Int J Clin Exp Pathol 2016;9(4):4260-4268
www.ijcep.com /ISSN:1936-2625/IJCEP0022448

*Original Article*

# Polymorphisms of drug-metabolizing enzyme CYP2J2 in Wa population from Yunnan Province of China

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Received December 22, 2015; Accepted March 20, 2016; Epub April 1, 2016; Published April 15, 2016

**Abstract:** Genetic variations in *CYP2J2* are known to contribute to inter-individual and inter-ethnic variability in response to clinical drugs, but there is no information about the genetic polymorphisms of *CYP2J2* in the Wa population. We used DNA sequencing to investigate the whole *CYP2J2* gene in 100 unrelated healthy Wa individuals. SIFT and PolyPhen-2 were used to predict the protein function of the novel non-synonymous mutation in *CYP2J2* coding regions. Fst anlyasis and Chi-square test were used to compare genotyping data of *CYP2J2* in our study Wa population with other populations around the world. We identified 14 different *CYP2J2* polymorphisms in the Wa Chinese population, including three novel variant (15016G>A, 19109C>T and 33885A>T). Variant 15016G>A was predicted to be possibly damaging on protein function by SIFT and PolyPhen-2. Using the Chi-square test, we found rs11572245 in the different frequency distributions when the Wa population was compared to other eleven populations. The frequencies of rs1155002 in the Wa people are higher than most of Asia populations, but lower than most of Europe populations. Our results provide basic information about *CYP2J2* alleles in Wa, which may help to optimize pharmacotherapy effectiveness by providing personalized medicine to this ethnic group.

**Keywords:** *CYP2J2*, ethnic groups, genetic polymorphism, Wa populations

## Introduction

Cytochrome P450 superfamily (CYP) is a large and diverse group of membrane-associated enzymes that can catalyze many various reactions, such as oxidation, peroxidation, hydroxylation and epoxidation, to metabolize both xenobiotic and endogenous compounds [1, 2]. These enzymes are involved in the phase I metabolism in humans, and are primarily responsible for the metabolism for clinical drugs [3]. As an important member of the cytochrome P450 superfamily, human cytochrome P450 2J2 (*CYP2J2*) is the only CYP enzyme found to be mainly expressed in the cardiovascular system [4]. *CYP2J2* can catalyze the epoxidation of arachidonic acid to epoxyeicosatrienoic acids (EETs), which play an essential role in the regulation of cardiovascular inflammation [5], and possess potent vasodilatory, anti-apoptotic properties in the cardiovascular system [6, 7].

Genetic polymorphisms in CYP genes are able to cause individual and population variations in the tolerance to toxins and drugs [8, 9]. To date, human *CYP2J2* has ten reported polymorphisms, which can significantly influence the metabolism of arachidonic acid [10]. The *CYP2J2*2* (T143A), *CYP2J2*3* (R158C), *CYP2J2*4* (I192N) and *CYP2J2*6* (N404Y) exhibited statistically significant reductions in the enzyme activity. Likewise, *CYP2J2*7* (G50T) polymorphism in the proximal promoter dis-

**Table 1.** Primers used to amplify regions of *CYP2J2*

| Primer name | Primer Sequence (5'-3') | Primer name | Primer Sequence (5'-3') | PCR product size (bp) |
|---|---|---|---|---|
| UTR&Exon1_F | ACAGCAAGATGAGACTACCGAG | UTR&Exon1_R | CCAGGTTACCAGCGTTAGCC | 783 |
| Exon2_F | CTCATGCCTTGCTCTAGGGAC | Exon2_R | CACGTTCCTCTGCTATAAATGGGT | 779 |
| Exon3_F | GTGCATTCCTAGTGTTTACCATAAC | Exon3_R | TGCCCATCTTTGTGTATTTACTTCT | 788 |
| Exon4_F | AGCATTGCATATGACAGAGGTGAG | Exon4_R | AGACTCAAGGGCAACAGCAAT | 856 |
| Exon5_F | AACACTCAACCAGTGCTCAGAT | Exon5_R | GAGAAGATGCTGTGCTTCTGG | 776 |
| Exon6_F | CAAATCTGTCTCGTTCACATCC | Exon6_R | ATACCAGACTAAAGTGCTTGAAC | 827 |
| Exon7_F | GAGCTGCCTCACTCCTTCTAC | Exon7_R | CTGACCTAGAACTGCTGCCTG | 850 |
| Exon8_F | CCAAGCCCTACTGAAACTGACC | Exon8_R | TTTCCAGAGGACAGAACACAGG | 688 |
| Exon9_F | CTTCTATGGTCCTACACCCTGC | Exon9_R | ACCACTTTGACTTGAGCTTCTC | 869 |
| Exon9&UTR_F | CCCAGCTCTACTGTCTCGTC | Exon9&UTR_R | GCAACGGAGCAAGACACTAC | 778 |

