1. Introduction

As one of the most important metabolic enzyme systems in humans, cytochrome P450 (CYP) enzymes are a central part in the metabolism of numerous drugs, especially in drug biotransformation processes.[1] CYP2J2 is the only member of CYP450 II J subfamily which transforms arachidonic acid (AA) to regioselective epoxyeicosatrienoic acids (EETs).[2] CYP2J2 plays important roles in maintaining homeostasis including the regulation of inflammation, relaxation of smooth muscles, angiogenesis, and vasodilatation. CYP2J2 is mainly expressed in the cardiovascular system[3] and is also found in the intestines, stomach, and other tissues.[4-6] CYP2J2 metabolizes many therapeutic agents, including ebastine, astemizole, perphenazine, diclofenac, and bufuralol.[6] Previous in vitro and in vivo studies have reported that AA which is a CYP2J2 substrate and ebastine strongly inhibited astemizole O-demethylation.[7] CYP2J2 catalyzes the metabolic conversion of AA to EETs, which are active drug compounds employed in the protection of the heart after ischemia.[8]

Tibetans are the 9th-largest minority group in China and inhabit the Tibetan plateau, which is geographically separated from other ethnic groups in the region. Tibet, known as the “world’s third pole,” is located in Southwest China at an average altitude of 4268 m.[9] Studies have shown that the Tibetans originated from east and central Asia.[10] Idiographic cold climates and hypoxia in Tibet make it an enriching natural laboratory to investigate human natural selection. The Tibetan people, with a population of 6,282,182 (according to the Chinese population survey 2010), are a well-known successful case of adaptation to high altitude. The Tibetans have a long history and have a lasting influence on the culture and traditions of Tibet. One study focusing on genomic variations of Tibetans reported that a considerable part of the Tibetan gene pool diverged from the Han ethnic group about 3000 years ago.[11]

Exome sequencing, genome-wide sequence variation analysis, and whole-genome genotyping have been used to reveal the important functional loci in the genetic adaptation to high altitude in Tibetans,[11-13] but studies have not focused on the polymorphisms of drug-metabolizing enzyme CYP2J2 in Tibetans. In this study, we systematically screened the whole CYP2J2 genes of 100 healthy, unrelated Tibetans to identify their polymorphisms, predict the enzyme function, and compare their frequencies with previous observations in other populations. Our results serve to provide a better knowledge of CYP2J2 variants and an available database to develop personalized medicine which will help devise new strategies for optimization of drug therapy in the Tibetan ethnic group.
2. Materials and methods

2.1. Subjects

Through a very detailed exclusion and recruitment criteria, a total of 100 healthy, unrelated Tibetan individuals (50 males and 50 females) were sampled for the population genetic research from the Tibet Autonomous Region. All the chosen subjects were judged to be Tibetans based on at least the past 3 generations of paternal ancestry. Individuals with chronic diseases involving vital organs (heart, brain, kidney, liver, and lung), and severe endocrinological, metabolic and nutritional diseases were excluded. The exclusion process aimed to minimize the known factors including environmental and therapeutics that interfere with genetic variation in the genes of interest.

Signed informed consents detailing the experimental procedures and the purpose of the study was obtained from the participants. The Human Research Committee of Xizang Minzu University for Approval of Research Involving Human Subjects approved the study protocol.

2.2. DNA sequencing

Peripheral blood samples were collected from each individual. Genomic DNA was extracted using the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Ltd, Xi’an, China) according to the manufacturer’s protocol. Polymerase chain reaction (PCR) primers were designed to amplify 2000 bp of the promoter and all exons of the CYP2J2 (Table 1). PCR was performed with 10 μL reactions, 1 μL template DNA and 5 μL HotStar Taq Master Mix (Qiagen, Germantown, MD), 0.5 μL primer (3 μM), and 3 μL deionized water. The thermal cycling conditions were initial denaturation for 15 minutes at 95°C followed by 35 cycles of denaturation at 95°C for 30 seconds, 55 to 64°C for 30 seconds, and 72°C for 1 minute, and a final extension step at 72°C for 3 minutes. PCR products were incubated with 0.5 μL shrimp alkaline phosphatase (Roche Diagnostics, Basel, Switzerland), 8 μL HotStar PCR product, and 1.5 μL deionized water (to a total volume of 10 μL), at 37°C for 30 minutes, followed by heat inactivation at 80°C for 15 minutes. Purified PCR products were directly sequenced using the ABI Prism BigDye Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems, Thermo Fisher Scientific, Inc., Waltham, MA) using an ABI Prism 3100 sequencer (Applied Biosystems, Thermo Fisher Scientific, Inc.).

