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Original Article

Analysis of *CYP2J2* mutations in the Chinese Uyghur population

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Abstract: Genetic characteristics of *CYP2J2* in different populations may be helpful to explore interethnic variability in drug response and disease susceptibility. There is no information about the genetic profile of *CYP2J2* in the Chinese Uyghur population. We used PCR and first-generation sequencing technology to investigate *CYP2J2* mutations in 100 unrelated healthy Chinese Uyghurs. The chi-square test was used to compare genotyping data of *CYP2J2* in the Chinese Uyghur population with other ethnic groups. The SIFT and PolyPhen-2 online tools were used to predict the protein function of the novel nonsynonymous mutations in *CYP2J2*. CADD software was used to predict pathogenicity of the mutations. We found twenty-eight mutations in *CYP2J2*, five new mutations, three alleles (**1*, **7* and **8*), and three genotypes (**1/*1*, **1/*7* and **1/*8*) in the Chinese Uyghur population. The allele frequencies of *CYP2J2 *1*, **7* and **8* were 96%, 3.45%, and 0.5%, respectively. Interethnic comparison found that subgenotype **1* in Uyghur was significantly higher than in Taiwanese and African Americans, and **7* frequency in Uyghur was slightly lower than that in Taiwanese and African Americans ($P<0.05$); *8 was only found in Chinese Uyghur and Korean populations, with frequencies of 0.5% and 0.8%, respectively. Furthermore, the protein prediction results revealed that the five nonsynonymous mutations could influence protein structure and function. The observations of this study give rise to useful information on *CYP2J2* mutations in Chinese Uyghurs, which may support future important clinical implications for the use of medications metabolized by *CYP2J2*.

Keywords: *CYP2J2*, genetics, mutations, Chinese Uyghur population, Polymorphisms

Introduction

The cytochrome P450 (CYP) enzymes play central roles in catalyzing oxidative reactions and bioactivation, and account for approximately 75% of the total drug metabolism [1]. The study of CYP enzymes has been the focus of toxicologists and pharmacologists. Currently, fifty-seven CYP genes involving three families (*CYP1*, *CYP2* and *CYP3*) have been demonstrated to contribute to the oxidative metabolism of various compounds [2]. CYP2J2, which belongs to the CYP family 2, subfamily J, polypeptide 2, is an important member of the CYP superfamily that catalyzes epoxide formation at the site of a carbon-carbon double bond in the substrate. This gene is located at human chromosome 1p31.3-p31.2 (approximately 40.3 kb), and contains nine exons and eight introns. Moreover, nine variants have been described in detail: *CYP2J2**2 to *10 (https://www.pharmvar.org/gene/*CYP2J2*), while many (8827) single-nucleotide polymorphisms (SNPs) in the 3'-untranslated region, 5'-regulatory region, and introns of *CYP2J2*, as well as missense SNPs, have also been reported (https://www.ncbi.nlm.nih.gov/snp/?term=CYP2J2) [3]. The CYP2J2 gene was found to be highly expressed in the cardiovascular system [4]. *CYP2J2* has a prominent role in cardiac protection because of its ability to catalyze arachidonic acid (AA) to epoxyeicosatrienoic acids that possess potent anti-inflammatory, vasodilatory, and fibrinolytic properties [5].

Variations in the coding regions of the *CYP2J2* gene may lead to changes in *CYP2J2* expression and/or enzymatic activity and result in altered CYP2J2-dependent metabolism of AA, which ultimately causes abnormal heart func-

tion. For example, the SNP rs890293 of *CYP2J2*7* is the most common known functional CYP2J2 variant. It can disrupt a binding site for the SP1 transcription factor, lead to both decreased promoter activity in vitro and reduced circulating levels of CYP2J2 epoxygenase metabolites and is associated with an increased risk of coronary artery disease (CAD) [6]. A recent study identified a novel polymorphism (*CYP2J2*8*) that results in a functional loss of enzyme catalytic activity [7]. In addition, interindividual variability in drug response depends on a number of genetic and environmental factors. Metabolic enzymes are well known for their contribution to this variability due to drug-drug interactions and genetic polymorphisms. The polymorphic nature of CYP genes affects individual drug responses and adverse reactions to a great extent [8]. It has been reported that allele G of rs2294950 (*CYP2J2*) was possibly associated with a decreased cardiotoxicity when treated with daunorubicin or doxorubicin in children with neoplasms compared to allele T [9]. A previous study showed that the genotype AA + AC of rs890293 (*CYP2J2*7*) was associated with an increased risk of nausea and vomiting when treated with tacrolimus in people with Kidney transplantation as compared to genotype CC [10].