rupts a Sp1 transcription factor binding site and leads to reduced *CYP2J2* transcription [11]. However, so far the detailed studies on *CYP2J2* genetic polymorphisms in the Chinese is not sufficient, especially in the ethnic minorities is still unknown and alluring.

The Wa is one of the oldest ethnic minorities in China, with a population of 429709 (according to population survey of China in 2010), live mostly in the backward mountains area of Yunnan Province, China. Geographically isolated from other ethnic groups in the mountains region result in Wa people rarely intermarries with other Chinese ethnic groups. Because information regarding *CYP2J2* polymorphisms is unknown in the Wa population, the main aim of this study was to investigate the distribution of *CYP2J2* genetic variations in Wa individuals, and the secondary aim was to compare their allelic frequencies with previous observations of other ethnic groups. Our results will provide a better understanding of *CYP2J2* variants in the Wa population.

## Materials and methods

### *Subjects*

The study subjects consisted of 100 unrelated Wa subjects (including 50 males and 50 females, age range 18-52) from Yunnan Province, China. The subjects selected were judged to be of good health by medical examination and had exclusively Wa ancestry for at least the last three generations. The subjects were also thought to be representative samples of the Wa population with regard to ancestry and environmental exposures. Volunteers with any type of medical illness, organ transplant, drug or alcohol addiction were excluded from the study.

The study was approved by Institutional Ethical Committee of Northwest University and conducted in compliance with the ethical principles for medical research involving human subjects of the Helsinki Declaration. All participants gave their written informed consent prior to enrollment in the study.

### *Genotyping of CYP2J2*

Genomic DNA was extracted from peripheral blood obtained from the subjects using the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag Ltd., Xi'an, China) according to the manufacturer's protocol. Primers listed in **Table 1** were designed to amplify the 5' flanking regions, all exons, and all introns of the *CYP2J2* gene. The polymerase chain reaction (PCR) was performed in a total volume of 10 mL containing 1 mL genomic DNA (20 ng/mL), 5 mL HotStar TaqMasterMix (QIAGEN, Germantown, MD), 0.5 mL each primer pair (5 mM), and 3 mL deionized water. The cycling protocol consisted of an initial denaturation step for 10 min at 95°C, followed by 35 cycles each consisting of the following steps: denaturation for 1 min at 95°C, then annealing for 45 s at 55-64°C, and extension for 1 min at 72°C, another last extension for 7 min at 72°C. The PCR products were purified by incubating with 0.5 mL shrimp alkaline phosphate (Roche, Basel, Switzerland), 1.5 mL deionized water, and 8 mL HotStar PCR product, for a total vol-

**Table 2.** Frequency distribution of *CYP2J2* polymorphisms in 100 Wa subjects

| NO. | SNP | Position | Nucleotide change | Region | Allele | Frequencies | Amino-acid effect |
|---|---|---|---|---|---|---|---|
| 1 | rs2229189 | 183 | C>T | Exon 1 | *1 | 0.04 | Phe61Phe[a] |
| 2 | rs3820538 | 10522 | C>T | Intron 1 | *1 | 0.07 | No translated |
| 3 | rs11572245 | 10982 | G>C | Intron 2 | *1 | 0.13 | No translated |
| 4 | rs149199403 | 10984 | G>A | Intron 2 | *1 | 0.06 | No translated |
| 5 | rs529370939 | 15016 | G>A | Exon 4 | Novel | 0.03 | Val188Ile[b] |
| 6 | rs1570693 | 15285 | A>C | Intron 4 | *1 | 0.15 | No translated |
| 7 | rs1155002 | 18644 | G>A | Intron 5 | *1 | 0.72 | No translated |
| 8 | rs2271800 | 18753 | T>G | Intron 5 | *1 | 0.19 | No translated |
| 9 | rs2229191 | 18919 | C>A | Exon 6 | *1 | 0.1 | Arg321Arg[a] |
| 10 | / | 19109 | C>T | Intron 6 | Novel | 0.01 | No translated |
| 11 | rs2271798 | 19114 | T>C | Intron 6 | *1 | 0.19 | No translated |
| 12 | rs4388726 | 33266 | C>T | 3'UTR | *1 | 0.07 | No translated |
| 13 | rs2280274 | 33345 | T>A | 3'UTR | *1 | 0.07 | No translated |
| 14 | / | 33885 | A>T | 3'UTR | Novel | 0.04 | No translated |

