RESEARCH ARTICLE

Genetic analysis of drug metabolizing phase-I enzymes CYP3A4 in Tibetan populations

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Abstract

The enzymatic activity of *CYP3A4* results in broad interindividual variability in response to certain pharmacotherapies. The present study aimed to screen Tibetan volunteers for *CYP3A4* genetic polymorphisms. Previous research has focussed on Han Chinese patients, while little is known about the genetic variation of *CYP3A4* in the Tibetan populations. Here, we adopted DNA sequencing to investigate the promoter, exons and surrounding introns, and 3′-untranslated region of the *CYP3A4* gene in 96 unrelated healthy Tibetan individuals. We identified 20 different *CYP3A4* polymorphisms in the Tibetan population, including two novel variants (21824 A>G and 15580 G>C). In addition, we also determined the allele frequencies of *CYP3A4**1A and *CYP3A4**1H were 82.29% and 28.13%, respectively. *CYP3A4**1P and *1G were relatively rare with frequencies of only 1.04% and 0.52%, respectively. Our results provide information on *CYP3A4* polymorphisms in Tibetan individuals which may help to optimize pharmacotherapy effectiveness by providing personalized medicine to this ethnic group.

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Introduction

Environment and hereditary factors can affect the individual's multiple drug metabolism. Many genes code the drug metabolizing enzymes and can influence the enzyme activity (Drögemöller *et al.* 2013; Fukuyoshi *et al.* 2016). Pharmacokinetic polymorphisms divide the population at least into two phenotypes: poor metabolizers (PMs) and extensive metabolizers (EMs). The PM condition can lead to an excessive or prolonged therapeutic effect or drug-related toxicity after a normal dose. While, EM populations may not achieve therapeutic levels of the drug administered at a standard dose resulting in the lack of a therapeutic effect.

The CYP3A locus consists of four genes: *CYP3A4*, *CYP3A5*, *CYP3A7* and *CYP3A43*, which reside in a 231 kb region of chromosome 7q21.1 (Drögemöller *et al.* 2013). *CYP3A4* is responsible for the metabolism of approximately 50–60% of clinical drugs that are used today, including acetaminophen (Jiang *et al.* 2015), codeine (Eissing *et al.* 2012), cyclosporine A (Cai *et al.* 2015), diazepam (Rezaee *et al.* 2014), and erythromycin (Boetsch *et al.* 2016). The most prevalent polymorphism in *CYP3A4* (CYP3A4*1B) (rs2740574) occurs in the 5′-flanking region of the gene, it involves an A>G transition at −293 position from the transcription start site (Lakhman *et al.* 2009). The frequency distribution of *CYP3A4**1B exists differently in some ethnic group. The frequency of *CYP3A4**1B in Caucasians

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was 0.02, while in Africans it was 0.82 (Garsa *et al.* 2005; Jin *et al.* 2005). *CYP3A4**5 and *CYP3A4**18 are the predominant mutations affecting the metabolism of certain drugs in Chinese people.

Tibetans are minority in China and have huge differences in the genetic structure, physiology, diet and lifestyle compared with the Han population. These variable factors may affect the drug metabolism. Thus, in this study, we explored the allelic and genotypic pattern of CYP3A4 in healthy Tibetan nationalities of the Chinese population.

Materials and methods

Subjects

In this study, we recruited 96 healthy Tibetans (48 males and 48 females) from the Xizang Minzu University, from October to December 2009, based on strict inclusion and exclusion criteria. All participants were from the Tibet Autonomous Region, and were Tibetan descent at least for three generations. The exclusion criteria were: (i) individual with any type of medical illness, (ii) organ transplant, (iii) pregnant, and (iv) smoking, drug/alcohol addiction. The purpose of exclusion was to minimize some factors that may have influenced genetic variation in the genes of interest.

When the samples were collected, the purpose and experimental procedures of our study were informed to all the participants, and a signed informed consent was obtained from them. The study protocol was performed in accordance with the Declaration of Helsinki and was approved by The Ethics Committees of Xizang Minzu University.