Therefore, exploration of CYP gene profiles in different populations will be useful for attenuating individual risk for adverse drug reactions. With the elucidation of the human genome sequence, *CYP2J2* genetic traits in different ethnic groups have been reported. There are still insufficient data available about *CYP2J2* genetic polymorphisms in China, especially in different Chinese ethnic minorities. Uyghurs are one of the oldest ethnic minorities in China. The population originated in Eurasia, with anthropometric and genetic characteristics of East and West Eurasia [11]. In this study, we systematically screened the promoter, the 3'-UTR and exon regions and partial sequences flanking exon-intron junctions of the *CYP2J2* gene from 100 unrelated healthy individuals of the Chinese Uyghur population to explore their mutations and compared their variability frequency with previous observations from other ethnic groups. We hope the present work will contribute to prediction of the potential risks of drug toxicity and individualized therapy based on *CYP2J2* genetic polymorphisms.

Materials and methods

Study population

A total of 100 unrelated healthy volunteers (aged 19-52 years), including 50 males and 50 females, were randomly recruited in this prospective study. The inclusion and exclusion criteria were as follows: The subjects selected were assessed to be in good health by medical examination, including biochemistry, hematology and immunology testing. All participants were Chinese Uyghurs, and their ancestors had lived in the Xinjiang Autonomous Region for at least three generations. Individuals who had any type of medical disease, were pregnant or lactating, had drug or alcohol addiction, and underwent organ transplant were excluded from the study.

Ethics approval and consent to participate

The study protocol and consent form were reviewed and approved by the Ethics Committee of Xizang Minzu University (No. 2019-12). This study was conducted in compliance with the ethical principles for medical research involving human subjects of the Helsinki Declaration. All participants provided written informed consent prior to study enrollment.

DNA extraction

Peripheral venous blood samples (5 mL) were taken into vacutainer tubes containing ethylenediaminetetraacetic acid (EDTA) from each subject during their routine health examination. Then, the blood samples were stored at -20°C until use. Genomic DNA was extracted from the blood samples using the GoldMag-Mini Whole Blood Genomic DNA Purification Kit (GoldMag. Co. Ltd., Xi'an, China) following the manufacturer's standard procedures. DNA concentration and purity were evaluated using a spectrophotometer (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, USA).

SNP genotyping

Amplification primers for the promoter region, the 3'-UTR, all exons and intron-exon boundaries of the *CYP2J2* gene were designed by online software Primer 3 Input (version 4.1.0) and synthesized by Sangon Biotech (Shanghai, China). Primers for PCR (polymerase chain reaction)

Table 1. Primers for *CYP2J2* gene amplification and sequencing

Fragment name	Forward primer sequence (5'-3')	Reverse primer sequence (5'-3')	PCR Product size (bp)
UTR&Exon1	ACAGCAAGATGAGACTACCGAG	CCAGGTTACCAGCGTTAGCC	783
Exon2	CTCATGCCTTGCTCTAGGGAC	CACGTTCCTCTGCTATAAATGGGT	779
Exon3	GTGCATTCCTAGTGTTTACCATAAC	TGCCCATCTTTGTGTATTTACTTCT	788
Exon4	AGCATTGCATATGACAGAGGTGAG	AGACTCAAGGGCAACAGCAAT	856
Exon5	AACACTCAACCAGTGCTCAGAT	GAGAAGATGCTGTGCTTCTGG	776
Exon6	CAAATCTGTCTCGTTCACATCC	ATACCAGACTAAAGTGCTTGAAC	827
Exon7	GAGCTGCCTCACTCCTTCTAC	CTGACCTAGAACTGCTGCCTG	850
Exon8	CCAAGCCCTACTGAAACTGACC	TTTCCAGAGGACAGAACACAGG	688
Exon9	CTTCTATGGTCCTACACCCTGC	ACCACTTTGACTTGAGCTTCTC	869
Exon9&UTR	CCCAGCTCTACTGTCTCGTC	GCAACGGAGCAAGACACTAC	778

UTR: Untranslated Region, PCR: Polymerase Chain Reaction.

amplification and sequencing are shown in **Table 1**. Each PCR was performed in a 10 μL volume system including 5 μL HotStar Taq Master Mix (Qiagen, Germany), 0.5 μL each primer (0.25 μM), 3 μL RNase-free water and 1 μL template genomic DNA (20 ng/μL). The PCR product that had a single band of the expected size, which was observed on agarose gel, was regarded as qualified. Subsequently, PCR products were purified using a TIANgel Midi Purification Kit (TIANGEN, Beijing, China) and sequenced using an ABI BigDye Terminator Cycle Sequencing Kit (version 3.1, Applied Biosystems, Thermo Fisher Scientific, Inc., USA) on an ABI Prism 3100 sequencer (Applied Biosystems, Thermo Fisher Scientific, Inc., USA).

