology, School of Medicine, The First Affiliated Hospital of Xi'an Jiaotong University, Xi'an, China. [*]Co-first authors.

Received November 26, 2015; Accepted January 27, 2016; Epub March 1, 2016; Published March 15, 2016

Abstract: Background: Different single nucleotide polymorphisms (SNPs) in gene may lead to different drug metabolic activity, and different SNPs may also be associated with genetic predisposition of certain diseases. *CYP3A5*, as one of the most active gene in CYP450 3A superfamily, has become a hot topic in medicine. Since the research about the genetic diversity of *CYP3A5* in Tibetans is rare, we carried the study to explore the genetic polymorphisms of it in one Tibetan population who living in Tibetan. Methods: DNA sequencing the promoter, exons and surrounding introns, and 3'-untranslated region of the *CYP3A5*1* gene in 96 healthy, unrelated volunteers of Tibetan by Direct Sequencing approach. Results: We finally identified 23 different *CYP3A5* polymorphisms, including three novel mutants in promoter, one novel nonsynonymous variant (C27122A) and four in the intron region. We found the allele frequencies of CYP3A5*1A, CYP3A5*1C, CYP3A5*1E and CYP3A5*3A were 0.328, 0.52, 0.182 and 0.484, respectively. Tfsitscan analyzed that some mutants leads the density of transcription factor binding varied in the different promoter regions. Conclusion: Our results obtain basic information of *CPY3A5* alleles in Tibetans and suggest that the diverse ethnic populations may have different enzymatic activities of CYP3A5.

Keywords: *CPY3A5*, genetic polymorphism, haplotype, tibetan, ethnic difference

Introduction

Cytochrome P450 (CYP) enzymes is one of the most important metabolizing enzyme system in humans, and play a central part of the metabolism of many compounds, specially the biotransformation of clinical medicine [1, 2]. Human CYPs contains 18 cytochrome gene families, involved in most drug metabolism is CYP1, CYP2, CYP3 three enzyme families, among them CYP3A subfamily member which is particularly prominent role, the abundant expressed and large substrate spectrum [3], is the most important drug metabolic reaction rate-limiting enzyme.

CYP3A5 gene is one of the four *CYP3A* genes which located on chromosome 7q22.1, the full-length of DNA sequence is 31.8 kb, contains 13exons, expressing about 502 amino-acids protein. CYP3A5 at 10% to 97% of the population expressed [4], in the crowd expressed their activity accounted for 2% to 60% of total activity in vivo CYP3A, and polymorphic expression, is considered the most important factor of the differences between individual CYP3A activity (a difference of about 10 to 40 times) [5]. CYP3A5 activity difference mainly is single nucleotide polymorphisms caused to shear abnormal and a truncated protein [5, 6], and finally caused to the different degrees alteration of metabolic corresponding substrate capacity and rate. The mutant of multiple allelic about CYP3A5 have been identified so far, CYP3A5*3 is the highest mutation frequency allele in all races among them, and different ethnic has the different mutation frequency. CYP3A5*3 is the A6986G allele mutation present in the third intron of *CYP3A5* gene, which is related with the expressed capacity and enzyme activity of

Table 1. Primers used for human CYP3A5 gene amplification

Region	Primers (5'>3')	Fragment size (bp)
Promoter_F	ACCCAAGTACTGGGAGCACA	926
Promoter_R	TAGCTGCAGCCAATAGCAAA	
Exon1_F	GCAGGTGAGAGGAGGGTTAAT	883
Exon1_R	CCAGGTCTACAGGGGCAATA	
Exon2_F	CCCAGTGACCCTCTGTGATT	916
Exon2_R	GGTGCGATCACAGCTCAATA	
Exon3_F	TGAAAAGGATACTGATGGAGGA	891
Exon3_R	ACAAAAACCACAACTTTGCAC	
Exon4_F	TTTCACTGACCTAATATTCTTTTTGA	925
Exon4_R	GCGATTCTCCTGTCTCAGACTAC	
Exon5_6_F	TAGAGGCAGGAGACCACACC	922
Exon5_6_R	GTAAATCCTGGCTCCTGTGC	
Exon7_F	GCCACCTTTCTTGAATCCAC	923
Exon7_R	TGAAACCTAAAGCCAGCAAAA	
Exon8_F	GGAAGTTGATGTACATATGCTGTG	904
Exon8_R	TCTTGGACTTAAAATGATTCTTTACC	
Exon9_F	GAAAATGCTCCCCCAAAAA	913
Exon9_R	CCTTGCGAACACACTTTCAA	
Exon10_F	GGGAAAAAGCCTACCCCATA	837
Exon10_R	TCTTCTCGCCACACTCCTTT	
Exon11_F	TTTGGTGGTAATGGTCATAGC	889
Exon11_R	AGGTCAGTGGATAGTTTCCTGTT	
Exon12_F	TGAGGCAGGAATCCACTTTT	875
Exon12_R	TTCTGCAGGTTCTGGTGATG	
Exon13_F	ATCCATGGCAATTTGCTTTC	905
Exon13_R	GCCCATCTTTATTTCAAGGTTTT	

CYP3A5 [7]. CYP3A5*3 genotype results in mRNA splicing changes, so that a premature termination codon and protein truncation, leading CYP3A5 activity decreased even disappeared, which is the most important gene polymorphisms of *CYP3A5* [8].