DNA sequencing of CYP3A4 variants

To extract genomic DNA from the 5 mL venous blood, we adopted GoldMag nanoparticles method (GoldMag, Xi'an, China), according to the manufacturer's instructions. We designed PCR primers to amplify the promoter, exons and the 3′-untranslated region of CYP3A4, the primers sequences are shown in table 1. PCR reaction system: 1 μL for genomic DNA (20 ng/μL), 5 μL for Hotstar Taq Master Mix, 0.5 μL for forward primer, 0.5 μL for reverse primer, and 3 μL for deionized water to make up 10 mL reaction system. The process of PCR reaction: 15 min at 95°C; 35 cycles of 30 s at 95°C, 30 s at 60°C and 1 min at 72°C; 3 min at 72°C; and 4°C hold. The result of amplicon was analysed on agarose gel electrophoresis, and used ABI PrismBigDye Terminator Cycle Sequencing kit ver. 3.1 (Applied Biosystems, Foster City, USA) on an ABI Prism3100 sequencer (Applied Biosystems) to be sequenced.

Data analysis

We analysed the sequencing results of CYP3A4, through Squencher4.10.1 (http://www.genecodes.com/) software. All the position of CYP3A polymorphism loci are reference *CYP3A4* gene sequences and protein sequences, the ID of

Table 1. Primers used to amplify regions of *CYP3A4*.

Primer name	Primer sequence (5′–3′)	DNA size for PCR (bp)
Promoter_F	GTGCAGAGACAGCAGCTGAG	882
Promoter_R	TCTCCTCTGAGTCTTCCTTTCA	
Exon1_F	CTTCCAACTGCAGGCAGAG	900
Exon1_R	GTTTGGAATGAGATCCGTCA	
Exon2_F	ATTCCTGCCTGAACCTCTCA	894
Exon2_R	GGTAAATACCTGGGCTCCCTA	
Exon3_F	AAGGATGACAAAGAGATAAAACACTG	898
Exon3_R	AAGACTCCGCAAAACTACAAGC	
Exon4_F	GGAGAATGGCATGGGAAATA	855
Exon4_R	CCACATGGAGACAGAGTGGA	
Exon5_6_F	CGCCCCACACAAATACATC	871
Exon5_6_R	TGTGCACAGGGGAGAAGAT	
Exon7_F	TGAGCCCCTTAGGAAGAGTT	900
Exon7_R	GCAGAAGAAAGAAAATGATACAGAC	
Exon8_F	TCTTGACTACCTACTATTTCTTGAACA	893
Exon8_R	TTGAAATGAGTCTTTACCAATTTATGA	
Exon9_F	CCCTTCAATAAATTGTCAGAGGA	927
Exon9_R	GTGGCTCCTGATTGGATGTT	
Exon10_F	ACATTTTTCTTGGGGGAGAG	928
Exon10_R	TAAGGGGACATCACACACCA	
Exon11_F	CAAAAGTCCTCCTTTTAGTGTGTG	912
Exon11_R	AAAAATATTCATTTGGGGGACA	
Exon12_F	TTCCCCTTCTCCTTCCTCAT	928
Exon12_R	CCAAGTTCTGGTTGGGAAGA	
Exon13_F	TTCAAAAACAGTTTGCCATCA	935
Exon13_R	GAATACTCCAGAGAAAACATGTGA	
3′-UTR_F	TTGGCTCCTCTGCTTCTCAC	880
3′-UTR_R	TTGGGTGTTGAGGATGGAAT	

gene sequences is AF208107 in GenBank data, the ID of protein sequences is P08684.

We calculated the allelic and genotypic frequencies through a statistical method and adopted chi-squared test to compare the differences of allele frequency with other ethnic populations (Adamec 1964). We also compared the frequency of the variants *CYP3A4* with 1000 Genome population frequencies. $P < 0.05$ was considered to represent statistical significance.

Finally, we used Haploview 4.1 (http://broad.mit.edu/mpg/haploview) software platform to analysis linkage disequilibrium (LD) and Hardy–Weinberg equilibrium for each genetic variant (Barrett *et al.* 2005). Meanwhile, we selected tSNPs of CYP3A4 to construct haplotype.