Statistical analysis

Microsoft Excel (Redmond, WA, USA) and SPSS 20.0 statistical packages (SPSS, Chicago, IL, USA) were used to perform statistical calculations. The initial analysis of the sequences, including base calling, fragment assembly, and detection of SNPs, insertions, and deletions, was performed by Sequencher 4.10.1 software (http://www.genecodes.com/). The main process includes creating a new project, importing data, trimming sequences, assembling a contig, viewing contig assembly, editing assembled chromatograms, finding heterozygotes, working with a reference sequence, translating sequences to amino acids, annotating a sequence, creating a variance table and report and creating a translation variance table. We used Blast online software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to screen the actual genetic differences. Specific steps: Selecting species (human), Nucleicacid BLAST in BLAST interface; Entering FASTA format sequence; Setting Blast parameters; Running; Alignment results. The *CYP2J2* variants were identified and named based on the *CYP2J2* nucleotide reference sequence NG_007931.1 in the National Center for Biotechnology Information (NCBI) database and CYP allele nomenclature (http://www.cypalleles.ki.se/). Statistical differences in the distributions of allele frequency between the Chinese Uyghur and other ethnic populations were evaluated using the chi-square test. A P value <0.05 was considered statistically significant, and all statistical tests were two-sided. We used the χ^2 test to examine Hardy-Weinberg equilibrium (HWE) for each genetic variant. Haploview software version 4.2 was used for analyses of linkage disequilibrium (LD) and haplotype reconstruction. The specific operation process is as follows: preparing ped (genotype data) and info files (rs-id and location); opening Hapolview software; importing data: "Data File (ped file), Locus Information File (info file)"; selecting "Do association test-Case/Control data"; saving the LD figure.

Prediction of phenotypic impact of identified variants

SIFT (sorting intolerant from tolerant, http://sift.bii.a-star.edu.sg/) and PolyPhen-2 (polymorphism phenotyping version 2, http://genetics.bwh.harvard.edu/pph2/) were used to predict the effects of coding nonsynonymous variants on protein function. Each variant was given a score based on its predicted impact on protein function. The SIFT output results were divided 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) [12]. The PolyPhen-2 results were divided into three categories: benign, possibly damaging and probably damaging [13]. We used combined annotation-dependent depletion (CADD) (https://cadd.gs.washington.edu/snv) to predict pathogenicity for variants in the *CYP2J2* gene. A high CADD score indicates that the variant may be associated with a higher probability of pathogenicity.

Results

Genetic variants

A total of twenty-eight different mutations in *CYP2J2* were determined by direct sequencing in the present study concerning the Chinese Uyghur population. The identified SNPs, as well as their position, region, nucleotide change, allele, amino acid impact, flanking sequence and the corresponding frequencies, are listed in **Table 2**. Rs1155002 (NM_000775.4: c.862-176 A>G) located in intron 5 was found to have the highest frequency (48%), followed by rs1570693 (NM_000775.4:c.684+147A>C) in intron 4, with a frequency of 34%. We found five novel mutations, the detailed chromosome number, genomic position and nucleotide change: 1:59927018 G>C, 1:59916083 G>A, 1:59911724 A>G, 1:59911684 A>G and 1:59893936 C>T, which have not previously been reported in either the NCBI database or the Human CYP Allele Nomenclature Committee tables. Among them, 1:59911724 A>G and 1:59911684 A>G within exon 4 caused amino acid alterations. Four SNPs were found to cause synonymous variants, and five resulted in nonsynonymous variants. The variant rs890293 located in the *CYP2J2* promotor at a frequency of 7% was reported to compromise the binding site of the Sp1 transcription factor and led to decreased transcription and consequently lower enzyme activity, as published before [5].

Allele and genotype frequencies

CYP2J2 alleles were detected in the Chinese Uyghur population (**Table 3**), including *CYP2J*1*, *CYP2J2*7* and *CYP2J2*8*. The reference allele, *CYP2J2*1,* had the highest frequency of 96.0%. *CYP2J2*7* had a frequency of 3.5%. In contrast, the frequency of *CYP2J2*8* was only 0.5% and was considered rare. We further identified three *CYP2J2* genotypes in the Chinese Uyghur population, including *CYP2J2*1/*1*, *CYP2J2*1/*7*, and *CYP2J2*1/*8*. Individuals with the wild-type *CYP2J2*1/*1* genotype have normal enzyme activity, and this genotype was found at a frequency of 92% in our study. The frequencies of heterozygous genotypes *CYP2J2*1/*7* and *CYP2J2*1/*8* were 7% and 1%, respectively. All allele and genotype distributions conformed to HWE.

Interethnic comparison

To understand the interethnic variabilities of *CYP2J2* genetic profiles, we further compared *CYP2J2* distribution patterns between our Chinese Uyghur population and previously published other major populations, including East Asians, Caucasians and Africans (**Table 4**). The allele frequency of *CYP2J2*1* in the Chinese Uyghur population was significantly higher than that in Taiwanese and African Americans ($P< 0.05$). Furthermore, we found that the allele frequency of *CYP2J2*7* in the Chinese Uyghur population was relatively lower than that in Taiwanese (12%) and African Americans (11.27% and 13.7%). The allele frequencies of *CYP2J2*7* in Mongolians (3.39%) and Tatars (3.65%) were both similar to our results. The allele frequency of *CYP2J2*8* was 0.5% and 0.8% in the Chinese Uyghur population and Korean population, respectively.