2.3. Statistical analysis

Sequencer 4.10.1 (https://www.genecodes.com/) was used to analyze sequences and SPSS 17.0 statistical packages (SPSS, Chicago, IL) were used to perform statistical calculations. CYP2J2 variants were analyzed based on the nucleotide reference sequence AF272142 (http://www.cypalleles.ki.se/) and the protein reference sequence P51589 (http://www.uniprot.org/). Allelic and genotypic frequencies were calculated through statistical methods. Comparisons of allelic frequencies among different ethnic populations were done using chi-squared tests with the significance level set at 0.05. Haplovew 4.2 was used to calculate linkage disequilibrium (LD) and Hardy–Weinberg equilibrium. Haplotypes were constructed from the selected tag single nucleotide polymorphisms (SNPs) and haplotype frequencies were derived for the study population.

2.4. Translational prediction

We adopted the 2 online tools PolyPhen-2 (Polyorphism Phenotyping v2, http://genetics.bwh.harvard.edu/pph2/) and Sorting Intolerant From Tolerant (SIFT, http://sift.bi.aster.edu.sg/) to analyze variants in the exon regions of CYP2J2 and predict the enzyme function of novel nonsynonymous SNPs. Each variant was given a score based on the extent of influence on enzyme function. The SIFT based on the score divided the results into 4 categories including tolerant (0.201–1.00), borderline (0.101–0.20), potentially intolerant (0.051–0.10), and intolerant (0.00–0.05). PolyPhen-2 results were divided into 5 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).

3. Results

3.1. Genetic variants

From the sequences, we identified a total of 24 CYP2J2 polymorphisms in the current Tibetan group that contained 4 novel SNPs. Among these polymorphisms, 4 synonymous mutations and 1 nonsynonymous mutation were identified. These mutants included 148C>T, 183C>T, 10782A>C, 15072T>C, and 18919C>A (Table 2).

3.2. Allelic and genotypic frequencies

We identified 2 alleles of CYP2J2 in Tibetans—CYP2J2*1 represented the wild allele and had the highest frequency (95.5%) and CYP2J2*7 with a frequency of 4.5%.

We detected 2 genotypes in the population. The homozygous CYP2J2*1/*1 is the wild one (91%). The heterozygous genotype *1/*7 (9%) resulted in a decreased enzyme activity. The allelic

| Table 1 |
| --- |
| **The primers used to amplify regions of CYP2J2.** |
| **Primer name** | **Primer sequence (5’-3’)** | **DNA size for PCR, bp** | **Primer sequence (3’-5’)** |
| UTR and Exon 1 | ACAGCGAGTAAAGAATCCAAAG | 783 | GCAGTTAACGCTGTTAGCC |
| Exon 2 | CTCACTGCACTTGTGAGAAAC | 779 | GCATCTCTCTCTGATATAAAGGGT |
| Exon 3 | GTGATTCTGTAGTTACCATAC | 788 | TCCTCTTTGATTGTCTTCTCTCTCT |
| Exon 4 | AGATTGATATGACAGAGGTG | 856 | AGACTACGGACACACGAAAA |
| Exon 5 | AACCTACACCCGCTGCTAGAT | 776 | GAGAAGAAAGCTGCTGCTG |
| Exon 6 | GAATACTGCTGGTCCATGAC | 827 | ATACACAGATACAGTGCTG |
| Exon 7 | TGCTAATCCATGCTTCCTTAC | 850 | CTTCTCAATCCATGCTTCCTTAC |
| Exon 8 | CCAGCCACTGACTAAAAATGACC | 688 | TTTCAGAGAGACAGAACAGAG |
| Exon 9 | CTTCTAGGGCTGCTCACACTGCCC | 869 | AAGACGTATGACAGGGTCTC |
| Exon 9 and UTR | CCAGGCTCTCTTGTGCTCCTTC | 778 | GCAGCAGAGACAGACATCC |

*PCR* = polymerase chain reaction.
and genotypic frequencies of CYP2J2 in Tibetan are shown in Table 3.

We further compared the distribution of CYP2J2 among different ethnic populations all over the world through literature surveys. We found that the distribution frequency is unique among different populations (Table 4), and identified that the CYP2J2 distribution in the Tibetan population is more similar to the Asian populations than other populations.