Transcriptional prediction

SIFT (http://sift.bii.a-star.edu.sg/) and PolyPhen-2 (a popular structure-based/sequence-based amino acid substitution prediction method) (http://genetics.bwh.harvard.edu/pph2/) may be useful in prioritizing changes that are likely to cause a loss of protein function. Here, we used these tools to predict the function, which nonsynonymous SNPs (nsSNPs) in CYP3A4 coding regions. The predicted results of SIFT can be divided into four categories: tolerant (0.201–1.00), borderline (0.101–0.20), potentially intolerant (0.051–0.10) and intolerant (0.00–0.05). Smaller score value shows that the mutation may have great influence on the function of the protein. The results of PolyPhen-2 can be divided into five categories: probably benign (0.000–0.999), borderline (1.000–1.249), potentially damaging (1.250–1.449), possibly damaging (1.500–1.999) and probably damaging (≥2.000). When the score is lower, the effect on protein is greater.

Results

Genetic variants

Preliminary analysis using Squencher4.10.1 on CYP3A4 sequencing in Tibetan, we detected 20 nucleotide change on the *CYP3A4* gene, there are two new mutations in the 20 nucleotide change (table 2). The first new mutations occurred in downstream position of 21824 (21824 A>G), which is a nonsynonymous mutations. Another mutation is a synonymous mutation in exon 7 (15580 G>C).

Allele and genotype frequencies

We detected four *CYP3A4* alleles in the Tibetan study population, *CYP3A4**1A, *CYP3A4**1H, *CYP3A4**1P and *CYP3A4**1G (table 3). The frequencies of *CYP3A4**1A, *CYP3A4**1H, *CYP3A4**1P and *CYP3A4**1G were 0.8229, 0.2813, 0.0104, 0.0052, respectively. The frequency of

Table 3. *CYP3A4* allele and genotype frequencies in Tibetan individuals.

Gene	Allele	Number (%)	Phenotype
	*1A	158 (82.29)	Normal
	*1G	1 (0.52)	–
	*1H	31 (16.15)	–
	*1P	2 (1.04)	–
CYP3A4	Genotype	Number (%)	Phenotype
	*1A/*1A	62 (64.58)	Normal
	*1A/*1G	1 (1.04)	–
	*1A/*1H	31 (32.29)	–
	*1A/*1P	2 (2.08)	–
Total	96		

Table 2. Frequency distribution of *CYP3A4* polymorphisms in 96 Tibetan subjects.

Nucleotide change	Position	Region	SNP	Allele	Amino acid effect	Frequencies (%)
A>W(T)	−789	Promoter			Not translated[a]	1.04
T>Y(C)	102	Intron 1	rs373014415		Not translated	2.08
T>K(G)	186	Intron 1			Not translated	1.04
C>Y(T)	3858	Intron 1	rs55913187		Not translated	8.33
A>M(C)	4303	Intron 2			Not translated	1.04
C>Y(T)	5843	Intron 2			Not translated	1.04
G>S(C)	15580	Exon 7		Novel 1	Gly178=	1.04
G>A	15727	Intron 7	rs55808838	CYP3A4*1P	Not translated	2.08
T>Y(C)	15977	Intron 7	rs2246709		Not translated	43.75
C>Y(T)	16613	Intron 7	rs4646437		Not translated	17.71
T>K(G)	17128	Intron 8	rs28371756		Not translated	1.04
G>R(A)	20230	Intron 10	rs2242480	CYP3A4*1G, 1H	Not translated	33.33
T>K(G)	21734	Intron 10			Not translated	1.04
A>R(G)	21824	Exon 11		Novel 2	Thr349Ala	1.04
T>K(G)	22121	Intron 11			Not translated	2.08
T>Y(C)	22140	Intron 11			Not translated	1.04
A>R(G)	22213	Intron 11	rs34382314		Not translated	1.04
G>R(A)	23383	Intron 12			Not translated	1.04
A>R(G)	25721	Intron 12	rs28988600		Not translated	37.50
C>M(A)	26206	3′UTR	rs59715127	CYP3A4*1G	Not translated	1.04

[a]Synonymous SNP mutations have no effect on protein sequence.

*CYP3A4**1A allele is higher than other allele, and *CYP3A4**1P and *1G were relatively rare.

We further compared the frequency of the variants *CYP3A4* with 1000 Genome population frequencies (table 4). Our results showed that the *CYP3A4**2 and *CYP3A4**18 were absent in our subjects. Compared with 1000 Genome population frequencies, *CYP3A4**2 occurs at a frequency of 1.01% in Finnish individuals from Finland. The frequencies of *CYP3A4**18 in CHS, JPT and KHV populations were 1.43, 1.44, 2.53%, respectively.

