Online journal ISSN - 1676-5680
GMR Genetics and Molecular Research
EVOLUTION AND TECHNOLOGY

Genetic polymorphisms of cytochrome P450 enzymes 2C9 and 2C19 in a healthy Mongolian population in China

Z.F. Yang[1], H.W. Cui[1], T. Hasi[1], S.Q. Jia[1], M.L. Gong[2] and X.L. Su[1]

[1]Clinical Medicine Research Center,
Inner Mongolia Medical College Affiliated Hospital,
Huhhot, Inner Mongolia, China
[2]Experimental and Clinical Research Center,
Max-Delbrueck-Center for Molecular Medicine, Berlin, Germany

The current address of Z.F. Yang is Affiliated People Hospital,
Inner Mongolia Medical College, Huhhot, Inner Mongolia, China.
Corresponding author: X.L. Su
E-mail: xlsu@hotmail.com / maolian@mdc-berlin.de

Genet. Mol. Res. 9 (3): 1844-1851 (2010)
Received June 24, 2010
Accepted July 18, 2010
Published September 14, 2010
DOI 10.4238/vol9-3gmr938

ABSTRACT. We examined the distribution of major allelic variants of *CYP2C9* and *CYP2C19* in the Mongolian population of China and compared it with that of other populations. The polymorphisms of *CYP2C9* (including the *CYP2C9*1*, *CYP2C9*2* and *CYP2C9*3* alleles) and *CYP2C19* (including the *CYP2C19*1*, *CYP2C19*2* and *CYP2C19*3* alleles) were analyzed in 280 healthy unrelated Chinese Mongolian subjects, using a PCR-RFLP assay. The frequencies of *CYP2C9*1*, **2* and **3* alleles were 0.97, 0.00 and 0.03, respectively. The frequencies of *CYP2C19*1*, **2* and **3* alleles were 0.72, 0.24 and 0.04, respectively. We did not find any differences in the allelic distribution of these two genes between age groups. However, the genotype frequency of *CYP2C9 *1/*3* was significantly higher in males than in females. Compared with other populations, we found that the allele frequencies of the *CYP2C9*2* and *CYP2C9*3* allelic variants in this Mongolian population of China were similar to those reported

 ©FUNPEC-RP www.funpecrp.com.br

for other Asian populations, with significant differences compared to Caucasians and African-Americans.

Key words: CYP2C9; CYP2C19; Mongolian population; Genetic polymorphism

INTRODUCTION

More and more studies have revealed that interethnic differences in drug response play an important role in pharmacogenetics. The existence of large population difference with small intra-patient variability is consistent with inheritance as determinant of drug response. It is estimated that genetics can account for 20-95% of variability in drug disposition and effects (Hitchen, 2006). The cytochrome P450 (*CYP450* genes) family enzyme system is a primary metabolic pathway for the metabolism of drugs (Gardiner and Begg, 2006). *CYP450* genes are highly polymorphic, and mutation in different genes can result in altered, reduced, increased, or no enzyme activity or changes in the amount of enzymes (Zhou et al., 2009a).

CYP2C9 catalyses the oxidation of clinically important drugs including phenytoin, tolbutamide, warfarin, and a large number of nonsteroidal anti-inflammatory drugs (Miners and Birkett, 1998; Zhou et al., 2009b; Kesavan et al., 2010). Presently, 34 different *CYP2C9* allelic variants have been identified (http://www.cypalleles.ki.se/cyp2c9.htm, January 2009). However, *CYP2C9*2* (Arg 144 to Cys, rs1799853) and *CYP2C9*3* (Ile 359 to Leu, rs1057910) are recognized as main *CYP2C9* variants in humans (Lee et al., 2002). *CYP2C9*2* and *CYP2C9*3* have reduced catalytic activity compared with the wild-type *CYP2C9*1* (Miners and Birkett, 1998; Goldstein, 2001).

Cytochrome P450 2C19 (CYP2C19) plays an important role in the metabolism of a wide range of therapeutic drugs including barbiturates, diazepam, lansoprazole, mephenytoin, omeprazole, proguanil, and propranolol (Goldstein, 2001; Desta et al., 2002). Several polymorphisms of *CYP2C19* have been identified that produce an inactive enzyme (http://www.cypalleles.ki.se/cyp2c19.htm, June 2009). Two variant alleles account for the majority of the poor metabolizer phenotypes. The first is *CYP2C19*2*, which carries a G>A nucleotide substitution in exon 5 resulting in an aberrant splice site (rs4244285; de Morais et al., 1994a). The second variant, *CYP2C19*3*, which carries a G>A nucleotide substitution at position 636 in exon 4, produces a premature stop codon (rs4986893; de Morais et al., 1994b).

China has 55 different minorities, accounting for approximately 100 million people. The different genetic backgrounds and diverse environments of the minor populations distinguish them from the Han majority. The Mongolian population represents one of the 15 largest ethnic minorities in China. It consists of 4.2 million people living mainly in the Inner Mongolian province in the northeastern region of China. Pharmacogenetic studies of the functional significance of polymorphisms in the *CYP450* genes and their roles as risk factors in drug-induced toxicities and therapeutic failures have been performed in some Chinese populations (Leung et al., 2001; Zhang et al., 2002; Liou et al., 2006). However, no data have been reported regarding the polymorphism of *CYP450* genes in the Mongolian population of China. The aim of this study was thus to investigate the al-

 ©FUNPEC-RP www.funpecrp.com.br

lele frequencies of *CYP2C9* (**2* and **3*) and *CYP2C19* (**2* and **3*) in a sufficiently large sample of the Mongolian population in China (280 subjects) and to compare our results with the data observed in other ethnic populations.

