Analysis of CYP2J2 polymorphisms in the Chinese Uyghur population

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Abstract

Background: Genetic characteristics of CYP2J2 in different populations may be helpful to explore inter-ethnic variability in drug response and disease susceptibility. There is no information about the genetic profile of CYP2J2 in the Chinese Uygur population.

Methods: We used PCR and direct sequencing to investigate the whole CYP2J2 in 100 unrelated healthy individuals of Chinese Uygur populations. Chi-square test was used to compare genotyping data of CYP2J2 in the Chinese Uygur population with other races. The SIFT and PolyPhen-2 online tools were used to predict the protein function of the novel non-synonymous mutations in CYP2J2. The CADD software was used to predict pathogenicity for mutations.

Results: We detected twenty-eight polymorphisms in the CYP2J2, including five new mutations, three alleles (*1, *7 and *8), and three genotypes (*1/*1, *1/*7 and *1/*8) of CYP2J2 in Chinese Uyghur population. The allele frequencies of CYP2E1*1, *7 and *8 were 96%, 3.45%, and 0.5%, respectively. Interethnic comparison found that *1 was significantly higher than Taiwanese and African-Americans; and*7 was relatively lower when compared with Taiwanese and African-Americans ($p < 0.05$). Furthermore, the protein prediction results revealed that the five non-synonymous mutations could influence the protein structure and function.

Conclusion: The observations of this study give rise to useful information on CYP2J2 polymorphisms in Chinese Uygur individuals. We hope the results will indicate important clinical implications for the use of medications metabolized by CYP2J2.

Introduction

The cytochrome P450 (CYP) enzymes play central roles in catalyzes oxidative reactions and bioactivation, accounting for almost 75% of the total drug metabolism (X. Liu et al., 2013; Shah & Breslin, 2010). Nowadays Cytochromes P450 have been the focus of study by toxicologists and pharmacologists. Currently fifty-seven CYP genes involving three families (*CYP1*, *CYP2* and *CYP3*) have been detected to contribute to the oxidative metabolism of various compounds (Arici & Özhan, 2017). The cytochrome P450, family 2, subfamily J, polypeptide 2 (CYP2J2), an important member of CYP superfamily, which is encoded by *CYP2J2* gene, is highly expressed in cardiovascular system (Delozier et al., 2007). The CYP2J2 has prominent role in cardiac protection because of its ability to catalyze arachidonic acid to epoxyeicosatrienoic acids that possess potent anti-inflammatory, vasodilatory, and fibrinolytic properties (Spiecker et al., 2004). Variations in the coding regions of *CYP2J2* gene may lead to changes in CYP2J2 expression and/or enzymatic activity, and result in altered CYP2J2-dependent metabolism of AA, and ultimately lead to abnormal heart function. For

example, *CYP2J2* SNP (single nucleotide polymorphism) such as G-76T, has been repeatedly considered an increased factor for coronary artery disease (S. Wu, Moomaw, Tomer, Falck, & Zeldin, 1996).

Exploration of *CYP* gene profiles in different populations might offer solutions to anticipating and decreasing individual risk for adverse drug reactions. With the elucidation of the human genome sequence, *CYP2J2* genetic trait in different ethnic groups was successively reported. There are still insufficient data in terms of China, especially in Chinses ethnic minorities. Uygur ethnic minority is one of the oldest ethnic minorities in China. But they are Eurasian population with Eastern and Western Eurasian anthropometric and genetic traits (Xu & Li, 2008).

Therefore, we systematically screened the whole *CYP2J2* gene from 100 unrelated healthy individuals of Chinese Uygur populations to explore their polymorphisms and compared their frequencies with previous observations of other ethnic groups. These genetic factors can be used to predict the drug metabolizing potential of patients before starting therapy. We hope the present work will contribute to prediction for potential risks of drug toxicity and individualized therapy based on *CYP2J2* genetic polymorphisms.