Han Chinese and 55 ethnic minorities which recognized by the People's Republic of China consists of the population of China. As the ninth-largest minorities in China, the Tibetans, with a 6282,000 population broad scattered in the Qinghai-Tibet Plateau and mainly live in the Tibet Autonomous Region (according the Chinese population survey 2010). Tibetan adaptation to the special environment of the plateau which average altitude of 4,000-5,000 meters, especially the significant hypoxia at this situation (60% of the oxygen concentration at sea level) [9]. The special language, culture and customs preserved throughout the ethnic history [10]. The distinctive living conditions and geographical environment might be expected a unique genetic polymorphisms.

We systematically screen 96 healthy, unrelated Tibetans to find out the more information for regarding CYP3A5 polymorphiams. And then compare the allelic frequencies with other previous observed ethnic populations. We hope to find corresponding CYP3A5 genotypes, phenotypes and the most optimal drug, offer a potential database for personalized medicine for the Tibetans.

Materials and methods

Sample collection

Ninety-six healthy, unrelated Tibetans (48 males and 48males) were randomly recruited between October and December 2009 from Xizang Minzu University. Participants were determined to be healthy by their medical history and a physical examination. We explained the purpose and experimental procedures of our research to them. All participants had at least three generations of Tibetan paternal ancestry, ensured no individuals share common ancestry by interview and provided the written informed consent before the sample collection and the subsequent analysis. The study pact was signed in accordance with the Declaration of Helsinki and was assented by The Ethics Committees of Xizang Minzu University.

DNA sequencing of CYP3A5 variants

We screened the CYP3A5 genetic polymorphisms of Tibetan population by DNA direct sequencing. Genomic DNA was extracted from whole peripheral blood using a GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Ltd., Xi'an, China) according to the manufacturer's recommendations, and NanoDrop 2000C (Thermo Scientific, Waltham, MA) was used to measure the concentration of DNA. **Table 1** showed the primers of PCR (Polymerase Chain Reaction) were designed using primer-Blast (http://frodo.wi.mit.edu/primer3/) to amplify the promoter, all exons and 3'-untranslated region of *CYP3A5* gene. PCR system in total 10 μl volume was consisted of 1 μl genomic DNA (20 ng/μl), 5 μl Hot Star Taq Master Mix, 0.5 μl each primer pair (5 μM), and 3 μl deionized water. PCR amplification cycling conditions consisted of an initial denaturation

Table 2. Basic information of CYP3A5 variants which detected in Tibetan populations