We also identified four *CYP3A4* genotypes, *1A/*1A, *1A/*1G, *1A/*1H and *1A/*1P, with frequencies ranging from 1.04 to 64.58% (table 3). The frequencies of *1A/*1A, *1A/*1G, *1A/*1H and *1A/*1P were 0.6458, 0.0104, 0.3229 and 0.0208. The heterozygous genotype *1A/*1P and *1A/*1H are relatively rare.

LD analysis

We used Haploview 4.1 software platform to analysis LD. The overall LD across the *CYP3A4* gene was found in one LD block (figure 1). In this block, we found that rs25721 and rs16613, rs20230 and rs16613 were very tightly correlated, the degree of linkage is close to 1, and rs25721 and rs20230 linkage degree up to 0.92.

Protein function prediction

Through the protein function of *CYP3A4* nonsynonymous mutation (21824 A>G), SIFT prediction results show that the score is 0.89 and the category is tolerant; PolyPhen-2 analysis predicted that the score is 0.001, and the mutation is benign (figure 2).

Table 4. *CYP3A4* allele frequencies in 1000 Genome population.

	ACB	ASW	BEB	CDX	CEU	CHB	CHS	CLM	ESN	FIN	GBR	GIH	GWD
1	0.9844	0.9754	0.9942	0.9946	0.9342	0.9951	0.9809	0.9521	0.9898	0.9241	0.8791	0.9902	0.9779
*2	–	–	–	–	–	–	–	–	–	0.0101	–	–	–
*3	–	0.0082	–	–	0.0152	–	–	0.0213	–	0.0101	0.011	–	–
*4	–	–	–	–	–	–	0.0048	–	–	–	–	–	–
*5	–	–	–	0.0054	–	0.0049	–	–	–	–	–	–	–
*6	–	–	–	–	–	–	–	–	–	–	–	–	–
*7	–	–	–	–	–	–	–	–	–	0.0051	–	–	–
*8	–	–	–	–	–	–	–	–	–	0.0051	–	–	–
*9	–	–	–	–	–	–	–	–	–	–	–	–	–
*10	–	–	0.0058	–	0.0051	–	–	–	–	–	0.0165	–	–
*11	–	–	–	–	–	–	–	–	–	–	0.0055	–	–
*12	–	–	–	–	–	–	–	–	0.0051	–	–	–	–
*15	0.0104	0.0164	–	–	–	–	–	–	0.0051	–	–	–	0.0221
*16	–	–	–	–	–	–	–	–	–	–	–	–	–
*18	–	–	–	–	–	–	0.0143	–	–	–	–	–	–
*19	–	–	–	–	–	–	–	–	–	–	–	0.0049	–
*22	0.0052	–	–	–	0.0455	–	–	0.0266	–	0.0455	0.0879	0.0049	–
	IBS	ITU	JPT	KHV	LWK	MSL	MXL	PEL	PJL	PUR	STU	TSI	YRI
1	0.9532	0.9853	0.9664	0.9545	0.9747	0.9824	0.9844	0.9823	0.9896	0.9327	0.9853	0.9579	0.9537
*2	–	–	–	–	–	–	–	–	–	–	–	–	–
*3	–	–	–	–	–	–	–	–	–	0.0048	–	–	–
*4	–	–	–	0.0202	–	–	–	–	–	–	–	–	–
*5	–	–	–	–	–	–	–	–	–	–	–	–	–
*6	–	–	–	–	–	–	–	–	–	–	–	–	–
*7	–	–	–	–	–	–	–	–	–	–	–	0.0047	–
*8	–	–	–	–	–	–	–	–	–	–	–	–	–
*9	–	–	–	–	–	–	–	0.0059	–	–	–	–	–
*10	0.0047	0.0098	–	–	–	–	–	–	–	0.0048	–	–	–
*11	–	–	–	–	–	–	–	–	–	–	–	–	0.0046
*12	–	–	–	–	0.0202	–	–	–	–	0.0048	–	–	–
*15	0.0047	–	–	–	0.0051	0.0176	0.0078	–	–	0.0048	–	–	0.0417
*16	–	–	0.0192	–	–	–	–	–	–	–	–	–	–
*18	–	–	0.0144	0.0253	–	–	–	–	–	–	–	–	–
*19	–	–	–	–	–	–	–	–	–	–	0.0049	–	–
*22	0.0374	0.0049	–	–	–	–	0.0078	0.0118	0.0104	0.0481	0.0098	0.0374	–