3.3. LD analysis

Haploview software was used to perform LD analysis and define blocks. In genetic association studies, LD, as the population-genomic feature, is used to locate the variants that predispose individuals to genetic diseases.[14] Figure 1 shows the extent of LD among the SNPs using the $D_0$ value. One LD block with a very strong linkage among 10984G>A, 15285A>C, 18644G>A, 18753T>G, 19114T>C, 33266C>T, and 33440A>G was identified among the 24 polymorphisms.

3.4. Protein function prediction of nonsynonymous mutations

Figure 2 shows the predicted functional consequence of the novel nonsynonymous mutations by PolyPhen-2 and SIFT. The 10782A>C was predicted by PolyPhen-2 to be benign. SIFT analysis data were consistent with PolyPhen-2 results.

4. Discussion

CYP2J2, as an enzyme responsible for the first-pass metabolism, is mainly expressed predominantly in the vascular endothelial cells and the heart tissue.[15] It is known that AA and linoleic acid are the endogenous substrates of CYP2J2.[16] CYP2J2 converts AA to regioselective EETS, which plays a significant role in maintaining the homeostasis of several human vital organs, such as the heart, lung, and kidney.[17] EETs mediate a number of biological processes including induction of membrane hyperpolarization and vasodilation, and reduction of inflammation by inhibition of transcription factors and increasing fibrinolysis activity.[18] It is quite

### Table 2

The frequencies and positions of CYP2J2 genetic variants in the Tibetans.

| SNP       | Position | Nucleotide change | Allele | Frequencies | Amino-acid effect | Region  |
|-----------|----------|-------------------|--------|-------------|-------------------|---------|
| rs890293  | −76      | G>T               | *7     | 0.09        | Decreased         | Promoter|
| rs11572191| 148      | C>T               |        | 0.01        | Leu50=            | Exon 1  |
| rs2229180 | 183      | C>T               |        | 0.08        | Phe61=            | Exon 1  |
| rs3820538 | 10522    | C>T               |        | 0.05        | No translated     | Intron 1|
| rs53720722| 10782    | A>C               |        | 0.01        | M116V             | Exon 2  |
| /         | 10814    | G>A               | Novel  | 0.02        | No translated     | Intron 2|
| /         | 10835    | G>A               |        | 0.03        | No translated     | Intron 2|
| /         | 10982    | G>C               |        | 0.18        | No translated     | Intron 2|
| /         | 10984    | G>A               |        | 0.1         | No translated     | Intron 2|
| /         | 14113    | C>T               | Novel  | 0.01        | No translated     | Intron 2|
| /         | 14705    | C>G               |        | 0.02        | No translated     | Intron 3|
| /         | 15072    | T>C               |        | 0.02        | Ser206=           | Exon 4  |
| /         | 15285    | A>C               |        | 0.33        | No translated     | Exon 4  |
| /         | 18644    | G>A               |        | 0.52        | No translated     | Intron 5|
| /         | 18740    | G>A               | Novel  | 0.02        | No translated     | Intron 5|
| /         | 18753    | T>G               |        | 0.31        | No translated     | Intron 5|
| /         | 18919    | C>A               |        | 0.07        | Arg321=           | Exon 6  |
| /         | 19114    | T>C               |        | 0.31        | No translated     | Intron 6|
| /         | 19228    | A>G               |        | 0.02        | No translated     | Intron 6|
| /         | 21581    | C>A               |        | 0.01        | No translated     | Intron 6|
| /         | 32899    | G>A               |        | 0.01        | No translated     | Intron 8|
| /         | 33266    | C>T               |        | 0.19        | No translated     | 3'-UTR |
| /         | 33440    | A>G               |        | 0.23        | No translated     | 3'-UTR |
| /         | 33472    | A>G               |        | 0.05        | No translated     | 3'-UTR |

SNP = single nucleotide polymorphism.

### Table 3

Allelic and genotypic frequencies of CYP2D6 in the Tibetan population.

| Gene     | Allele | Number | Phenotype | Frequency, % |
|----------|--------|--------|-----------|--------------|
| CYP2D6   | *1     | 191    | Normal    | 95.5         |
|          | *7     | 9      | Decreased | 4.5          |
| Total number |       | 200    |           | 100.0        |

| Genotype | Number | Phenotype | Frequency, % |
|----------|--------|-----------|--------------|
| *1/*1    | 91     | Normal    | 91.0         |
| *1/*7    | 9      | Decreased | 9.0          |
| Total number |       | 100      | 100.0        |
meaningful to study the polymorphisms of CYP2J2 in view of the function of the enzyme.