Region	Position	Nucleotide change	Allele	SNP	Frequencies	Flanking Sequence	Amino-acid effect
Promoter	-833	T>C	Novel	/	1.04%	TGGGACACAG Y GTGGCTGCAT	No translated
Promoter	-795	T>A		rs3823812	35.42%	ATTAGTCTAT W GCTATCACCA	No translated
Promoter	-418	G>R(A)	Novel	/	1.04%	CCAGGCAGAG R GAATTTCGTG	No translated
Promoter	-74	C>Y(T)	CYP3A5*1C	rs28371764	1.04%	AAGCTCCAGG Y AAACAGCCCA	No translated
Promoter	-67	C>Y(T)	Novel	/	1.04%	AGGCAAACAG Y CCAGCAAACA	No translated
Intron 1	205	C>Y(T)		rs375494568	1.04%	ACGCTACAAA Y GCATAGAAGT	No translated
Intron 1	5210	C>Y(T)		rs28365067	4.17%	TGAAAGATAG Y AGGCCTGATT	No translated
Intron 3	5643	A>R(G)	Novel	/	1.04%	GTTTTGAGAT R ATTATTGTTA	No translated
Intron 3	6981/6986*	A>R(G)	G(CYP3A5*3A) A(1A)	rs776746	96.88%	TTGTCTTTCA R TATCTCTTCC	Splicing effect
Intron 5	13023	G>R(A)		rs538264311	1.04%	ATCACAGTCC R TTTCCAAGGG	No translated
Intron 6	13364	G>R(A)		rs564026994	1.04%	CCATGGAGTC R ACAGTCGCAC	No translated
Intron 6	14381	A>M(C)		rs148149634	3.13%	TGTCTCCATC M CCCCCAGCAT	No translated
Intron 7	14948	C>T		rs2040992	100.00%	ACAAACCCCA T TGCCCTAAGC	No translated
Intron 8	16988	C>S(G)		rs58708491	1.04%	CACTTTCTGC S AAAGAAATCT	No translated
Intron 9	17158/17163*	G>K(T)	CYP3A5*1E	rs4646453	36.46%	TGTGCAGGAA K TATTCCAGGA	No translated
Intron 9	19064	G>R(A)		rs41303334	1.04%	ATGATTTTGC R TCATCCTGGC	No translated
Exon 11	27122	C>M(A)	Novel	/	1.04%	TTCCAGGCAC M ACCTACCTAT	P231Q
Intron 11	27505	A>R(G)	Novel	/	1.04%	CAAACCACAG R CTAGAAAAAA	No translated
Intron 11	27521	C>Y(T)		rs6976017	4.17%	AAAAACGAAA Y TACATCCATC	No translated
Intron 12	30032	G>R(A)	Novel	/	1.04%	ACACAGATTA R CATGACATGA	No translated
3'-UTR	31606/31611*	C>Y(T)	T(CYP3A5*3A) C(1A)	rs15524	96.88%	ATTCTAAGGA Y TTCTACTTTG	Splicing defect
3'-UTR	31900	A>R(G)		rs534819182	1.04%	TCATCAGAGA R TAAATATTTC	No translated
3'-UTR	32245	T>Y(C)	Novel	/	36.46%	ATTCATAGTT Y CATTCTGCCT	No translated

*indicated the first digit before "/" is according the reference sequence NG_007938.1, another figure is corresponding NG_000004.3 (old version sequence).

at 95°C for 15 min, followed by 35 cycles of 95°C for 30 s, 55-64°C for 30 s, 72°C for 1 min, and a final extension step at 72°C for 3 min. We sequenced the purified PCR products on an ABI Prism 3100 sequencer (Applied Biosystems Inc., Foster City, CA, USA) using the ABI Prism BigDye Terminator Cycle Sequencing Kit version 3.1.

Statistical analysis

Sequencher 4.10.1 software was performed to assemble and analyses the sequences of PCR products. Based on the nucleotide reference sequence CYP3A5--NG_007938.1 and the Human Cytochrome *P450 (CYP)* Allele Nomenclature Committee (http://www.cypalleles.ki.se/), we assigned the CYP3A5 variants we detected, and we divided the samples into three phenotypic groups: poor metabolizer (PM), extensive metabolizer (EM), and ultra-rapid metabolizer (UM) depending on the metabolic activity of *CYP3A5* [11]. Haploview software (version 4.2) was used to assess Hardy-Weinberg equilibrium and Linkage disequilibrium (LD) between loci pairs. Across all detected SNPs, we based on the LD analyses to select out haplotypes which were defined in accordance to the Gabriel definition (D' N 0.9; minimum allele frequency, 5%) [12]. Allele and genotype frequencies were calculated by the counting method. We using Chi-squared test with a significance level set at 0.05 to compare the allele frequencies difference among different geographic populations or other ethnic populations.

Transcriptional prediction

The online tool Tfsitescan (www.ifti.org/cgi-bin/ifti/Tfsitescan.pl) and the transcription factor binding sites database were used to analyses the potential effects of promoter-region variants on transcription [13]. We analyzed the wild-type and allelic variants separately. We contrasting analyzed the wild-type and all allelic variants that we found on promoter-region to observe the alteration.

Functional prediction of nonsynonymous mutation

We used online tool SIFT (http://sift.bii.a-star.edu.sg/) and PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/) to analyses and predict the potential effect of nonsynonymous mutations in the exon-region variants on CYP3A5

Table 3. Allele and genotype frequencies of CYP3A5 mutants in Tibetans

Allele	Total (n=192)	Phenotype	Frequency (%)
CYP3A5*1A	63	Normal	0.328125
CYP3A5*1C	1		0.0052083
CYP3A5*1E	35		0.1822917
CYP3A5*3A	93	Decrease	0.484375
Genotype	Total (n=96)	Phenotype	Frequency (%)
*1A/*1A	22	Normal	0.2291667
*1A/*1C	1		0.0104167
*1A/*3A	18		0.1875
*1E/*3A	35		0.3645833
*3A/*3A	20	Decrease	0.2083333

protein function [14, 15]. The outcomes of SIFT prediction can be classified as fellow: tolerant (0.201-1.00), borderline (0.101-0.20), potentially intolerant (0.051-0.10) and intolerant (0-0.05). PolyPhen-2 divide the consequence in five categories: probably benign (0-0.999), borderline (1.000-1.249), potentially damaging (1.250-1.499), possibly damaging (1.500-1.999) and probably damaging (≥2).