[a]synonymous mutations; [b]non-synonymous mutations.

ume of 10 mL, at 38°C for 30 min, followed by heat inactivation at 80°C for 15 min. The purified PCR products were directly sequenced using an ABI Prism BigDye Terminator Cycle Sequencing Kit version 3.1 (Applied Biosystems Inc., Foster City, CA), on an ABI Prism 3100 sequencer (Applied Biosystems).

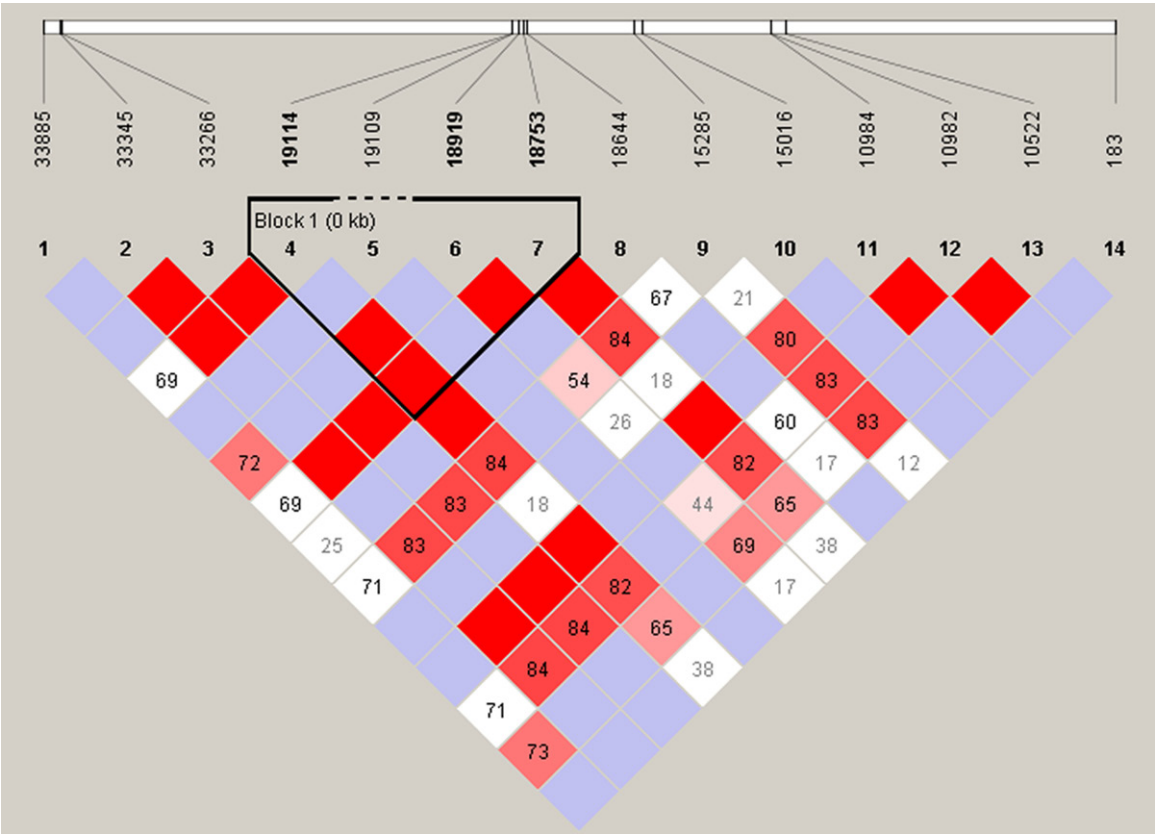
*HapMap genotype data*

The genotype data of individuals from eleven populations were downloaded from the International HapMap Project web site (HapMap_release127) at http://hapmap.ncbi.nlm.nih.gov/biomart/martview/. The eleven populations included those of (1) African ancestry in Southwest USA (ASW); (2) Utah, USA residents with Northern and Western European ancestry from the CEPH collection (CEU); (3) Han Chinese in Beijing, China (CHB); (4) Chinese in metropolitan Denver, CO, USA (CHD); (5) Gujarati Indians in Houston, Texas, USA (GIH); (6) Japanese in Tokyo, Japan (JPT); (7) Luhya in Webuye, Kenya (LWK); (8) Mexican ancestry in Los Angeles, California, USA (MEX); (9) Maasai in Kinyawa, Kenya (MKK); (10) Toscani in Italy (TSI); and (11) Yoruba in Ibadan, Nigeria (YRI).

*Statistical analysis*

We used Microsoft Excel (Redmond, WA, USA) and SPSS 17.0 statistical packages (SPSS, Chicago, IL, USA) to perform statistical calculations. The Sequencher 4.10.1 (http://www.genecodes.com/) software (Gene Codes Corporation, Ann Arbor, MI) was used for our initial analysis of the sequences including base calling, fragment assembly, and detection of SNPs, insertions, and deletions. The *CYP2J2* variants were named based on NCBI Reference Sequence: AF272142.1 and CYP allele nomenclature (http://www.cypalleles.ki.se/). Haploview software (version 4.2) was used to assess linkage disequilibrium (LD) and Hardy-Weinberg equilibrium for each genetic variant [12].

The Arlequin (version 3.1) software was used to calculate the value of F-statistics (Fst) to infer the pairwise distance between Wa and the other eleven populations [13]. Fst is directly related to the variance in allele frequency among populations and to the degree of resemblance among individuals within populations. If Fst is small, it means that the allele frequencies within each population are similar; if it is large, it means that the allele frequencies are different. Additionally, we calculated and compared the genotype frequencies of the variants in the Wa data with those in the eleven populations separately using the chi-squared test [14]. All *p* values obtained in this study were two-sided, and Bonferroni's adjustment for multiple tests was applied to the level of significance, which was set at $P<0.05/(5*11)$. The purpose of the chi-squared test was to discover sites with significant differences.




**Fiugre 1.** Linkage disequilibrium analysis of *CYP2J2*. 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.

Afterwards, we downloaded the SNP allele frequencies from the Allele FREquency Database (http://alfred.med.yale.edu, ALFRED) and observed the global distribution of genetic variation at specific loci.

*Transcriptional prediction*

We analyzed novel non-synonymous SNP in the *CYP2J2* coding regions to predict the corresponding protein function. Two online tools, SIFT (Sorting Intolerant From Tolerant, http://sift.bii.a-star.edu.sg/) and PolyPhen-2 (Polymorphism Phenotyping v2, http://genetics.bwh.harvard.edu/pph2/), were used to perform the functional prediction of non-synonymous SNPs [15]. Each variant was given a score based on the impact of its mutation on protein function. The SIFT divided results into four categories based on these scores: 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 three categories: benign, possibly damaging and probably damaging.

## Results

*Genetic variants*

Fourteen different *CYP2J2* polymorphisms were determined in our study subjects, three of which were novel: 15016G>A, 19109C>T and 33885A>T. 15016G>A was a non-synonymous mutation identified in exon 4, 19109C>T and 33885A>T were not translated. All of the three variants have not previously been reported in the NCBI database or in the Human CYP Allele Nomenclature Committee tables (**Table 2**).

*Linkage disequilibrium analysis*

We performed LD analysis using Haploview with confidence intervals to define LD blocks. The extent of LD for each pair of SNPs was measured by the D' value, By default, our meth-