ACB, African Carbbean Barbados; ASW, HapMap African ancestry individuals from SW US; BEB, Bengali in Bangladesh; CDX, Chinese Dai in Xishuangbanna, China; CEU, CEPH individuals; CHB, Han Chinese in Beijing; CHS, Han Chinese South; CLM, Colombian in Medellin, Colombia; FIN, HapMap Finniah individuals from Finland; GBR, British individuals from England and Scotland; GIH, HapMap Gujarati India individuals from Texas; GWD, Gambian in Western Division – Mandinka; IBS, Iberian populations in Spain; JPT, Japanese individuals; KHV, Kinh in Ho Chi minh City, Vietnam; LWK, Luhya individuals; MSL, Mende in Sierra Leone; MXL, HapMap Mexican individuals from LA California; PEL, Peruvian in Lima, Peru; PUR, Puerto Rican in Puerto Rico; STU, Sri Lankan Tamil in the UK; TSI, Toscan individuals; YRI, Yoruba individuals.

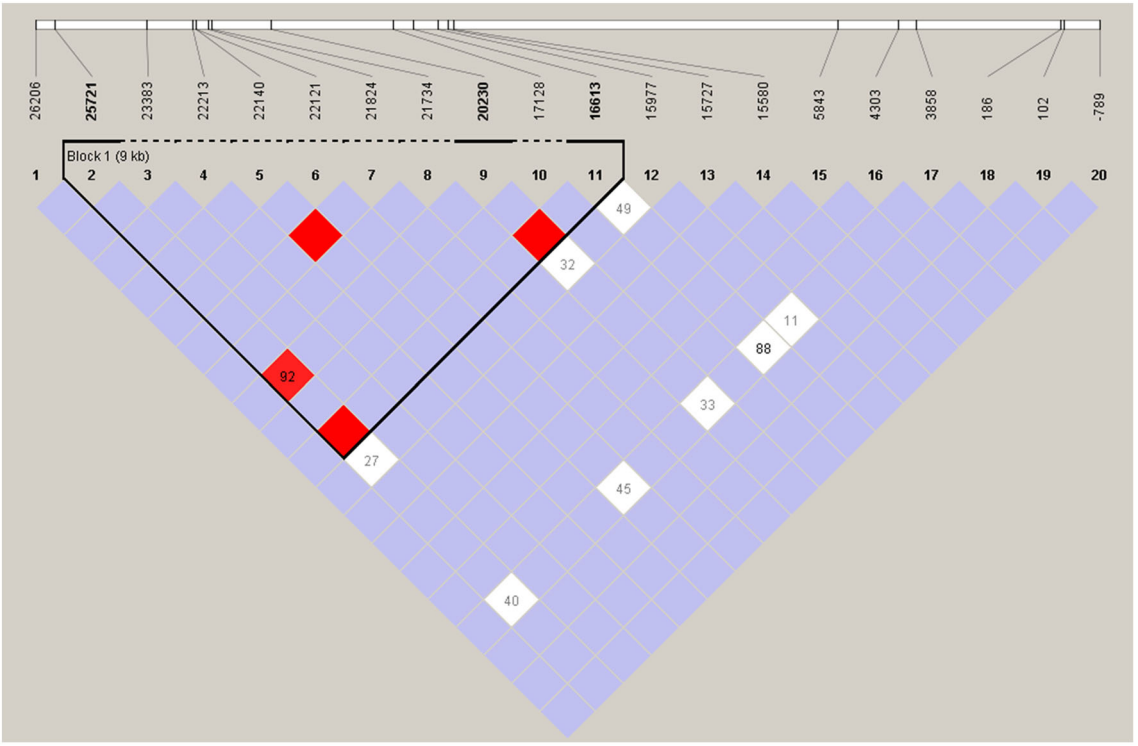

Figure 1. LD analysis of CYP3A4. LD is displayed by standard colour schemes, with bright red for very strong LD (LOD > 2, D′= 1), pink red (LOD > 2, D′ < 1) and blue (LOD < 2, D′= 1) for intermediate LD, and white (LOD < 2, D′ < 1) for no LD.