Results

Genetic variants

From our results, we identified twenty-three different polymorphisms of *CYP3A5* in Tibetan population, include 1 non-synonymous mutation (C27122A) and 7 have not been previous reported were novel which showed in **Table 2**.

Allele and genotype frequency

In the Tibetan samples, we searched four *CYP3A5* alleles included: three subtype of the *CYP3A5* wild-type allele, *CYP3A5*1A*, *CYP3A5*1C* and *CYP3A5*1E*, found in 32.81%, 0.52% and 18.23% of the group, separately. We identified the other one common allele, *CYP3A5*3A*, comprised 48.44% of the variants.

Meanwhile, we detected five genotypes of *CYP3A5* in the Tibetans (**Table 3**). The wild-type *1A/*1A and *1A/*1C genotypes account for 22.92% and 1.04% of the samples which have normal enzyme activity. Other genotypes contained *1A/*3A, *1E/*3A and *3A/*3A (18.75%, 36.46% and 20.83%) leads to decreased enzyme activity. All alleles and genotypes frequencies accorded with Hardy-Weinberg equilibrium.

Inter-population comparisons

We compared CYP3A5 allele frequencies between Tibetans and other ethnic groups to better understand the distributional patterns. From the frequencies of CYP3A5*1, *3 and *6 in the Tibetans was different from the other different populations (**Table 4**). Three major alleles, the wild-type allele CYP3A5*1, the prevalent allele CYP3A5*3 and CYP3A5*6, were analyzed in those groups. We found that the allele frequency of CYP3A5*1 was significantly higher ($P<0.05$) in Tibetans compared with Japanese, Chinese Han and Caucasians. In contrast, CYP3A5*3 frequency was significantly lower ($P<0.05$) in Tibetans than in Caucasians, Japanese and Chinese Han. CYP3A5*6 allele frequency was not detect out except African-American, Africans and German.

Linkage disequilibrium analysis

We performed LD analysis to define the relationship between the SNPs by Haploview 4.2 using D' values (**Figure 1**). We typically ignored markers with minor allele frequencies (MAF) <0.05 which were almost no effect to detect LD. We found the LD blocks and extended haplotypes in the sequenced data. T32245C, C31606T, G17158T, A6981G and T-795A consisted of the big block, and between them obviously linked with high D' (D'≥0.96).

Genetic variants in the transcription factor binding site

In promoter region, we found five SNPs including -833T>C, -795T>A, -418G>A, -74C>T and -67C>T. Tfsitescan analyzed the loci in transcription factor binding domains and found the -833T>C and -67C>T mutation changed the original binding sites of transcription factors. Thus potentially affecting the binding proteins and resulting in the corresponding gene expression activity abnormal. Two new polymorphism mutants -74C>T, -795T>A and -418G>A have no effect on the binding of the transcription factors (**Table 5**).

Protein function prediction of non-synonymous mutation

The SIFT scores for the amino acid substitutions P231Q (Pro231Gln, C27122A) was 0.16 and was predicted as being borderline which not likely to alter protein function. We used

Table 4. Comparison of allele frequencies of CYP3A5*1, *3 and *6 in different ethnic populations

Ethnic group	Study population No. of alleles	Frequency			Reference
		*1	*3	*6	
Tibetan	192	0.5156	0.4844	0	Current study
Asian					
Chinese	216	0.25	0.75	0	[18]
Koreans	388	0.276	0.724	0	[20]
Japanese	530	0.26	0.74	0	[19]
Indian	180	0.41	0.59	0	[18]
Malays	196	0.39	0.61	0	[18]
Jordanian	458	0.072	0.9258	0.0022	[23]
Caucasians	54	0.15	0.85	0	[5]
Europe	190	0.06	0.94	0	[22]
German	1000	0.082	0.917	0.001	[25]
French	NR	NR	0.813	NR	[24]
African-Americans	40	0.45	0.48	0.08	[5]
African	190	0.72	0.12	0.16	[22]
Ghanaians	406	0.71	0.15	0.14	[27]
Brazilians	1818	0.21	0.73	0.04	[21]
White	616	0.15	0.82	0.02	[21]
Brown	592	0.26	0.68	0.05	[21]
Black	610	0.32	0.52	0.11	[21]

PolyPhen-2 to predict P231Q, the score was 0.225 in HumDiv and 0.109 in HumVar that means was benign (**Figure 2**). The protein function prediction of P231Q from SIFT and PolyPhen-2 analysis were consistent.