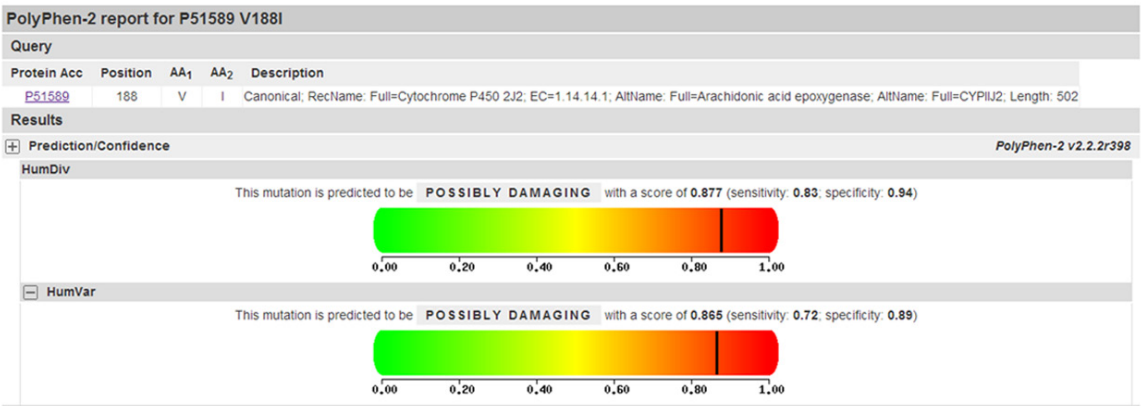



**Figure 2.** Predicted protein function of the novel mutation 15016G>A by PolyPhen-2.

**Table 3.** Fst values between population pairs

| | Wa | ASW | CEU | CHB | CHD | GIH | JPT | LWK | MEX | MKK | TSI | YRI |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Wa | 0 | | | | | | | | | | | |
| ASW | 0.41913 | 0 | | | | | | | | | | |
| CEU | 0.36014 | 0.15228 | 0 | | | | | | | | | |
| CHB | 0.45241 | 0.1207 | 0.02383 | 0 | | | | | | | | |
| CHD | 0.46915 | 0.15961 | 0.02388 | -0.00206 | 0 | | | | | | | |
| GIH | 0.52069 | 0.22181 | 0.07257 | 0.02811 | 0.01668 | 0 | | | | | | |
| JPT | 0.44688 | 0.0687 | 0.06471 | 0.01738 | 0.03628 | 0.06414 | 0 | | | | | |
| LWK | 0.4341 | -0.00204 | 0.1872 | 0.15188 | 0.19247 | 0.2539 | 0.09659 | 0 | | | | |
| MEX | 0.39433 | 0.11493 | 0.02233 | 0.00404 | 0.00842 | 0.02913 | 0.01473 | 0.14693 | 0 | | | |
| MKK | 0.39552 | 0.0023 | 0.14625 | 0.11855 | 0.151 | 0.20436 | 0.08298 | 0.00117 | 0.12017 | 0 | | |
| TSI | 0.33262 | 0.11406 | -0.00075 | 0.02997 | 0.03682 | 0.0968 | 0.05879 | 0.14462 | 0.02496 | 0.11138 | 0 | |
| YRI | 0.46746 | 0.00786 | 0.22248 | 0.17736 | 0.21678 | 0.26663 | 0.11161 | 0.00171 | 0.17445 | 0.01796 | 0.18491 | 0 |

od ignores markers with MAF<0.05; SNPs with lower frequencies have little power to detect LD. Haplotype analysis identified one LD blocks within *CYP2J2*, and very strong linkage was found between rs2271800, rs2229191, novel variant 19109C>T, and rs2271798 (**Figure 1**).

*Predicted protein function of the non-synonymous mutation*

Three novel variants were detected in our study, only one mutation (15016G>A) was non-synonymous (Val188Ile). Analysis using SIFT of the Val188Ile variant indicated that it was intolerant (score=0.05). PolyPhen-2 results for Val188Ile reveal that it was possibly damaging. PolyPhen-2 utilized two models (HumDiv and HumVar), in which the latter is more rigorous in its false discovery rate. So the HumVar dataset was usually used to predict protein function (**Figure 2**). The protein function prediction results from SIFT and PolyPhen-2 analysis of the Val188Ile were highly consistent.

*Inter-ethnic differences*

We downloaded the genotyping data of five loci of eleven populations from Hapmap, and compared them with our data. Pairwise Fst values were calculated for all population comparisons across loci. As shown in **Table 3**, we found that pairwise Fst values for comparisons of the Wa population with the other 11 populations ranged from 0.36014 to 0.52069. The value of Fst for the Wa and CEU populations was the smallest.