Materials And Methods

Study populations

A total of 100 unrelated healthy volunteers (aged 19-52 years) included 50 males and 50 females were randomly recruited in our study. The inclusion and exclusion criteria were as follows: the subjects selected were judged to be of good health by medical examination; all of these participants were Chinese Uygur populations; their ancestors had lived in the Xinjiang Autonomous Region for at least three generations; volunteers who had any type of medical disease, being pregnant or lactating, drug or alcohol addiction, and organ transplant were excluded from the study.

Ethics approval and consent to participate

The study protocol and consent form were reviewed and approved by Ethics Committee of the Xizang Minzu University and 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 sample (5 mL) was taken into vacutainer tubes containing EDTA (Ethylene diamine tetra-acetic acid) from each subject on an empty stomach during their routine health examination. Then, the 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 region, all exons and intron-exon boundaries of *CYP2J2* gene were designed by online software Primer 3 Input (version 4.1.0) and synthesized by the Sangon Biotech (Shanghai, China). Primers for PCR (polymerase chain reaction) amplification and sequence are shown in Table 1. Each PCR was performed in 10ul volume system including 5μl HotStar Taq Master Mix (Qiagen, Germany), 0.5μl each primer (0.25uM), 3μl RNase-free water and 1ul template genomic DNA (20ng/ul). PCR product that have single band with prospective size in agarose gel was regarded as qualified. Subsequently, PCR products were purified using TIANgel Midi Purification Kit (TIANGEN, Beijing, China) and sequenced using ABI BigDye Terminator Cycle Sequencing Kit (version 3.1, Applied Biosystems, Thermo Fisher Scientific, Inc., USA) on an ABI Prism3100 sequencer (Applied Biosystems, Thermo Fisher Scientific, Inc., USA).

Statistical analysis

The 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 were performed by Sequencher 4.10.1 software (http://www.genecodes.com/). We used Blast online software (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to screen the actual genetic differences. The *CYP2J2* variants were identified and named based on the wildtype *CYP2J2* nucleotide sequence NG_007931.1 in NCBI (national center for biotechnology information) database and CYP allele nomenclature (http://www.cypalleles.ki.se/). Statistical differences in the distributions of allele frequency between the Chinese Uygur and other ethnic populations were evaluated using Chi-square test. P value < 0.05 was considered statistically significant, and all statistical tests were two sided. We used χ^2 test to examine HWE (hardy-weinberg equilibrium) for each genetic variant. Haploview software version 4.2 was used for analyses of linkage disequilibrium and haplotype construction.

Tanscriptional prediction

The 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/) online tools were used to predict the effects of coding non-synonymous variants on protein function. Each variant was given a score based on the impact of its mutation 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) (Kumar, Henikoff, & Ng, 2009). The PolyPhen-2 results were divided into three categories: benign, possibly damaging and probably damaging (Adzhubei et al., 2010). We used the CADD online tool (combined annotation-dependent depletion, https://cadd.gs.washington.edu/snv) to predict pathogenicity for mutations in the *CYP2J2* gene. The high CADD score indicates that the mutation is a higher probability of pathogenicity.

Results

Genetic variants

A total of twenty-eight different SNPs in *CYP2J2* were determined by direct sequence in the present study Chinese Uygur population. The identified SNPs, position, region, nucleotide change, allele, amino-acid effect, flanking sequence and the corresponding frequencies are

listed in Table 2. Rs1155002 (18644 G>A) locating in intron 5 was found to have the highest frequency of 48%, followed by rs1570693 (15285 A>C) in intron 4 with the frequency of 34%. We found five novel polymorphisms, -273 G>C, 10664 G>A, 15023 A>G, 15062 A>G and 32811 C>T, which have not previously been reported in either the NCBI database or the Human Cytochrome P450 Allele Nomenclature Committee tables. Among of them, 15023 A>G and 15062 A>G within coding region exon 4, caused the amino acid alterations. Four SNPs were found to cause synonymous mutations and five resulted in non-synonymous mutations. The mutation -76 G>T locating in *CYP2J2* promotor with a frequency of 7%, was reported to lose the binding site of the Sp1 transcription factor and led to a decreased enzyme activity referring to published studies (Spiecker et al., 2004).