Discussion

The present study is the first to report the gene polymorphisms of *CYP3A5* in Tibetans of southwest China with 96 unrelated volunteers. We systematically screened the *CYP3A5* gene sequences by direct sequencing approach, used Sequencher 4.10.1 software to analysis the polymorphism distribution and checked the allele frequencies seriously. Twenty-three genetic variants were found out including eight novel polymorphisms. Three of them in the promoter region and leads to some alteration on the transcription factors binding. Only one novel variant within exon 11 results in a nonsynonymous mutation and makes no effect on protein function. Form the data, we observed the special polymorphisms distribution within Tibetans and a significant difference in frequency distribution among different ethnic through combined with other studies on research *CYP3A5* gene polymorphisms. In short, the data of present study provide a foundational theory for *CYP3A5* in Tibetans, and is benefit for the development of personalized medicine.

CYP3A5 shows significant polymorphisms in different ethnics. *CYP3A5*3* (A6986G) allele located in intron 3 caused a splicing alternation and resulted in a truncated protein with a decreased CYP3A5 expression [5]. The different frequencies of alleles in different groups suggested that the CYP3A5 protein expression activity exist difference [16]. CYP3A enzymes are the important metabolism proteins of 50% current drugs approved by the US FDA (food and drug administration) in the human liver [17]. The population expression content differences and widespread clinical drug metabolism of CYP3A5 may have significant consequence which could arise because of different genetic and environmental background [18], and hence need corresponding dosages of different drug to achieve the best therapeutic effects. In the present study, we found the most important allele CYP3A5*3 varied significantly in different ethnic which detected in 48.44% in Tibetans, 71-75% in East Asian populations (Chinese, Japanese and Koreans) [18-20], 55-65% in South Asian populations (Malay and Indians) [18], majority of Caucasians more than 90% [5, 21-25]. A person with a CYP3A5*1 allele is four times expression activity in total CYP3A compared with other people with CYP3A5*3 homozygous [5]. In short, we verified about 20.83% of CYP3A5*3/*3 genotype frequency in Tibetans related with decreased/disappeared enzyme activity, is significant lower than Chinese, Japanese and Caucasians, very close to African-American. When carried on the drug treatment among different populations, we recommended the dosage of corresponding drugs should be calculated according the different genotypes.