Discussion

In this study, we adopted the direct sequencing to screen *CYP3A4* gene polymorphism in the Tibetan population, and statistics the frequency of allele and genotype. We detected 20 polymorphisms on the *CYP3A4* gene, including two new mutations in the coding region (21824 A>G and 15580 G>C).

We further compared *CYP3A4* allele frequencies between our data and previously published data from different countries and ethnic groups. Our results showed that the frequency of the wild-type allele, *CYP3A4**1, in our study group was significantly lower than in Han, Uighur, Hui and Mongolian populations. The frequencies of *CYP3A4**1 in Han, Uighur, Hui and Mongolian populations were 0.8162, 0.8598, 0.8079, 0.7975, respectively (Zuo *et al.* 2012). The differences could be attributed to the ethnic origin and geographical distribution of these populations, their cultural and dietary habits, or other environmental factors. It is of potential clinical importance to identify individuals from different areas of China who have altered pharmacokinetics for *CYP3A4* substrates so that appropriate dosage strategies for these drugs can be adopted and adverse drug reactions can be avoided.

Recently, several *CYP3A4* variant alleles have been identified. *CYP3A4**1B (rs2740574 A>G) is known to be the polymorphism that increases expression by changing the transcription factor binding affinity (Amirimani *et al.* 2003;

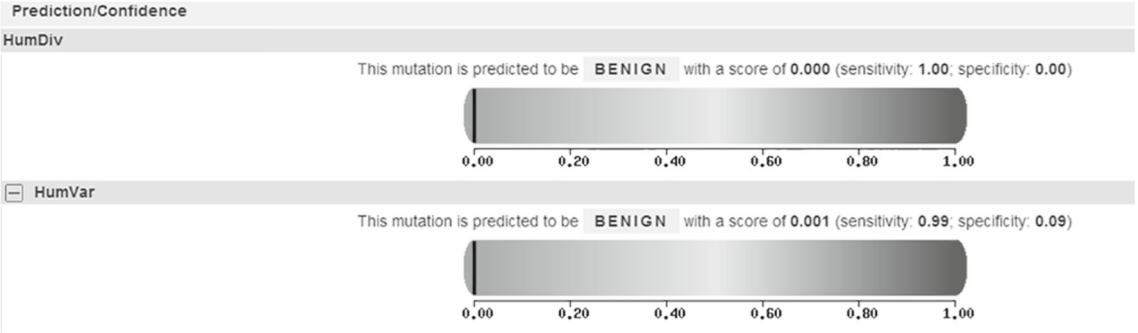

Figure 2. PolyPhen-2 prediction of functional change resulting from an amino acid mutation at position 349.

Georgitsi *et al.* 2011). We did not detect the *CYP3A4**1B gene in our study, while 0.042 were noticed in European–Americans, 0.271 in African–Americans (Lee *et al.* 2013). CYP3A4*1B carriers showed higher drug clearance for anticancer agents, such as docetaxel and cyclophosphamide, than wild-type subjects (Tran *et al.* 2006). Midazolam (MDZ), which can be administrated both intravenously and orally, is selectively metabolized by CYP3A4 and CYP3A5 to its primary metabolite, 1′-hydroxymidazolam, and is not a substrate of P-glycoprotein. In healthy volunteers for midazolam content detection results showed that CYP3A4*1B homozygous mutant of midazolam clearance rate decreased 30% compared to homozygous wild (Matsumura *et al.* 2004).

Additional alleles include *CYP3A4**2 (S222P), that occurs at a frequency of 2.75% in Finnish Caucasians, while absent in Black and Chinese subjects, and *CYP3A4**3 (M445T) that occurs with an allele frequency of 2.2% in Dutch Caucasians, and *CYP3A4**4 (I118V), *CYP3A4**5 (P218R) and *CYP3A4**6 (17776A ins) demonstrating frequencies of 1.47, 0.98 and 0.5%, respectively, in a Chinese population (Lamba *et al.* 2002). CYP3A4*2 was found in White population and had altered catalytic activity towards nifedipine and testosterone compared with the wild-type CYP3A4*1 P450. Sata *et al.* (2000) revealed that the CYP3A4*2 P450 had a lower intrinsic clearance for the CYP3A4 substrate nifedipine compared with the wild-type enzyme, and the clearance rate was reduced by 36%.