Allele and genotype frequencies

There *CYP2J2* alleles were detected in the Chinese Uygur population (Table 3), which including *CYP2J2**1, *CYP2J2**7 and *CYP2J2**8. The wildtype allele, *CYP2J2*1* had the highest frequency of 96.0%. *CYP2J2*7* had a frequency of 3.5%. By contrast, the frequency of *CYP2J2*8* was just 0.5% and was considered as rare. We further identified three *CYP2J2* genotypes in the Chinese Uygur population, included *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 in a highest frequency of 92% in our study. The frequencies of heterozygous genotype *CYP2J2**1/*7 and *CYP2J2**1/*8 were 7.0% and 1.0%, respectively. All allele and genotype distributions conformed to HWE.

Interethnic comparison

In order to understand the interethnic variabilities of *CYP2J2* genetic profiles, we further compared *CYP2J2* distribution patterns between Chinese Uygur population and previously published other major races including East Asians, Caucasians and Africans (Table 4). The allele frequency of *CYP2J2**1 in the Chinese Uygur population was significantly higher than Taiwanese and African-Americans ($p < 0.05$). Furthermore, we found that the allele frequency of *CYP2J2**7 in the Chinese Uygur population was relatively lower when compared with Taiwanese (12%) and African-Americans (11.27% and 13.7%). The allele frequency of

*CYP2J2**7 in Mongolians (3.39%) and Tatars (3.65%) were both similar to our results. However, the allele frequency of *CYP2J2*8* is currently not reported in other races.

Linkage disequilibrium analysis

We used Haploview to evaluate LD between SNPs with a minor allele frequency higher than 5%. The D' values on the square is a measure of the LD extent for each pair of SNPs (Figure 1). 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 strong LD in block 2.

Predicted protein function for non-synonymous SNPs

We found five non-synonymous SNPs in the present study (15023 A>G, 15052 C>T, 15062 A>G, 18892 G>A, and 21748 G>A). They caused the amino acid substitutions (Asn190Ser, Arg200Cys, Tyr203Cys, Gly312Arg and Ala355Thr respectively). The protein prediction results of the five nonsynonymous variant from the SIFT analysis indicated that all the missense mutations were predicted to be intolerant with scores of 0.00-0.05 (Table 5). The CADD analysis also indicated that all the missense mutations were predicted to be pathogenicity with scores of 4.21, 5.26, 5.93, 2.52 and 13.29, respectively (Table 5). The result performed by PolyPhen-2 analysis showed that 15023 A>G, 15052 C>T, 15062 A>G, and 18892 G>A seemed to be probably damaging (Figure 2). HumVar values of 15052C>T and18892 G>A were 1.000, while 15023 A>G and 18892 G>A were close to 1.000. However, 21748 G>A was classified to benign mutation with a PolyPhen-2 value of 0.015.

Discussion

In the present study, we sequenced the whole *CYP2J2* gene in 100 Chinese Uyghur populations. The results 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 Chinese Uyghur population. We also compared the allele frequencies of *CYP2J2**1, *CYP2J2**7 and *CYP2J2**8 with previous observations of other ethnic populations, and found

that *CYP2J2**1 was significantly higher than Taiwanese and African-Americans; *CYP2J2**7 was relatively lower when compared with Taiwanese and African-Americans ($p < 0.05$). Furthermore, the protein prediction results revealed that the variants (15023 A>G, 15052 C>T, 15062 A>G, 18892 G>A, and 21748 G>A) could influence the protein structure and function.

P450 pharmacogenetics has been one of the important areas of pharmacogenetic variability. Genetic trait of cytochrome P450 genes subfamily offers the opportunity to identify sources of inter-individual variability in drug disposition and response (Fohner et al., 2013). On the other hand, functional polymorphisms in P450 genes can affect the enzyme activity and relate to human disease. Hundreds of *CYP2J2* polymorphisms have been identified currently. *CYP2J2*7* resulted in the loss of binding of the Sp1 transcription factor to the *CYP2J2* promoter and decreased *CYP2J2* promoter activity (Spiecker et al., 2004). Besides coronary artery disease, it has been implicated in other cardiovascular disease. Ping-Yen Liu *et al* demonstrated that *CYP2J2*7* T allele was synergistically associated with the risk of premature MI (myocardial infarction), particularly in smokers. Evidence about associations between *CYP2J2*7* genotype and hypertension coronary, artery disease, and ischemic stroke were also reported (Dreisbach et al., 2005; Li et al., 2015; P. Y. Liu et al., 2007). The *CYP2J2*8* variant exhibited almost complete loss of enzymatic activity as determined by CYP2J2-catalysed astemizole O-demethylation and ebastine hydroxylation (King et al., 2002; Lee et al., 2005a).