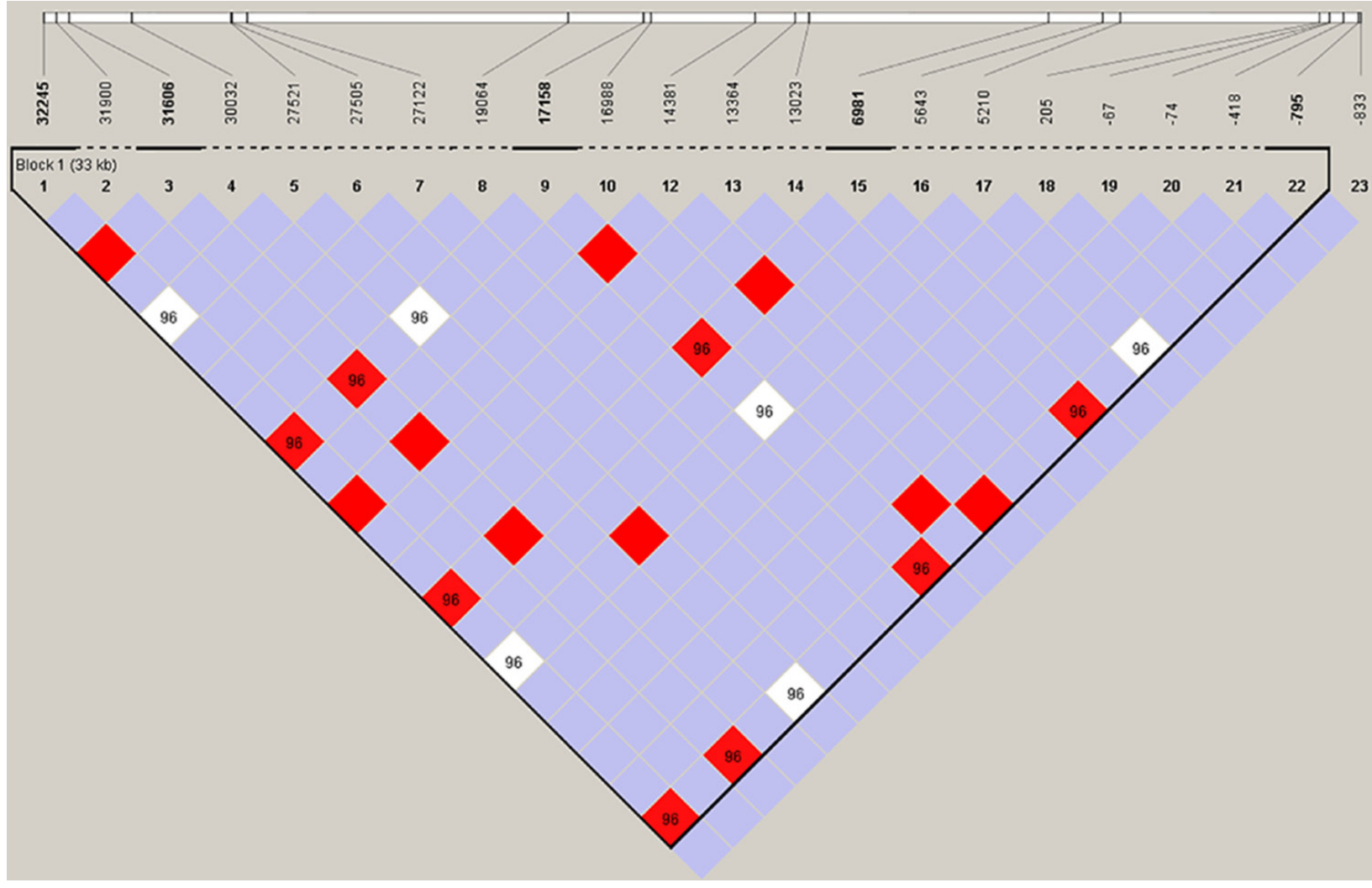

Figure 1. Linkage disequilibrium analysis of CYP3A5 in Tibetans. LD is displayed by standard color schemes with bright red for very strong LD (LOD42, D0 ¼ 1), pink red (LOD42, D051), blue (LOD52, D0 ¼ 1) for intermediate LD and white (LOD52, D051) for no LD.

Table 5. Results from Tfsitescan of CYP3A5 promoter region with the original and mutant sequence

Promoter SNPs	Original sequence					Mutant sequence				
	Site (Length)	Position	Score (Gaps)	Occur-rence	Exp Value	Site (Length)	Position	Score (Gaps)	Occur-rence	Exp Value
T-833C (256)						XRE_CS1'(6)	283	6 (0)	1	6.42E-01
						Rb-CAMLG'(7)	284	7 (0)	1	1.20E-01
C-67T (1023)	AP-2-alpha/gamm'(9)	1036	7 (0)	1	9.90E-01					
	AP2-Hmt2a2'(12)	1036	9 (0)	1	4.61E-03	HS-40_GATA1(d)'(11)	1040	8 (0)	1	1.69E-02
	HNF-3-EGFR'(7)	1043	7 (0)	1	1.20E-01	FREAC-2_CS2(7)	1043	7 (0)	1	2.26E-01
	HNF-3-rEGFR'(7)	1043	7 (0)	1	1.20E-01	FREAC_core_moti(7)	1043	7 (0)	1	4.01E-01
	HNF-5_CS'(7)	1043	7 (0)	1	4.01E-01	FoxO_CS(6)	1044	6 (0)	1	8.72E-01
	HNF-5_site(7)	1043	7 (0)	1	4.01E-01					
	57bp-URS-heptam(7)	1047	7 (0)	1	2.26E-01					

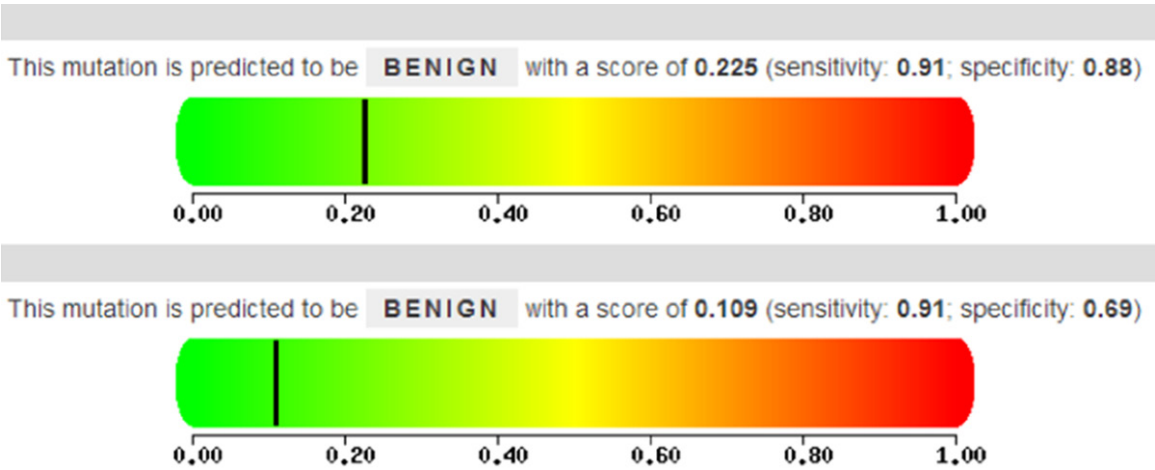

Figure 2. Protein prediction of the variants 27122C>A (novel mutation).