Linkage disequilibrium analysis

We used Haploview to evaluate linkage disequilibrium (LD) between SNPs with a minor allele frequency higher than 5%. Two LD blocks in the *CYP2J2* gene were determined. Strong LD was observed between each pair of rs4388726 (33266 T>A), rs2280273 (33440 A>G) and rs2271798 (19114 T>C) in block 1 (**Figure 1**). The three SNPs rs3820538 (10522 C>T), rs11572245 (10982 G>C) and rs1570693 (15285 A>C) were also found to have strong LD in block 2. The LD between each pair of SNPs is the standardized deviation (D'). Bright red corresponds to a very strong LD, blue corresponds to intermediate LD, and white corresponds to no LD.

Predicted protein function for nonsynonymous mutations

We found five nonsynonymous mutations in the present study, including 1:59911724 A>G (p. Asn190Ser), rs201070738 (p. Arg200Cys),

Table 2. Frequencies distributions of *CYP2J2* genetic polymorphisms in the Uygur population

SNP-ID	Gene position	Chr: genomic position	HGVS name	Amino-acid change	Region	Allele	Flanking Sequence	Frequency (%)
/	-273	1:59927018	/	No translated	Promoter	Novel	TCATAGGAGA S ACGGTGATTG	1
rs890293	-76	1:59926822	NG_007931.1:g.4930G>T	Decreased	Promoter	*7	GGCTGGGAGC K AGGCGGGGCG	7
rs11572191	148	1:59926599	NM_000775.4:c.148C>T	p.Leu50=[a]	Exon 1		GCCCTGGCGC Y TGCCCTTCCT	5
rs2229189	183	1:59926564	NM_000775.4:c.183C>T	p.Phe61=[a]	Exon 1		TTGTGGACTT Y GAGCAGTCGC	2
rs778101462	242	1:59926505	NM_000775.4:c.210+32T>C	No translated	Intron 1		TAGCGTGTCC Y GACCCTAACT	2
rs3820538	10522	1:59916225	NM_000775.4:c.211-125C>T	No translated	Intron 1		CACACACACA Y GTACACACAC	14
/	10664	1:59916083	/	p.Gly76=[a]	Exon 2	Novel	AGAAATATGG R AACCTTTTTA	1
rs3738474	10835	1:59915912	NM_000775.4:c.373+26G>A	No translated	Intron 2		AACGAAAGGT R AGTGTTTGAT	5
rs11572245	10982	1:59915765	NM_000775.4:c.373+173G>C	No translated	Intron 2		GTCACTCCCT S AGAAGATTTG	17
rs149199403	10984	1:59915763	NM_000775.4:c.373+175G>A	No translated	Intron 2		TCACTCCCTGA R AAGATTTGAT	3
/	15023	1:59911724	/	p.Asn190Ser[b]	Exon 4	Novel	GCAGTTTCCA R TATCATTTGC	1
rs201070738	15052	1:59911694	NM_000775.3:c.598C>T	p.Arg200Cys[b]	Exon 4		CTTCGGAGAA Y GCTTTGAGTA	1
/	15062	1:59911684	/	p.Tyr203Cys[b]	Exon 4	Novel	CGCTTTGAGT R CCAGGATAGT	1
rs1570693	15285	1:59911461	NM_000775.4:c.684+147A>C	No translated	Intron 4		TATTTGAAAT M AATCTATTGA	34
rs1155002	18644	1:59908103	NM_000775.4:c.862-176A>G	No translated	Intron 5		GGGCAGGACA R TGCTAATGAT	48
rs2271800	18753	1:59907994	NM_000775.4:c.862-67T>G	No translated	Intron 5		TGAAGCCCCT K TGTGTTACGG	39
rs150461093	18892	1:59907855	NM_000775.4:c.934G>A	p.Gly312Arg[b]	Exon 6	*8	CTTCTTTGCC R GAACCGAGAC	1
rs2229191	18919	1:59907855	NM_000775.4:c.961C>A	p.Arg321=[a]	Exon 6		CACAACTCTG M GATGGGCTCT	10
rs2271798	19114	1:59907633	NM_000775.4:c.1003+153T>C	No translated	Intron 6		ACATTCTTCA Y ATTTTCTGTC	29
rs79222846	19228	1:59907519	NM_000775.4:c.1003+267A>G	No translated	Intron 6		ACATTGAGAT R GTTCCAGGAA	1
rs144856672	21748	1:59904999	NM_000775.3:c.1063G>A	p.Ala355Thr[b]	Exon 7		GAGCACAGCC R CCCGGGAGTC	2
rs11572304	25391	1:59901356	NM_000775.4:c.1192-253G>A	No translated	Intron 7		AAGGAAGCTTC R ATCCTGCAGT	2
/	32811	1:59893936	/	No translated	Intron 8	Novel	CTGGGGCCTA Y AGGCCCTTCC	1
rs201638221	33146	1:59893604	NM_000775.3:c.*47G>A	No translated	3'UTR		GACATGGCAC R TGTTCTGAAA	1
rs4388726	33266	1:59893484	NM_000775.3:c.*167C>T	No translated	3'UTR		TCTACTGTCT Y GTCCGAATTA	10
rs41287722	33386	1:59893364	NM_000775.3:c.*287T>A	No translated	3'UTR		TCAAAAGAAA W GGTGAGCTTT	1
rs2280273	33440	1:59893310	NM_000775.3:c.*341A>G	No translated	3'UTR		AGTTCTATCT R TAGTGTGCCT	18
rs11572327	33472	1:59893278	NG_007931.1:g.38474A>G	No translated	Downstream		CCTTTGTGAG R TATGTGTTTG	8