CYP3A4*18 variant allele in the exon 10 of CYP3A4 involves T to C transition at position 169068 (in GenBank NG-000004) that changes leucine −293 to proline, which was identified by direct sequencing in 24 Asians. Dai *et al.* (2001) indicated that CYP3A4*18 showed significantly a higher turnover number for both testosterone and insecticide chlorpyrifos *in vitro*. Hu *et al.* (2005) determined the allelic frequency of the CYP3A5*3 and CYP3A4*18 in a group of 302 Chinese subjects by using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays and found that the frequency of CYP3A4*18 variant allele in Chinese population was 0.01. Zuo *et al.* (2012) compared the frequencies of *CYP3A4* allelic variants in Han (Liaoning), Uighur, Hui and Mongolian people in the Chinese population. The frequencies of *CYP3A4**18 in Han, Uighur, Hui and Mongolian populations were 0.1838, 0.1402, 0.1921 and 0.2025, respectively. The frequencies of CYP3A4*18 in CHS, JPT and KHV populations were 1.43, 1.44, 2.53%, respectively.

Through analysis of the protein function of *CYP3A4* nonsynonymous mutation, which results in an amino acid change from Thr to Ala (21824 A>G), we found that this mutation may not influence the protein function. The novel genetic variants identified here should be confirmed by other means in future studies. Another novel polymorphisms is synonymous mutation in exon 7 (15580 G>C, Gly178=Gly). Synonymous mutations are mutations that do not change the encoded amino acids. It is generally assumed that synonymous mutations are evolutionary neutral and they have no effects on the phenotype. Therefore, may not affect the function of the encoded protein and drug metabolism. SIFT uses sequence homology to predict whether an amino acid substitution will affect protein function and hence potentially alter phenotype. The result predict by SIFT existed 20% false positive error and 31% false negative error (Ng and Henikoff 2003). The predict result of PolyPhen exist 9% false positive error and 31% false negative error (Ng and Henikoff 2006).

In conclusion, we detected 20 polymorphisms on the *CYP3A4* gene, including two new mutations in the coding region (21824 A>G and 15580 G>C). Our results provide a basic profile of *CYP3A4* in the Tibetan population, and can be used to determine optimal dosage recommendations leading to individualized medicine.

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References

Adamec C. 1964 Example of the use of the nonparametric test. Test ×2 for comparison of 2 independent examples. *Cesk. Zdrav.* **12**, 613–619.

Amirimani B., Ning B., Deitz A. C., Weber B. L., Kadlubar F. F. and Rebbeck T. R. 2003 Increased transcriptional activity of the CYP3A4* 1B promoter variant. *Environ. Mol. Mutagen.* **42**, 299–305.

Barrett J. C., Fry B., Maller J. and Daly M. J. 2005 Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21**, 263–265.

Boetsch C., Parrott N., Fowler S., Poirier A., Hainzl D., Banken L. *et al.* 2016 Effects of cytochrome P450 3A4 inhibitors-ketoconazole and erythromycin-on bitopertin pharmacokinetics and comparison with physiologically based modelling predictions. *Clin. Pharmacokinet.* **55**, 237–247.

Cai N., Li B., Huang X., Xu K., Feng H., Cheng Z. *et al.* 2015 A non-invasive CYP3A4 biomarker and body mass index predict cyclosporine dosage requirements in Chinese renal transplant recipients. *Pharmazie* **70**, 815–818.

Dai D., Tang J., Rose R., Hodgson E., Bienstock R. J., Mohrenweiser H. W. *et al.* 2001 Identification of variants of CYP3A4 and characterization of their abilities to metabolize testosterone and chlorpyrifos. *J. Pharmacol. Exp. Ther.* **299**, 825–831.

Drögemöller B., Plummer M., Korkie L., Agenbag G., Dunaiski A., Niehaus D. *et al.* 2013 Characterization of the genetic variation present in CYP3A4 in three South African populations. *Front. Genet.* **4**, 17.