Haploview software calculated out a big block in the genomic structure of CYP3A5 in Tibetans. The haplotype on a block means to the tightly linked genetic markers on the chromosome area always inherited together [26]. The twenty-three SNPs of *CYP3A5* in Tibetans remained a weak linkage and only one block consisted of five SNPs. The situation indicated that the genetic mode of different variants is relatively independent. The weak linkage might be a reason why majority of the alleles in CYP3A5 don't alter the expression content of CYP3A5 in human.

We analyzed the all SNPs of promoter region, 833T>C and -67C>T changed some transcription binding site of promoter region. The -833T >C leading to two new binding sites XRE_CS1' AND Rb_CAMLG' appeared. -67C>T variant, leading to the loss of AP-2-alpha/gamm', AP2-Hmt2a2', HNF-3-EGFR', HNF-3-rEGFR', HNF-5_CS', HNF-5_site and 57bp-URS-heptam but appearance of several new sites: HS-40_GATA1(d)', FREAC-2_CS2, FREAC_core_moti, FoxO_CS and so on. The variants make great effect on the promoter region regulation process, and give some explains why the expression content of CYP3A5 differed different individuals.

The mutants in coding region predicted by SIFT and PolyPhen-2 showed that 27122C>A makes no effect on the function and construction of CYP3A5 protein. The prediction of the software based on some different database and a series of algorithms. The accuracy of the result is limited which needed to get a more reliable conclusion by combined with more experiments.

Conclusion

In summary, these findings provide some of the first information on the genetic polymorphisms of Tibetans. The results also confirm that there are important interethnic differences in the distribution of CYP3A5 variants, which may result

in major differences among different individual respond to drug treatment. The information should be favorable to the establishment of appropriate personalized treatment strategies for Tibetan people. Besides, Future studies will focus on identifying CYP3A5 variants in a larger sample size of Tibetans, thereby promoting the enhanced application of personalized medicine in Tibetans.

Acknowledgements

This work is supported by Natural Science Foundation of Xizang (Tibet) Autonomous Region (20152R-13-11) and Major Training Program of Tibet University for Nationalities (No. 13myZP06).

Disclosure of conflict of interest

None.

Address correspondence to: Dongya Yuan and Dr. Longli Kang, Key Laboratory of High Altitude Environment and Genes Related to Diseases of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang 712082, China; Key Laboratory for Basic Life Science Research of Tibet Autonomous Region, School of Medicine, Xizang Minzu University, Xianyang 712082, Shaanxi, China. Tel: +86-29-33755000; Fax: +86-29-33755000; E-mail: dyyuanxzmz@gmail.com (DYY); kanglongli6211@163.com (LLK)

References

[1] Rochat B. Role of Cytochrome P450 Activity in the Fate of Anticancer Agents and in Drug Resistance. Clin Pharmacokinet 2005; 44: 349-366.

[2] van der Weide J and Hinrichs JW. The influence of cytochrome P450 pharmacogenetics on disposition of common antidepressant and antipsychotic medications. Clin Biochem Rev 2006; 27: 17.

[3] Topletz AR, Dennison JB, Barbuch RJ, Hadden CE, Hall SD and Renbarger JL. The relative contributions of CYP3A4 and CYP3A5 to the metabolism of vinorelbine. Drug Metab Dispos 2013; 41: 1651-1661.

[4] Koch I, Weil R, Wolbold R, Brockmöller J, Hustert E, Burk O, Nuessler A, Neuhaus P, Eichelbaum M and Zanger U. Interindividual variability and tissue-specificity in the expression of cytochrome P450 3A mRNA. Drug Metab Dispos 2002; 30: 1108-1114.

[5] Kuehl P, Zhang J, Lin Y, Lamba J, Assem M, Schuetz J, Watkins PB, Daly A, Wrighton SA and Hall SD. Sequence diversity in CYP3A promoters and characterization of the genetic basis of polymorphic CYP3A5 expression. Nat Genet 2001; 27: 383-391.

[6] Lin YS, Dowling AL, Quigley SD, Farin FM, Zhang J, Lamba J, Schuetz EG and Thummel KE. Co-regulation of CYP3A4 and CYP3A5 and contribution to hepatic and intestinal midazolam metabolism. Mol Pharmacol 2002; 62: 162-172.

[7] Busi F and Cresteil T. CYP3A5 mRNA degradation by nonsense-mediated mRNA decay. Mol Pharmacol 2005; 68: 808-815.