The present result was quite different from our previous analysis in the Chinese Wa and Zhuang population (Zhang, Chen, et al.; Zhang, Cheng, et al.). A total of fourteen *CYP2J2* genetic varieties were identified in the Chinese Wa population, twelve of which were also found in the Chinese Uighur population. Only one allele *CYP2J2**1 was identified in the Chinese Wa population and no common synonymous and nonsynonymous mutations were found. We identified seventeen SNPs in the Chinese Zhuang population, including two common synonymous mutations associated with the Chinese Uighur population. We found that the most common polymorphism in the Chinese Uygur population, rs1155002, is also the highest among the Chinese Wa and Zhuang population. Its frequency in Chinese Uygur

population (48%) and Zhuang (34%) population were much less than that in the Chinese Wa population (72%). This common intron SNP is an important SNP that has been recognized as a risk factor for hypertension (S. N. Wu et al., 2007).

*CYP2J2**7 is an allele that has been receiving attention and has appeared in many races. Wang et al. first analyzed the *CYP2J2*7* single nucleotide polymorphism in the Chinese population (Wang et al., 2006). They indicated that the allele frequency of *CYP2J2*7* was 2.6% in Chinese Han, 17% in Africa, 5.49% in White, 13% in Asian and 4.23% in Korean population. The allele frequency was comparable to that of Korean, but significantly lower than those of African and White. Here we expanded the groups and further expound that *CYP2J2*7* variant represents a relatively rare polymorphism in most East Asians. But in Taiwanese, the allele frequency of *CYP2J2*7* was distinctly higher and comparable to African-Americans. This indicates that although in the same continent, there are still differences among diverse ethnic groups. Possible explanations for these differences include: genetic background, cultural variants and other factors, such as living environment and lifestyles.

Five non-synonymous mutations Asn190Ser, Arg200Cys, Tyr203Cys, Gly312Arg and Ala355Thr were observed in the Chinese Uygur population. Asn190Ser and Tyr203Cys were never identified before. Gly312Arg was predicted as probably damaging and affecting protein function. Sang et al. reported that Gly312Arg variant was co-expressed with NADPH-cytochrome P450 reductase in Sf9 cells and its catalytic activities were quantified. Compared with the wild-type *CYP2J2,* recombinant *CYP2J2* Gly312Arg variant showed almost complete loss of enzymatic activity (Lee et al., 2005a).

In addition to Ala355Thr, protein functional analysis of the other four mutations showed consistency. However, polyPhen-2 protein function predicted results showed that Ala355Thr was benign, whereas SIFT predicted it was damaging. We deem that the inconsistency is reasonable because algorithms of different bioinformation tools are based on different training data. PolyPhen-2 indicates benign variants occurring at residues that were polymorphic across multiple species. SIFT assess the pathogenicity of a missense variant based on the sequence homology and a conservation value (Lai et al., 2015; J. Wu, Li, & Jiang, 2014). As to chemical properties, the replacement from alanine to threonine was non-

conservative. According to related analysis, the sensitivity of SIFT and PolyPhen was both reasonably high (69% and 68%) but their specificity was low (13% and 16%) (Flanagan, Patch, & Ellard, 2010). Therefore, we think predictions should be interpreted with caution before reporting novel missense changes and further experimental evidence should be sought.

In this study, some limitations should be taken into consideration. First, the sample size included 100 individuals is relatively small, therefore, it is needed to continue collecting samples to confirm our findings in the future study. Second, other genes, which are equally important for CYP450 enzyme activity, such as *CYP2S1* and *CYP2R1*, should be studied in the further research. Finally, the function analyses of mutations in the *CYP2J2* gene were not performed. Therefore, further studies are warranted to investigate the function of mutation site.