Chr: chromosome; HGVS: Human Genome Variation Society; SNP: single nucleotide polymorphisms; Asn: Asparagine; Ser: Serine; Arg: Argnine; Cys: Cysteine; Tyr: Tyrosine; Gly: Glycine; Arg: Argnine; Ala: Alanine; Thr: Threonine; S: Ser, Serine; K: Lys, Lysine; Y: Tyr, Tyrosine; R: Arg, Argnine; W: Trp, Tryptophane; M: Met, Methionine; Amino-acid change: decreased, reduced transcription due to loss of Sp1 binding site; No translated, these mutations have no effect on protein sequence; [a], synonymous mutations; [b], non-synonymous mutations.

Table 3. Allele and genotype frequencies of *CYP2J2* variants in the Uygur population

Variants		Number	Phenotype	Frequency (%)
Allele	*CYP2J2*1*	192	Normal	96.0
	*CYP2J2*7*	7	Decreased	3.5
	*CYP2J2*8*	1	-	0.5
Genotype	**1/*1*	92	Normal	92.0
	**1/*7*	7	Decreased	7.0
	**1/*8*	1	-	1.0

Table 4. Alleles of *CYP2J2* frequencies distributions in different ethnic populations

Races	Study population no.	*1	*7	*8	Reference
East Asians					
Chinese Uygur	100	0.960	0.035	0.005	Current study
Chinese Zhuang	100	0.955	0.045	/	[20]
Chinese Wa	100	1.000	/	/	[19]
Chinese Han	384	0.974	0.026	/	[22]
Taiwanese	200	0.880*	0.120*	/	[15]
Mongolians	118	0.966	0.034	/	[27]
Japanese	338	0.938	0.062	/	[27]
Koreans	271	/	0.042	/	[7]
	563	/	/	0.008	
Caucasians					
Russian	217	0.952	0.048	/	[28]
Spanish	89	0.933	0.067	/	[29]
Germans	960	0.935	0.065	/	[30]
Germans	255	0.945	0.055	/	[31]
Bashkirs	102	0.985	0.015	/	[28]
Tatars	178	0.964	0.037	/	[28]
Americans	116	0.901	0.099	/	[32]
Africans					
African-Americans	102	0.887	0.113*	/	[16]
African-Americans	73	0.863*	0.137*	/	[32]
Ovambos	186	0.933	0.067	/	[27]

*$P<0.05$, compared with the data of the present study.

1:59911684 A>G (p. Tyr203Cys), rs15046-1093 (p. Gly312Arg) and rs144856672 (p. Ala355Thr). The predicted protein function for the five nonsynonymous variants from the SIFT analysis indicated that all the missense variants were likely to be predicted to be pathogenic with scores of 0.00-0.05 (**Table 5**). The CADD analysis also indicated that all the missense variants were predicted to be pathogenic, with scores of 4.21, 5.26, 5.93, 2.52 and 13.29, respectively (**Table 5**). The results generated by PolyPhen-2 analysis showed that 1:59911724 A>G (p. Asn190Ser), rs201070738 (p. Arg200Cys), 1:59911684 A>G (p. Tyr203Cys), rs150461093 (p. Gly312Arg), and rs14485-6672 (p. Ala355Thr) seemed to be damaging (**Figure 2**). HumVar values of 1:59911724 A>G (p. Asn190Ser) and rs15-0461093 (p. Gly312Arg) were 1.000, while 1:59911724 A>G (p. Asn190Ser) and 1:59911-684 A>G (p. Tyr203Cys) were close to 1.000. However, rs14-4856672 (p. Ala355Thr) was classified as a benign variant with a PolyPhen-2 value of 0.015.