Using the chi-squared test with the Bonferroni correction for multiple hypotheses and multiple comparisons (**Table 4**), we found that rs11572245 in the different frequency distributions when the Wa population was compared to the ASW, CEU, CHB, CHD, GIH, JPT, LWK, MEX,

**Table 4.** Significant variants in Wa compared to the 11 populations, as determined by Chi-square test

| SNP | Chi-square test *p* values (after Bonferroni correction) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | ASW | CEU | CHB | CHD | GIH | JPT | LWK | MEX | MKK | TSI | YRI |
| rs1155002 | / | / | 4.666 E-04 | 5.548 E-04 | 4.462E-09 | 1.467E-08 | 3.762E-05 | / | / | / | 9.377E-08 |
| rs11572245 | 1.332E-28 | 1.266E-37 | 5.272E-38 | 3.178E-38 | 2.117E-38 | 1.804E-36 | 1.278E-34 | 1.779E-27 | 2.71E-46 | 1.721E-32 | 3.446E-41 |
| rs2271798 | 2.142E-08 | / | / | / | / | / | 3.976E-13 | / | 6.88E-13 | / | 1.468E-15 |
| rs2271800 | 4.083E-11 | / | / | / | / | / | 8.004E-16 | / | 4.922E-15 | / | 1.709E-16 |
| rs4388726 | / | / | / | / | / | / | / | / | / | / | / |