Eissing T., Lippert J. and Willmann S. 2012 Pharmacogenomics of codeine, morphine, and morphine-6-glucuronide: model-based analysis of the influence of CYP2D6 activity, UGT2B7 activity, renal impairment, and CYP3A4 inhibition. *Mol. Diagn. Ther.* **16**, 43–53.

Fukuyoshi S., Kometani M., Watanabe Y., Hiratsuka M., Yamaotsu N., Hirono S. *et al.* 2016 Molecular Dynamics simulations to investigate the influences of amino acid mutations on protein three-dimensional structures of cytochrome P450 2D6.1, 2, 10, 14A, 51, and 62. *PLoS One* **11**, e0152946.

Garsa A. A., McLeod H. L. and Marsh S. 2005 CYP3A4 and CYP3A5 genotyping by Pyrosequencing. *BMC Med. Genet.* **6**, 19.

Georgitsi M., Zukic B., Pavlovic S. and Patrinos G. P. 2011 Transcriptional regulation and pharmacogenomics. *Pharmacogenomics* **12**, 655–673.

Hu Y. F., He J., Chen G. L., Wang D., Liu Z. Q., Zhang C. *et al.* 2005 CYP3A5*3 and CYP3A4*18 single nucleotide polymorphisms in a Chinese population. *Clin. Chim. Acta* **353**, 187–192.

Jiang Y., Fan X., Wang Y., Chen P., Zeng H., Tan H. *et al.* 2015 Schisandrol B protects against acetaminophen-induced hepatotoxicity by inhibition of CYP-mediated bioactivation and regulation of liver regeneration. *Toxicol. Sci.* **143**, 107–115.

Jin M., Gock S. B., Jannetto P. J., Jentzen J. M. and Wong S. H. 2005 Pharmacogenomics as molecular autopsy for forensic toxicology: genotyping cytochrome P450 3A4*1B and 3A5*3 for 25 fentanyl cases. *J. Anal. Toxicol.* **29**, 590–598.

Lakhman S. S., Ma Q. and Morse G. D. 2009 Pharmacogenomics of CYP3A: considerations for HIV treatment. *Pharmacogenomics* **10**, 1323–1339.

Lamba J. K., Lin Y. S., Thummel K., Daly A., Watkins P. B., Strom S. *et al.* 2002 Common allelic variants of cytochrome P4503A4 and their prevalence in different populations. *Pharmacogenetics* **12**, 121–132.

Lee J. S., Cheong H. S., Kim L. H., Kim J. O., Seo D. W., Kim Y. H. *et al.* 2013 Screening of genetic polymorphisms of CYP3A4 and CYP3A5 genes. *Korean J. Physiol. Pharmacol.* **17**, 479–484.

Matsumura K., Saito T., Takahashi Y., Ozeki T., Kiyotani K., Fujieda M. *et al.* 2004 Identification of a novel polymorphic enhancer of the human CYP3A4 gene. *Mol. Pharmacol.* **65**, 326–334.

Ng P. C. and Henikoff S. 2003 SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res.* **31**, 3812–3814.

Ng P. C. and Henikoff S. 2006 Predicting the effects of amino acid substitutions on protein function. *Annu. Rev. Genomics Hum. Genet.* **7**, 61–80.

Rezaee M. M., Kazemi S., Kazemi M. T., Gharooee S., Yazdani E., Gharooee H. *et al.* 2014 The effect of piperine on midazolam plasma concentration in healthy volunteers, a research on the CYP3A-involving metabolism. *Daru* **22**, 8.

Sata F., Sapone A., Elizondo G., Stocker P., Miller V. P., Zheng W. *et al.* 2000 CYP3A4 allelic variants with amino acid substitutions in exons 7 and 12: evidence for an allelic variant with altered catalytic activity. *Clin. Pharmacol. Ther.* **67**, 48–56.

Tran A., Jullien V., Alexandre J., Rey E., Rabillon F., Girre V. *et al.* 2006 Pharmacokinetics and toxicity of docetaxel: role of CYP3A, MDR1, and GST polymorphisms. *Clin. Pharmacol. Ther.* **79**, 570–580.

Zuo J., Xia D., Jia L. and Guo T. 2012 Genetic polymorphisms of drug-metabolizing phase I enzymes CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in Han, Uighur, Hui and Mongolian Chinese populations. *Pharmazie* **67**, 639–644.

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