Conclusions

In conclusion, we detected twenty-eight polymorphisms in the *CYP2J2*, including five new mutations, three alleles (*1, *7 and *8), and three genotypes (*1/*1, *1/*7 and *1/*8) of *CYP2J2* in the Chinese Uyghur population. Five non-synonymous mutations among them could influence the protein structure and function. The allele the allele frequency of *1 and *7 were significantly differences compared with other races. Overall, our results provide a basic profile of *CYP2J2* in the Chinese Uygur population. We hope the data may be helpful to plan candidate gene-trait association studies and population-specific research on pharmacogenetics.

Declarations

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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Tables

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	783bp
Exon2	CTCATGCCTTGCTCTAGGGAC	CACGTTCCTCTGCTATAAATGGGT	779bp
Exon3	GTGCATTCCTAGTGTTTACCATAAC	TGCCCATCTTTGTGTATTTACTTCT	788bp
Exon4	AGCATTGCATATGACAGAGGTGAG	AGACTCAAGGGCAACAGCAAT	856bp
Exon5	AACACTCAACCAGTGCTCAGAT	GAGAAGATGCTGTGCTTCTGG	776bp
Exon6	CAAATCTGTCTCGTTCACATCC	ATACCAGACTAAAGTGCTTGAAC	827bp
Exon7	GAGCTGCCTCACTCCTTCTAC	CTGACCTAGAACTGCTGCCTG	850bp
Exon8	CCAAGCCCTACTGAAACTGACC	TTTCCAGAGGACAGAACACAGG	688bp
Exon9	CTTCTATGGTCCTACACCCTGC	ACCACTTTGACTTGAGCTTCTC	869bp
Exon9&UTR	CCCAGCTCTACTGTCTCGTC	GCAACGGAGCAAGACACTAC	778bp

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

SNP	Gene position	Region	Nucleotide change	Allele	Amino-acid effect	Flanking Sequence	Frequency (%)
/	-273	Promoter	G>C	Novel	/	TCATAGGAGA S ACGGTGATTG	1
rs890293	-76	Promoter	G>T	*7	Decreased[a]	GGCTGGGAGC K AGGCGGGGCG	7
rs11572191	148	Exon 1	C>T		Leu50=[b]	GCCCTGGCGC Y TGCCCTTCCT	5
rs2229189	183	Exon 1	C>T		Phe61=[b]	TTGTGGACTT Y GAGCAGTCGC	2
rs778101462	242	Intron 1	T>C		No translated[c]	TAGCGTGTCC Y GACCCTAACT	2
rs3820538	10522	Intron 1	C>T		No translated	CACACACACA Y GTACACACAC	14
/	10664	Exon 2	G>A	Novel	Gly76=[b]	AGAAATATGG R AACCTTTTTA	1
rs3738474	10835	Intron 2	G>A		No translated	AACGAAAGGT R AGTGTTTGAT	5
rs11572245	10982	Intron 2	G>C		No translated	GTCACTCCCT S AGAAGATTTG	17
rs149199403	10984	Intron 2	G>A		No translated	TCACTCCCTGA R AAGATTTGAT	3
/	15023	Exon 4	A>G	Novel	Asn190Ser[d]	GCAGTTTCCA R TATCATTTGC	1
rs201070738	15052	Exon 4	C>T		Arg200Cys[d]	CTTCGGAGAA Y GCTTTGAGTA	1
/	15062	Exon 4	A>G	Novel	Tyr203Cys[d]	CGCTTTGAGT R CCAGGATAGT	1
rs1570693	15285	Intron 4	A>C		No translated	TATTTGAAAT M AATCTATTGA	34
rs1155002	18644	Intron 5	G>A		No translated	GGGCAGGACA R TGCTAATGAT	48
rs2271800	18753	Intron 5	T>G		No translated	TGAAGCCCCT K TGTGTTACGG	39
rs150461093	18892	Exon 6	G>A	*8	Gly312Arg[d]	CTTCTTTGCC R GAACCGAGAC	1
rs2229191	18919	Exon 6	C>A		Arg321=[b]	CACAACTCTG M GATGGGCTCT	10
rs2271798	19114	Intron 6	T>C		No translated	ACATTCTTCA Y ATTTTCTGTC	29
rs79222846	19228	Intron 6	A>R		No translated	ACATTGAGAT R GTTCCAGGAA	1
rs144856672	21748	Exon 7	G>A		Ala355Thr	GAGCACAGCC R CCCGGGAGTC	2
rs11572304	25391	Intron 7	G>A		No translated	AAGGAAGCTTC R ATCCTGCAGT	2
/	32811	Intron 8	C>T	Novel	No translated	CTGGGGCCTA Y AGGCCCTTCC	1
rs201638221	33146	Intron 9	G>A		No translated	GACATGGCAC R TGTTCTGAAA	1
rs4388726	33266	3'UTR	T>A		No translated	TCTACTGTCT Y GTCCGAATTA	10
rs41287722	33386	3'UTR	A>G		No translated	TCAAAAGAAA W GGTGAGCTTT	1
rs2280273	33440	3'UTR	A>G		No translated	AGTTCTATCT R TAGTGTGCCT	18
rs11572327	33472	3'UTR	A>G		No	CCTTTGTGAG R	8