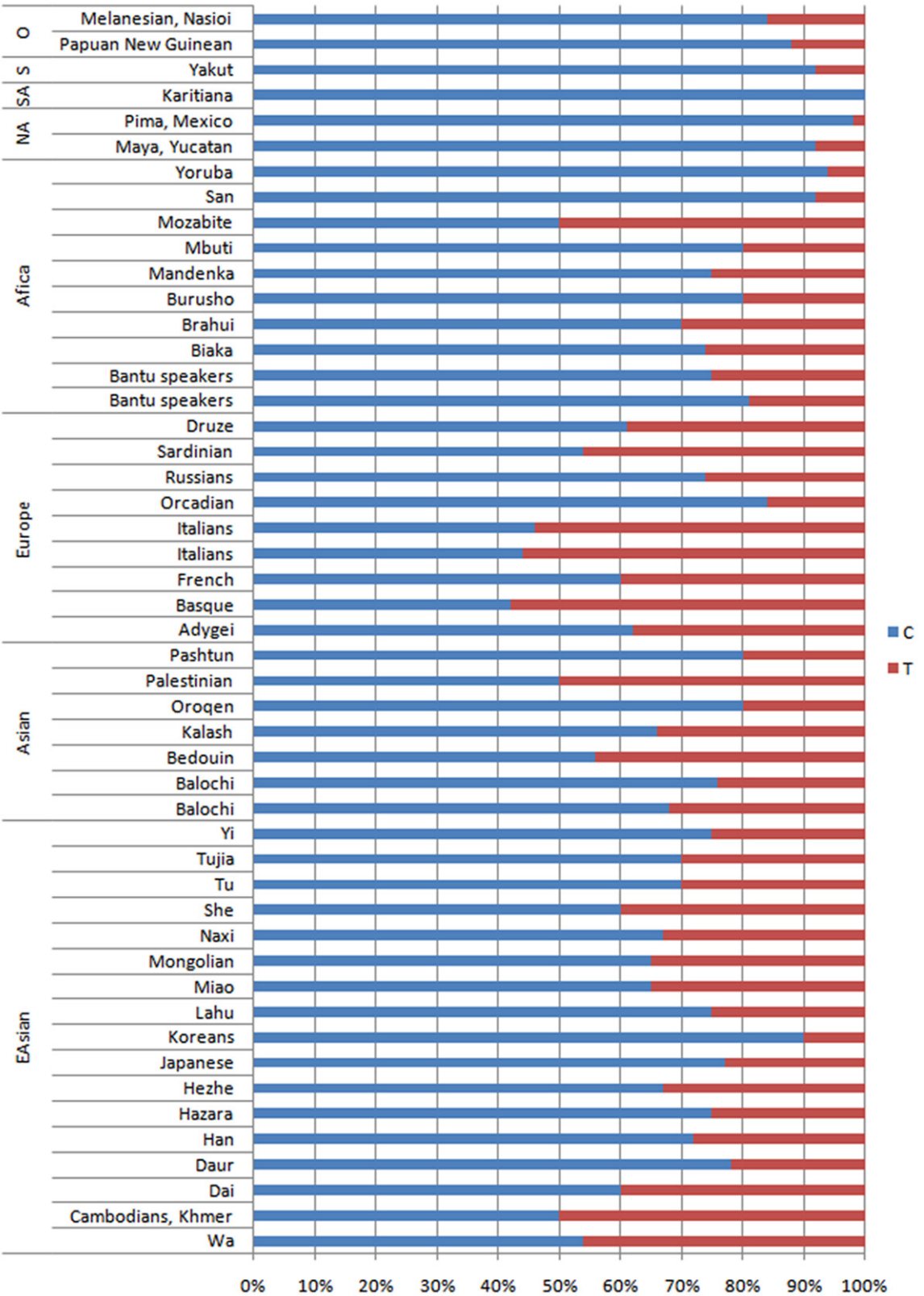



**Figure 3.** Rs1155002 frequencies in populations from different regions of the world. EAsia, East Asia; NA, North America; SA, South America; S, Siberia; O, Oceania.

MKK, TSI, and YRI populations, respectively (P< 0.00091).

Then we downloaded the data pertaining to rs1155002 from ALFRED, and combined our new data for a global analysis (**Figure 3**). These data indicate that the frequencies of this variant in the Wa people are higher than most of Asia populations, but lower than most of Europe populations. The frequencies in American, Oceania, and Siberia are relatively lower compared to other populations.

## Discussion

To better understand the distribution of *CYP2J2* allele frequency in the Wa population, we sequenced the whole *CYP2J2* gene in 100 Wa subjects from Yunnan Province of China. Our study, for the first time, systematically screened for variants of *CYP2J2* by directly sequencing among the Wa and compared the results with other ethnic populations around the world. Our results provide a better understanding of *CYP2J2* variants and a potential database for promoting personalized medicine in the Wa population.

We identified 14 genetic variants including three novel polymorphisms in our study Wa Chinese population. Analysis of novel genetic polymorphisms in the coding region revealed that variant 15016G>A could influence the protein structure and function, and the results of SITF and PolyPhen-2 were highly consistent. The prediction accuracy of PolyPhen-2 and SIFT is only 75% and 63% [15, 16], so the results identified here should be confirmed by other means in further studies. We also analyzed the pattern of LD in *CYP2J2* among Wa subjects and observed one block. In order to see the differences in the LD structure, we compared the LD plots between our study and a previous study of Caucasians [17], but the LD plots did not have much in common. This may be explained by the fact that Wa people live in mountains area and have very different lifestyles, culture, and history from Caucasians.