[8] Lamba J, Hebert JM, Schuetz EG, Klein TE and Altman RB. PharmGKB summary: very important pharmacogene information for CYP3A5. Pharmacogenet Genomics 2012; 22: 555.

[9] Song D, Li LS, Arsenault PR, Tan Q, Bigham AW, Heaton-Johnson KJ, Master SR and Lee FS. Defective Tibetan PHD2 binding to p23 links high altitude adaption to altered oxygen sensing. J Biol Chem 2014; 289: 14656-14665.

[10] Ye Y, Gao J, Fan G, Liao L and Hou Y. Population genetics for 23 Y-STR loci in Tibetan in China and confirmation of DYS448 null allele. Forensic Sci Int Genet 2015; 16: e7-e10.

[11] Meyer UA. Pharmacogenetics and adverse drug reactions. Lancet 2000; 356: 1667-1671.

[12] Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, Defelice M, Lochner A and Faggart M. The structure of haplotype blocks in the human genome. Science 2002; 296: 2225-2229.

[13] Duan P, Zhang Y, Han X, Liu J, Yan W and Xing Y. Effect of neuronal induction on NSE, Tau, and Oct4 promoter methylation in bone marrow mesenchymal stem cells. In Vitro Cell Dev Biol Anim 2012; 48: 251-258.

[14] Ng PC and Henikoff S. Predicting the effects of amino acid substitutions on protein function. Annu Rev Genomics Hum Genet 2006; 7: 61-80.

[15] Sim NL, Kumar P, Hu J, Henikoff S, Schneider G and Ng PC. SIFT web server: predicting effects of amino acid substitutions on proteins. Nucleic Acids Res 2012; 40: W452-W457.

[16] Roy JN, Lajoie J, Zijenah LS, Barama A, Poirier C, Ward BJ and Roger M. CYP3A5 genetic polymorphisms in different ethnic populations. Drug Metab Dispos 2005; 33: 884-887.

[17] Rodriguez-Antona C and Ingelman-Sundberg M. Cytochrome P450 pharmacogenetics and cancer. Oncogene 2006; 25: 1679-1691.

[18] Balram C, Zhou Q, Cheung YB and Lee EJ. CYP3A5*3 and *6 single nucleotide polymorphisms in three distinct Asian populations. Eur J Clin Pharmacol 2003; 59: 123-126.

[19] Hiratsuka M, Takekuma Y, Endo N, Narahara K, Hamdy S, Kishikawa Y, Matsuura M, Agat-

suma Y, Inoue T and Mizugaki M. Allele and genotype frequencies of CYP2B6 and CYP3A5 in the Japanese population. Eur J Clin Pharmacol 2002; 58: 417-421.

[20] Park S, Kang Y, Jeong M, Yoon H and Han K. Frequencies of CYP3A5 genotypes and haplotypes in a Korean population. J Clin Pharm Ther 2008; 33: 61-65.

[21] Suarez-Kurtz G, Vargens DD, Santoro AB, Hutz MH, de Moraes ME, Pena SD, Ribeiro-dos-Santos Â, Romano-Silva MA and Struchiner CJ. Global pharmacogenomics: distribution of CYP3A5 polymorphisms and phenotypes in the Brazilian population. PLoS One 2014; 9: e83472.

[22] Garsa AA, McLeod HL and Marsh S. CYP3A4 and CYP3A5 genotyping by Pyrosequencing. BMC Med Genet 2005; 6: 19.

[23] Yousef AM, Bulatova NR, Newman W, Hakooz N, Ismail S, Qusa H, Zahran F, Ababneh NA, Hasan F and Zaloom I. Allele and genotype frequencies of the polymorphic cytochrome P450 genes (CYP1A1, CYP3A4, CYP3A5, CYP2C9 and CYP2C19) in the Jordanian population. Mol Biol Rep 2012; 39: 9423-9433.

[24] Quaranta S, Chevalier D, Allorge D, Lo-Guidice J, Migot-Nabias F, Kenani A, Imbenotte M, Broly F, Lacarelle B and Lhermitte M. Ethnic differences in the distribution of CYP3A5 gene polymorphisms. Xenobiotica 2006; 36: 1191-1200.

[25] van Schaik RH, van der Heiden IP, van den Anker JN and Lindemans J. CYP3A5 variant allele frequencies in Dutch Caucasians. Clin Chem 2002; 48: 1668-1671.

[26] Ge J, Budowle B, Planz JV and Chakraborty R. Haplotype block: a new type of forensic DNA markers. Int J Legal Med 2010; 124: 353-361.

[27] Kudzi W, Dodoo AN and Mills JJ. Genetic polymorphisms in MDR1, CYP3A4 and CYP3A5 genes in a Ghanaian population: a plausible explanation for altered metabolism of ivermectin in humans? BMC Med Genet 2010; 11: 111.