In the study, we systematically sequenced CYP2J2 using Sequencher 4.10.1 and analyzed the polymorphism distribution and allele frequencies of CYP2J2. From the 24 SNPs, we identified 4 novel all-intron polymorphisms. We found the 10782A>C was a nonsynonymous mutation with no effect on the function of the enzyme. Nonsynonymous mutations directly change the sequence and structure of the protein and have a direct impact on its function. Increasing evidence suggests that nonsynonymous mutations occur after translation and could influence the protein function or even damage it. We explored the distribution of CYP2J2 in the different population and identified a diverse frequency distribution. Our results provide the basic information about CYP2J2 polymorphisms in the Tibetan population and contribute to the development of individualized medication.

Owing to its important function, CYP2J2 has been comprehensively studied. Earlier studies have identified polymorphisms of the CYP2J2 gene in different populations and have focused on the association between CYP2J2 polymorphisms (rs890293) and cardiovascular risk, but this has provided inconsistent results. Zhu et al reported that rs890293 does not have a significant association with coronary artery disease (CAD).[19] Spiecker et al[16] and Liu et al[20] showed that the functionally relevant polymorphism rs890293 was independently associated with an increased risk of CAD in younger groups in Tainan. A decreased risk of CAD was associated with rs890293 in African-Americans while no significant association was found in Caucasians.[21] The above differences could be due to different dietary habits and geographical factors between different populations. Other studies have demonstrated that CYP2J2 is the major enzyme responsible for the metabolism of many different drugs. Wu et al proved that CYP2J2 is an important enzyme for the pharmacokinetics of anthelmintics in human liver. Christina Westphal demonstrated that the overexpression of CYP2J2 is a protective factor against arrhythmia susceptibility in a mouse model of cardiac hypertrophy.

Our study revealed that 9% of the sampled Tibetans carried the mutant CYP2J2 with decreased activity. We also identified

### Table 4

| Population                  | Sample size | Allele frequency, % |
|-----------------------------|-------------|---------------------|
| Tibetan                     | 100         | 95.50               |
| Russian                     | 227         | 95.16               |
| Tatars                      | 178         | 96.35               |
| Bashkirs                    | 102         | 98.53               |
| Ovambs                      | 186         | 93.28               |
| Mongolians                  | 118         | 96.61               |
| Japanese                    | 338         | 93.79               |
| Americans                   | 116         | 90.09               |
| Germans                     | 960         | 93.54               |
| Spanish                     | 89          | 93.26               |
| African-Americans           | 102         | 88.73               |
| Chinese                     | 384         | 97.40               |
| Taiwanese                   | 200         | 88                  |
| American Ancestry           |             |                     |
| ACB                         | 96          | 89.06               |
| ASW                         | 61          | 81.97               |
| ESN                         | 99          | 81.82               |
| GWD                         | 113         | 84.52               |
| LWK                         | 99          | 85.85               |
| MSI                         | 85          | 78.24               |
| YRI                         | 108         | 84.26               |
| CLM                         | 94          | 94.68               |
| MNL                         | 64          | 96.87               |
| PEL                         | 85          | 99.41               |
| PUR                         | 104         | 93.75               |
| CDX                         | 93          | 98.39               |
| CHB                         | 103         | 95.63               |
| CHS                         | 105         | 93.81               |
| JPT                         | 104         | 96.16               |
| KHV                         | 99          | 97.98               |
| CEU                         | 99          | 94.95               |
| FIN                         | 99          | 92.93               |
| GBR                         | 91          | 95.05               |
| IBS                         | 107         | 93.93               |
| TSI                         | 107         | 94.86               |
| BEB                         | 86          | 99.24               |
| GHT                         | 103         | 94.66               |
| ITU                         | 102         | 93.63               |
| PJL                         | 96          | 92.19               |
| STU                         | 102         | 90.20               |

Reference

[19] Zhu et al
[20] Spiecker et al
[21] Liu et al
[22] Present study
[23] 1000 Genomes Project
[24] Wu et al
[25] Christina Westphal
[26] Cao et al. Medicine (2018) 97:40 Medicine
the polymorphisms of CYP2J2 and their genetic frequencies in a Tibetan population. It is necessary to carry a more in-depth study for exploring the distribution of the polymorphisms and their effect on drug clearance rates and adverse drug reactions.

5. Conclusion

In conclusion, we systematically analyzed the variants of CYP2J2 by directly sequencing the gene in a Tibetan population and compared its distribution in other ethnic groups. The study offers some useful information for the establishment of a database of Tibetan population CYP2J2 genetic polymorphisms, which would offer a theoretical basis for individualized medical treatment and drug genomics studies in Tibet. We need a larger sample size for validating our study, which would be useful for the advancement of personalized medicine.

**Author contributions**

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