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SNP communication A

Novel single nucleotide polymorphisms (SNPs) of *CYP2W1* gene in Chinese Uygur and Han populations☆

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ABSTRACT

Cytochrome P450 2W1 (CYP2W1) is expressed specially in certain cancers and could metabolize some substances into cytotoxic antitumor drugs in Caucasian ethnic population. To investigate the genetic polymorphism of *CYP2W1* in Chinese, all the nine exons and exon–intron junctions were sequenced by dideoxy chain termination method among 385 Chinese subjects (including 223 Han and 162 Uygur). The present results showed that 40 single nucleotide polymorphisms (SNPs) were detected (14 in the exons and 26 in the introns), 10 were novel variants of which in Chinese. There were 7 novel SNPs in the exons and other 3 novel SNPs in the introns. Four of the 6 novel non-synonymous variations in exons, 131T > C (Leu44Pro), 1289C > A (Ala88Glu), 2027G > A (Arg187Gln) and 5070C > T (Thr383Met) were computationally predicted to affect CYP2W1 protein function, in spite of these variants were heterozygotes. Moreover, the allele frequencies in 6 known SNPs including *CYP2W1*2* (2008G > A) and *CYP2W1*3* (173A > C) were analyzed, which were significantly lower in Chinese Han (2.9% and 0.0%, respectively) and Uygur (5.2% and 0.0%, respectively) individuals, than those reported previously in Caucasians (9.1% and 33.1%, respectively, $P < 0.05$). These data provide useful information on the pharmacogenetic studies of *CYP2W1* among Chinese and other ethnic populations.

1. Introduction

Cytochrome P450 2W1 (CYP2W1) was demonstrated to be expressed in fetal tissues, predominantly the colon, but silenced after birth and could not be detected in adult tissues [1]. However, CYP2W1 was re-expressed in some transformed tissues, like colorectal, hepatic and adrenal tumors [2,3]. Initially, CYP2W1 was considered to be an orphan enzyme, whereas, some potential substrates of it had been found recently, such as aromatic amines [4], lysophospholipids [5] and duocarmycin analogs [6]. Promisingly CYP2W1 might be an anticancer drug target because it could metabolize some duocarmycin analogs into cytotoxic substances and subsequently inhibit the growth of cancer cells.

CYP2W1 locates on chromosome 7p22.3, spanning over 5.5 kb with nine exons. The open-reading frame is 1473-nucleotide long and encodes a 490-amino acid long polypeptide. Until now there are seven alleles of *CYP2W1* (*CYP2W1*1A*, **1B*, **2–*6*) in the CYP allele nomenclature webpage (http://www.cypalleles.ki.se/cyp2w1.htm), including five non-synonymous single nucleotide polymorphisms (SNPs): 173A > C (Glu58Ala), 2008G > A (Ala181Thr), 5432G > A (Val432Ile), 5584G > C (Gln482His), and 5601C > T (Pro488Leu), and one synonymous SNP: 166C > T [7]. Association of *CYP2W1* variants with colorectal cancer risk had been investigated in Caucasians [8,9]. However, genetic polymorphisms of *CYP2W1* in Chinese had not been reported yet.

Until recently the systematic investigation of genetic variants of *CYP2W1* had only been performed in one Japanese population. Besides a Han majority group, Chinese Uygur is a larger minority group with the population of approximately 10.07 million people (2010 census), who reside in the Silk Road of northwestern China with genetic admixture between Orientals and Caucasians and our previous studies showed that intermediate allele frequencies of *MDR1*, *CYP3A5*, *CYP2C19* and *CYP2E1* were observed in Chinese Uygur between Han and Caucasians populations [10,11]. To investigate the genetic polymorphism of *CYP2W1* among Chinese, all the

☆ On September 1, 2015, the variation was not found on the CYP allele nomenclature webpage (http://www.cypalleles.ki.se/cyp2w1.htm), the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/SNP/), or the PharmGKB (http://www.pharmgkb.org/) database.

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nine exons and exon–intron junctions were sequenced in 385 healthy Chinese subjects (including 223 Han and 162 Uygur).

2. Materials and methods

Three hundred and eighty-five unrelated mainland Chinese healthy volunteers, including 223 Chinese Han and 162 Chinese Uygur, were recruited for the present study. Each subject had the same ethnic origin for at least three generations, i.e., on both mothers' and fathers' sides, also the status of the grandparents. The present study protocol was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Beijing University, Beijing, China. Written informed consents were obtained from all subjects.

Genomic DNA was extracted from blood leukocytes by standard methods. The primers sequences amplifying *CYP2W1* are shown in Supplemental Table 1. Polymerase chain reaction (PCR) was performed in a total volume of 25 μL containing 1 μL genomic DNA (20 ng/μL), 12.5 μL 2 × Taq PCR Mix, 0.25 μL each primer pair (20 μM), and 11 μL deionized water. The detailed information regarding primers, conditions and products of PCR amplification are listed in Supplemental Table 1. PCR products were sequenced by ABI 3730XL (Applied Biosystems, Foster City, CA, USA). The sequencing primers are listed in Supplemental Table 2. Sequence chromatograms were analyzed using Sequence Scanner v1.0 (Applied Biosystems). The comparison with the inference sequence of *CYP2W1* (NCBI Reference Sequence: NC_000007.14) was performed by Align in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi?PAGE_TYPE=BlastSearch&BLAST_SPEC=blast2seq&LINK_LOC=align2seq) and verified through manual inspection.