translated TATGTGTTTG

a, decreased: reduced transcription due to loss of Sp1 binding site; b, synonymous mutations; c, no translated: these mutations have no effect on protein sequence; d, non-synonymous mutations.

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

Allele Frequencies			
Allele	Number (n=200)	Phenotype	Frequency
*CYP2J2*1*	192	Normal	96.00%
*CYP2J2*7*	7	Decreased	3.50%
*CYP2J2*8*	1	-	0.50%
Genotype Frequencies			
Genotype	Number (n=100)	Phenotype	Frequency
**1/*1*	92	Normal	92.00%
**1/*7*	7	Decreased	7.00%
**1/*8*	1	-	1.00%

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

Races	Study population no.	*1	*7	*8	Reference
East Asians					
Chinese	100	0.960	0.035	0.005	Current study
Uygur Chinese	100	0.955	0.045	/	(Zhang, Chen, et al.)
Zhuang Chinese	100	1.000	/	/	(Zhang, Cheng, et al.)
Wa Chinese	384	0.974	0.026	/	(Wang et al., 2006)
Han Taiwanese	200	0.880*	0.120*	/	(P. Y. Liu et al., 2007)
Mongolians	118	0.966	0.034	/	(Takeshita, Tsubota, Takatsuka, Kunito, & Fujihara, 2008)
Japanese	338	0.938	0.062	/	(Takeshita et al., 2008)
Koreans	271	0.958	0.042	/	(Lee et al., 2005b)
Caucasians					
Russian	217	0.952	0.048	/	(Korytina, Kochetova, Akhmadishina, Viktorova, & Victorova, 2012)
Spanish	89	0.933	0.067	/	(Gervasini, Vizcaino, Carrillo, Caballero, & Benitez, 2006)
Germans	960	0.935	0.065	/	(Hoffmann et al., 2007)
Germans	255	0.945	0.055	/	(Banas, 1992)
Bashkirs	102	0.985	0.015	/	(Korytina et al., 2012)
Tatars	178	0.964	0.037	/	(Korytina et al., 2012)
Americans	116	0.901	0.099	/	(King et al., 2005)
Africans					
African-Americans	102	0.887	0.113*	/	(Dreisbach et al., 2005)
African-Americans	73	0.863*	0.137*	/	(King et al., 2005)
Ovambos	186	0.933	0.067	/	(Takeshita et al., 2008)

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

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

SNP	SNP position	Reference	Alternate	Amino-acid effect	SIFT score	CADD score
15023 A>G	59911723	G	A	Asn190Ser	0.00	4.21
rs201070738 (15052 C>T)	59911694	T	C	Arg200Cys	0.00	5.26
15062 A>G	59911704	G	A	Tyr203Cys	0.00	5.93
rs150461093 (18892 G>A)	59907855	A	G	Gly312Arg	0.00	2.52
rs144856672 (21748 G>A)	59904999	A	G	Ala355Thr	0.01	13.29