Previous studies showed that population genetic structure is important to the study of DNA forensics, complex diseases, and human origins [18], and Fst analyses is common in population genetic studies [19]. Using the Fst calculations, we found that pairwise Fst values for comparisons of the Wa population with the other 11 populations ranged from 0.36014 to 0.52069. These data suggested that Wa population is not similar with other eleven populations, even the CHB populations. Although the value of Fst for the Wa and CEU populations was the smallest 0.36014, we don't think that the genetic backgrounds of these two populations are similar.

From the chi-squared test, we found clear evidence that the allele characteristics of the rs11572245 variant in the Wa population is quite different from that in other ethnic groups. However, there have been no previous studies showing the population difference on this SNP; in fact, studies on this loci is rare, so the result identified here should be used in the further pharmacogenomics studies.

Rs1155002 is an important mutation in *CYP2J2* that have been identified. In a previous study on genetic polymorphisms on *CYP2J2* associated with hypertension [20], the researchers pointed out that TT homozygote of rs1155002 is a risk factor for hypertension (P=0.014). In another association study on aneurysmal subarachnoid hemorrhage [21], the authors selected rs1155002 as a tag SNP, but they have found no significant results on this SNP. In our study, we combined our genotyping data of rs1155002 with the data downloaded from ALFRED datebase, sorted these data by geographical distribution. The result showed that although in the same continent, there are some differences among diverse ethnic groups. This suggests that ancestry should be considered when determining dosages for different patients.

In conclusion, our data provide new information regarding *CYP2J2* genetic polymorphisms in Wa individuals. Future studies will focus on identifying *CYP2J2* variants in a larger sample size of Wa and some other ethnic minorities in China, leading to the improved application of personalized medicine in different group in China.

## Acknowledgements

This work is supported by Major science and technology research projects of Xizang (Tibet) Autonomous Region (2015) and The National Natural Science Foundations (No. 81560516).

## Disclosure of conflict of interest

None.

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