Discussion

In the present study, we sequenced the promoter and exon regions and partial sequences flanking exon-intron junctions of the *CYP2J2* gene in 100 Chinese Uyghur individuals. The analysis identified *CYP2J2* genetic variants, including five novel polymorphisms, three alleles (**1*, **7* and **8*), and three genotypes **1/*1*, **1/*7* and **1/*8*) of *CYP2J2* in the Chinese Uyghur population. We also compared the allele frequencies of *CYP2J2*1*, *CYP2J2*7* and *CYP2J2*8* in the Chinese Uyghur population with previous observations from other ethnic populations and found that in the Chinese Uyghur population, *CYP2J2*1* was significantly higher than in Taiwanese and African Americans; *CYP2J2*7* was slightly lower than that in Taiwanese and African Americans ($P<0.05$). Furthermore, the protein prediction results revealed that the identified missense variants (1:59911724 A>G, rs201070738, 1:59911684 A>G, rs150-461093, and rs144856672) could potentially influence protein structure and function.

CYP pharmacogenetics has been an important area of research. The CYP subfamily of genes

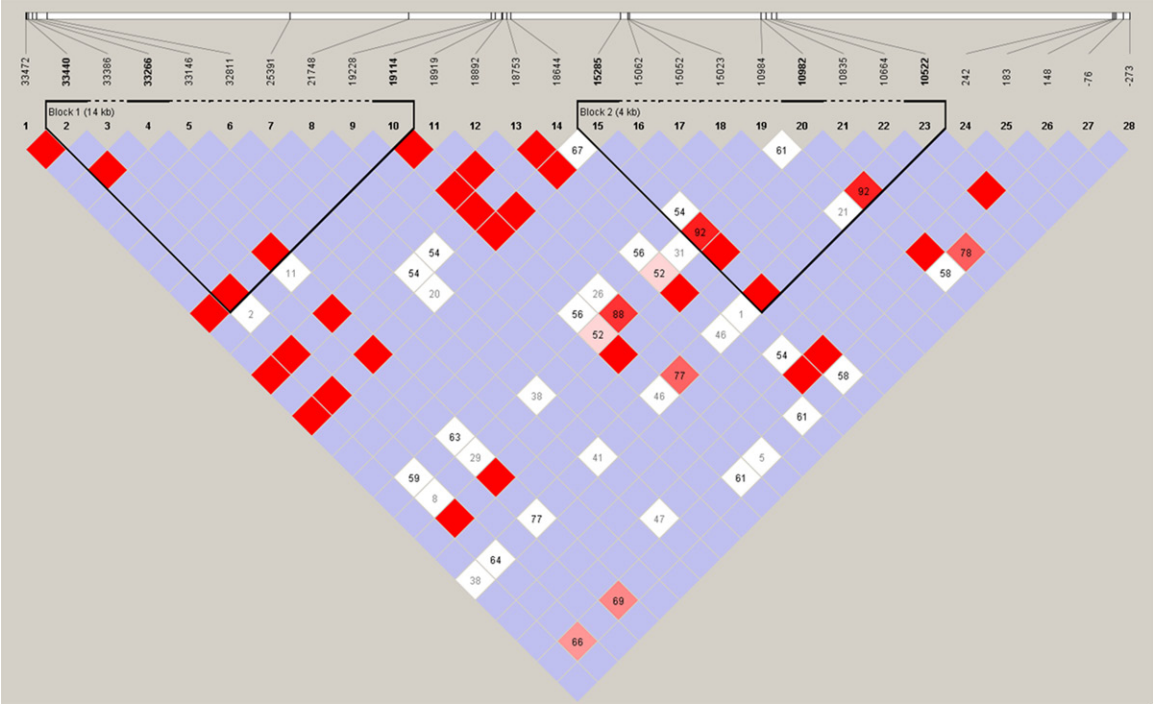

Figure 1. Haplotype block map for SNPs in *CYP2J2*.

Table 5. SIFT score and CADD score of the five non-synonymous mutations

Chr: genomic position	SNP-ID	Ref	Alt	Amino-acid effect	SIFT score	CADD score
1:59911724	/	G	A	Asn190Ser	0.00	4.21
1:59911694	rs201070738	T	C	Arg200Cys	0.00	5.26
1:59911684	/	G	A	Tyr203Cys	0.00	5.93
1:59907855	rs150461093	A	G	Gly312Arg	0.00	2.52
1:59904999	rs144856672	A	G	Ala355Thr	0.01	13.29

Chr: chromosome; SNP: single nucleotide polymorphisms; Ref: Reference; Alt: Alternate; Ala: alanine; Arg: arginine; Asn: asparagine; Cys: cysteine; Gly: Glycine; Ser: serine; Thr: threonine; Tyr: tyrosine.