Ala: alanine; Arg: arginine; Asn: asparagine; Cys: cysteine; Gly: Glycine; Ser: serine; Thr: threonine; Tyr: tyrosine

Figures

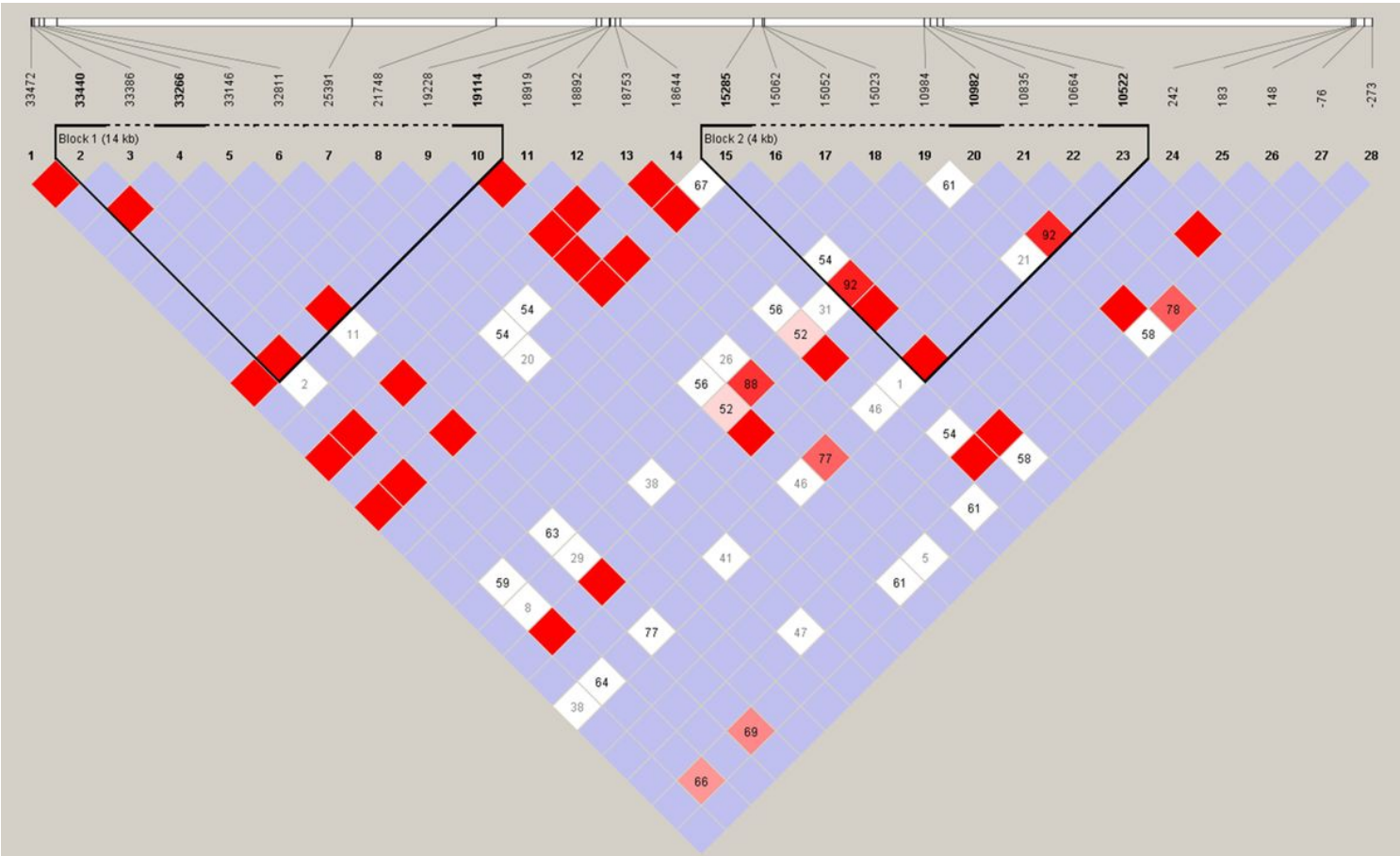

Figure 1

Haplotype block map for SNPs in CYP2J2 The linkage disequilibrium (LD) between each pair of SNPs is standardized deviation (D'). The bright red corresponds to a very strong LD; blue corresponds to intermediate LD, and white corresponds to no LD.