Functional prediction of the non-synonymous SNPs was performed by software PolyPhen-2 [12], SIFT [13] and MutationTaster-2 [14] respectively. Functional prediction of the synonymous and noncoding SNPs was performed by MutationTaster-2. Allele and genotype frequencies were calculated by the counting method, and comparisons between different populations were performed using Fisher's exact test, and $P < 0.05$ was considered to be statistically significant. Statistical testing was carried out using GraphPad Prism v5 (http://www.graphpad.com). Linkage disequilibrium (LD) and Hardy–Weinberg equilibrium were assessed for each genetic variant using HAPLOVIEW 4.1 (http://broad.mit.edu/mpg/haploview).

3. Results and discussion

Ten novel SNPs were found as follows:

1) SNP: 150901Zhang/Qi001; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-TCGTCGGGAACCT/CGCACTTGCTGCG-3′.
2) SNP: 150901Zhang/Qi002; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-CTGGGCCTGTCGG/TCTGCCTGCTCAG-3′.
3) SNP: 150901Zhang/Qi003; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-CCCCCCTCGGCCC/GTCAGGTGTTCAA-3′.
4) SNP: 150901Zhang/Qi004; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-CGGTCAAAGAGGC/AGCTGGCGGGCCC-3′.
5) SNP: 150901Zhang/Qi005; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-TTTTCATCCCAAG/ATCAAGAGGGGCC-3′.
6) SNP: 150901Zhang/Qi006; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-TTCCCGCTGGCCC/TTACTGGGCTGGG-3′.
7) SNP: 150901Zhang/Qi007; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-TCTTCGGCCGCCG/AATTTGACTACCG-3′.
8) SNP: 150901Zhang/Qi008; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-GGACGCCCTGATC/GCAGCAGGGACAG-3′.
9) SNP: 150901Zhang/Qi009; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-CCCCACAGGGCAC/TGCCCGTGATTCC-3′.
10) SNP: 150901Zhang/Qi010; GENE NAME: *CYP2W1*; ACCESSION NUMBER: NC_000007.14; LENGTH: 25 bases; 5′-TTTGTGAAGCGGG/AAGGCCTTCCTGC-3′.

A total of 40 genetic variations were detected in the *CYP2W1* of 385 Chinese participants. The positions of each variant, minor allele frequencies and Hardy–Weinberg equilibrium *P*-values are shown in Supplemental Table 3. Totally 10 variations were newly discovered and reported in Chinese, of which 7 sites were located in the exons, and 3 in the introns. Of the 7 novel variations in the exons, 6 were non-synonymous. One non-synonymous variation, 5529C > T (Pro464Leu, rs149976257), and two synonymous variations, 150G > A (rs182768976) and 2055G > A (rs201860979), were also found in the present study but had not been reported in the CYP allele nomenclature webpage. The sequencing graphs of the 10 discovered coding SNPs in the present study which had not been reported in the CYP allele nomenclature webpage are shown in Supplemental Figs. 1–10.

All of the six novel non-synonymous variations were found as individual heterozygotes, of which 131T > C (Leu44Pro), 3574C > G (Ile269Met), 5070C > T (Thr383Met) and 5183G > A (Glu421Lys) with a frequency of 0.2% respectively in Chinese Han population, 1289C > A (Ala88Glu) and 2027G > A (Arg187Gln) with a frequency of 0.3% respectively in Uygur population. The novel synonymous mutation, 1975C > T, was found as a heterozygote in one Han individual with a frequency of 0.2%. We also found 5529C > T, with a frequency of 0.6% in Uygur population, 150G > A with a frequency of 0.2% in Han population, and 2055G > A with a frequency of 0.6% and 0.2% respectively in Uygur and Han population.

As shown in Supplemental Table 4, except those of *CYP2W1*1B* and *CYP2W1*2*, the allele frequencies of *CYP2W1*4* and *CYP2W1*6* were different in Han and Uygur populations. *CYP2W1*3* and *CYP2W1*5* were not found in our study. As shown in Table 1, the allele frequencies of the common known *CYP2W1* genetic variants, 166T, 2008A and 5601T, were different in Chinese Uygur and Han, Japanese and Caucasians populations.

Functional prediction of all known and newly discovered SNPs was performed in the present study by PolyPhen-2, SIFT and MutationTaster-2. As shown in Table 2, the four out of six newly discovered missense SNPs, 131T > C, 1289C > A, 2027G > A and 5070C > T were found to affect protein function by all the three programs. The other two novel missense SNPs, 3574C > G and 5183G > A, were not found to affect protein function. Of all the known missense mutations, only 173A > C (Glu58Ala) was found to affect protein function. In addition, 5584G > C (Gln482His) was found to affect protein function by MutationTaster-2, but not by PolyPhen-2 or SIFT. The computationally prediction of protein function of *CYP2W1* 2008G > A and 5601C > T was consistent with the experimental results of the two CYP2W1 variants [9]. All the synonymous mutation and SNPs in the introns found in the present study were not found to affect CYP2W1 protein function by MutationTaster-2.

Linkage disequilibrium analysis was performed by Haploview in Chinese Uygur, Han and total Chinese populations, using those SNPs found in the present study (Supplemental Figs. 11–13). The levels of LD were different between Chinese Uygur and Han