offers the opportunity to identify sources of interindividual variability in drug disposition and response [14]. On the other hand, functional polymorphisms in *CYP* genes can also affect enzyme activity and are relate to human diseases. Hundreds of *CYP2J2* polymorphisms have been identified to date. The *CYP2J2*7* allele affects the binding of the Sp1 transcription factor to the *CYP2J2* promoter, leading to decreased *CYP2J2* promoter activity [5]. Liu et al. demonstrated that the *CYP2J2*7* allele was synergistically associated with the risk of premature myocardial infarction (MI), particularly in smokers. Evidence about associations between *CYP2J2*7* genotype and hypertension coronary, artery disease, and ischemic stroke was also reported [15-17]. The *CYP2J2*8* variant exhibits almost complete loss of enzymatic activity, as determined by CYP2J2-catalyzed astemizole O-demethylation and ebastine hydroxylation measurements [7, 18].

The present result was different with our previous analysis in the Chinese Wa and Zhuang populations [19, 20]. A total of fourteen *CYP2J2* genetic variants were identified in the Chinese Wa population, twelve of which were also found in the Chinese Uyghur population. Only one allele, *CYP2J2*1,* was identified in the Chinese Wa population, and no common synonymous or nonsynonymous variants were found. We identified seventeen SNPs in the Chinese Zhuang population, including two common synonymous variants associated with the Chinese Uyghur

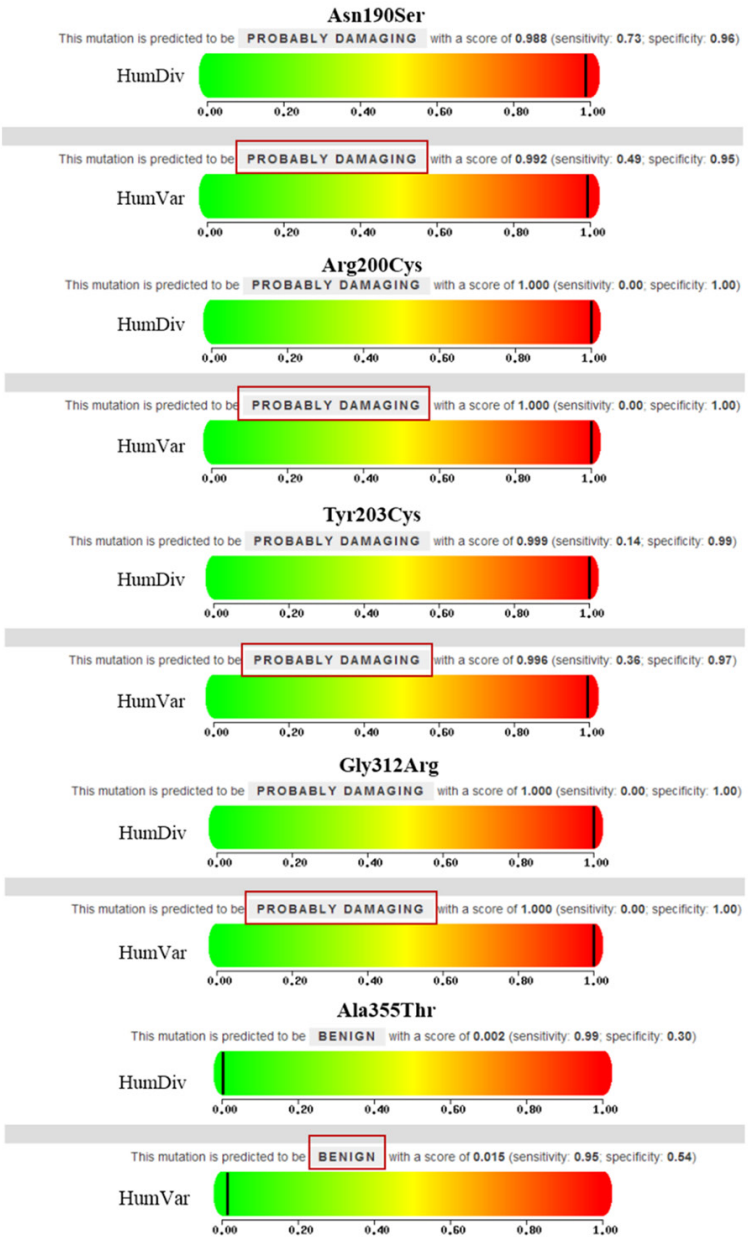

Figure 2. PolyPhen-2 prediction of functional change resulting from amino acid mutation at position 190, 200, 203, 312, and 355, respectively.

population. We found that the most common polymorphism in the Chinese Uyghur population, rs1155002, is also the most frequent among the Chinese Wa and Zhuang populations. Its frequency in the Chinese Uyghur population (48%) and Zhuang (34%) population was much lower than that in the Chinese Wa population (72%). This common intronic SNP is important, as it has been recognized as a risk factor for hypertension [21].