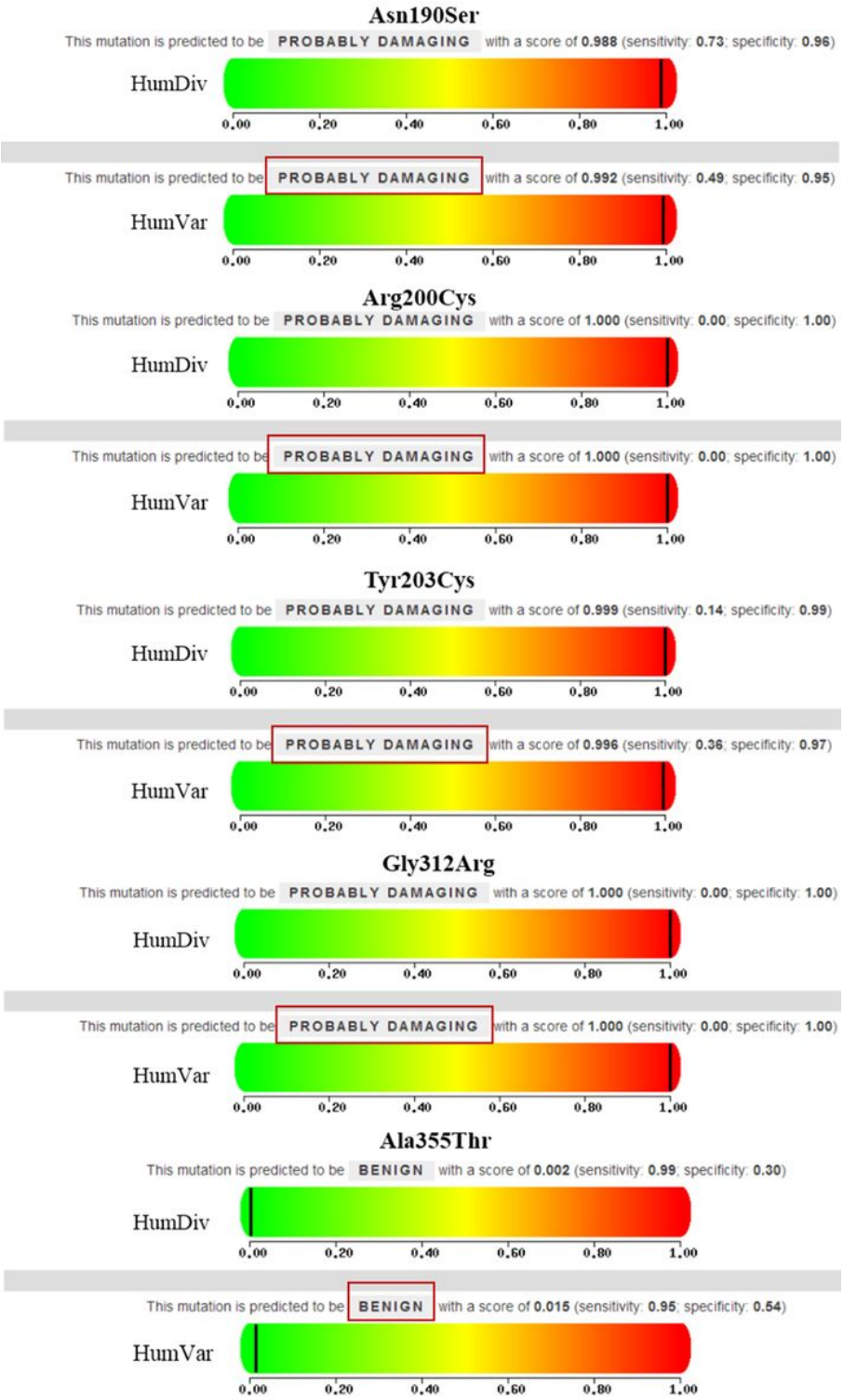

Figure 2

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