*CYP2J2*7* is an allele that has received attention, as it is found in many populations. Wang et al. first analyzed the *CYP2J2*7* allele in the Chinese population [22]. They also reviewed the allele frequency of *CYP2J2*7* to be 2.6% in the Chinese Han population, 17% in Africa, 5.49% in Caucasians, 13% in Asians and 4.23% in the Korean population. The allele frequency was comparable to that of Koreans but significantly lower than those of Africans and Caucasians. Here, we expanded the groups and further determined that the *CYP2J2*7* variant represents a relatively rare polymorphism in most East Asians. However, in Taiwanese individuals, the allele frequency of *CYP2J2*7* was markedly higher and comparable to that in African Americans. This indicates that although on the same continent, there are still differences among diverse ethnic groups. Possible explanations for these differences include genetic background, living environment and lifestyles.

Five nonsynonymous variants, Asn190Ser, Arg200Cys, Tyr203Cys, Gly312Arg and Ala355Thr, were observed in the Chinese Uyghur population of this study. Asn190Ser and Tyr203Cys have not been identified before. Gly312Arg was predicted to be damaging and may affect protein function. Sang et al. reported that the Gly312Arg variant was coexpressed with NADPH-CYP reductase in Sf9 cells, and its catalytic activities were quantified. Compared with wild-type *CYP2J2,* the recombinant *CYP2J2*8* (Gly312Arg) variant showed almost complete loss of enzymatic activity, and nine subjects with heterozygous *CYP2J2*8* were found from 563 Korean subjects with an allele frequency

of 0.8%, but no further subjects with this variant were identified from 192 Chinese, 159 Vietnamese, 100 African-American and 99 Caucasian DNAs [7]. In our study, we found that the allele frequency of *CYP2J2*8* was 5% in the Chinese Uyghur population.

In addition to Ala355Thr, protein functional analysis of the other four variants generally showed consistency across the different algorithms used in this study. However, polyPhen-2 protein function prediction results showed that Ala355Thr was benign, whereas SIFT predicted that it was damaging. This inconsistency could be explained, as algorithms of different bioinformatics tools are based on different training datasets. PolyPhen-2 assem includes benign variants occurring at residues that are polymorphic across multiple species. SIFT assesses the possible pathogenicity of a missense variant based on sequence homology and a conservation value [23]. Regarding their chemical properties, the replacement of alanine with threonine is nonconservative. Comparing the two computational tools, the sensitivity of SIFT and PolyPhen was reasonably high (69% and 68%), but their specificity was rather low (13% and 16%) [24]. Therefore, we believe that predictions should be interpreted with caution before reporting novel missense changes, and further experimental evidence should be sought.

Some studies on polymorphisms of the *CYP2J2* gene in the Uygur population, such as Kang et al. [25] sequenced all of the exons, exon-intron boundaries and 1 kb 5-flanking regions of the *CYP2J2* gene in 150 Chinese subjects to investigate the genetic variations of the gene and their polymorphic distribution in different Chinese populations. They identified 15 genetic variants in the *CYP2J2* gene and found that the genetic polymorphisms of *CYP2J2* showed significant differences in three Chinese populations (Han, Tibetan and Uighur). The seven SNPs (rs890293, rs11572191, rs3738474, rs2271800, rs2229191, rs4388726, and rs2280273) they identified were consistent with the results we found. In addition, Zhu et al. [26] assessed the association between human *CYP2J2* gene polymorphisms and CAD in a Han and Uygur population of China. They found that the distribution of rs2280275 genotypes showed a significant difference between CAD and control participants in the Uygur population. However, further studies on the association between *CYP2J2* genetic polymorphisms and disease susceptibility and studies about the function of *CYP2J2* genetic polymorphisms are required.

In this study, some limitations should be taken into consideration. First, the sample size of 100 individuals was small; therefore, it is necessary to continue collecting samples to confirm our findings in future studies. Second, other genes that are equally important for CYP enzyme activity, such as *CYP2S1* and *CYP2R1*, should be studied in future research. Third, this study only selected the 3 regions for exploration, including the promoter, the 3'-untranslated region, and exons of the *CYP2J2* gene. Finally, functional analyses of SNPs in the *CYP2J2* gene should be validated experimentally. Therefore, further studies are warranted to investigate the function of each identified variant.

In conclusion, we detected twenty-eight polymorphisms in *CYP2J2*, including five new variants, three alleles (**1*, **7* and **8*), and three genotypes (**1/*1*, **1/*7* and **1/*8*) of *CYP2J2* in the Chinese Uyghur population. Five nonsynonymous SNPs could influence protein structure and function. The allele frequencies of **1* and **7* were significantly different compared with other populations reported in the literature. Overall, our results provide a basic profile of *CYP2J2* in the Chinese Uyghur population. We hope these data will help to plan candidate gene-trait association studies and population-specific research in pharmacogenetics.

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Disclosure of conflict of interest

None.

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