MATERIAL AND METHODS

Subjects

Inner Mongolia, on the northern frontier of China, stretches all the way from Eastern to Western China. In this study, we estimated the distribution of common variants of *CYP2C9* and *CYP2C19* in a sample of 280 unrelated healthy Mongolian volunteers (119 males and 161 females aged between 18 and 51 years; with a mean age of 33.0 ± 9.0 years) from Xilinguole Meng located in northeastern Inner Mongolia. They are still nomadic people living on the relatively limited grazing land, thus, they rarely communicate or interact with other cultural groups in Inner Mongolia of China. They have their own ancient language and their own unique cultural traits. All subjects were informed both verbally and in writing about the procedures and purpose of the study. Written informed consents were obtained from all of them. All subjects were healthy and had no abnormalities, as shown by routine histories and physical examinations. The subjects were carefully interviewed and were considered to belong to the Mongolian nationality, by lineage and birth. For all investigated subjects, at least three generations of their family members are from the same nationality. This clinical protocol was approved by the Institution Review Board of Human Medical University and is in compliance with Department of Health and Human Services (DHHS) regulations for protection of human research subjects.

DNA extraction and PCR-RFLP

Three milliliters venous blood was obtained in Vacutainer tubes containing ethylene diamine tetraacetic acid (EDTA), from each subject. Genomic DNA was extracted from peripheral blood leukocytes using a DNA extraction kit from Promega (Germany). The extracted DNA was dissolved in sterile distilled water and stored at -20°C until polymerase chain reaction (PCR) analysis. The amplification procedure was performed in a PCR system gradient master cycler. PCR was performed in a 25-µL reaction mixture containing 2X Taq PCR buffer, 0.4 mM dNTPs, 20 µM of each specific forward and reverse primer, 1.25 U Taq DNA polymerase, and 500 ng template DNA. The amplification program was as follows: 1) initial denaturation at 94°C for 5 min, 2) 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 45 s, and 3) final extension at 72°C for 10 min. The amplification primers were the same as previously described (Kimura et al., 1987; Niu et al., 2004). The PCR products of *CYP2C9*2*, *CYP2C9*3*, *CYP2C19*2*, and *CYP2C19*3* were digested with *Ava*II, *Nsi*I, *Sma*I, and *Bam*HI, respectively. Digested PCR products were analyzed by 3% agarose gels stained with ethidium bromide and then visualized and documented.

Statistical analysis

Allele distributions were compared using χ^2 and Fisher exact tests. $P < 0.05$ was con-

sidered to be significant. These analyses were performed by SPSS (SPSS Inc., Chicago, USA, for Windows, version 11.000).

RESULTS

The allele and genotype frequencies of *CYP2C9* and *CYP2C19* in the Chinese Mongolians are summarized in Tables 1 and 2. For *CYP2C9*, 97% of the studied subjects were found to be the wild type. The *CYP2C9*2* allele was not detected in this study and the allele frequency for *CYP2C9*3* was 0.03. The allele frequencies of *CYP2C19*1*, *2, and *3 were 0.72, 0.24, and 0.04, respectively. Of the 22 poor metabolizers of CYP2C19, 18 were found to be homozygous of the *CYP2C19*2* variant, 2 were homozygous of the *CYP2C19*3* variant, and 2 were heterozygous of both variants.

Table1. Allele frequencies of *CYP2C9* and *CYP2C19* in the Mongolian population.

Gene	Variant allele	Frequency (N = 560, %)
CYP2C9	*CYP2C9*1*	0.97%
	*CYP2C9*2*	0.00%
	*CYP2C9*3*	0.03%
CYP2C19	*CYP2C19*1*	0.72%
	*CYP2C19*2*	0.24%
	*CYP2C19*3*	0.04%

N = number of alleles.

Further analysis showed that the low-activity allele of *CYP2C9*3* (slow metabolizer) was significantly more common in men than in women ($P < 0.005$); no other gender difference was detected for the two genes in the studied population (Table 2). No significant difference was detected in different age groups for the allele and genotype frequencies of these two genes (Table 2).

The allele frequencies of these two genes in the Mongolian population were compared with those in the other populations (Table 3). In contrast to the significantly lower frequency of the *CYP2C9*2* allele in the Mongolians than that in the African-American and American populations, the frequency of the *CYP2C9*3* allele in the Mongolians was significantly higher than that in both populations. Allelic distributions of *CYP2C9*2* and *CYP2C9*3* were similar among Mongolian, Han Chinese, and Japanese populations. For the gene *CYP2C19*, the allele frequency of *CYP2C19*2* was significantly higher than that in Caucasians, but was similar to that in Africans and other Asians. The allele frequency of the *CYP2C19*3* was significantly higher than that in African-American and American populations. No significant difference of *CYP2C19*3* frequency was found among the Asian groups.

DISCUSSION

In our study, *CYP2C9*2* was absent in the Chinese Mongolian population, which was consistent with the previous reports in Orientals (Sullivan-Klose et al., 1996; Xie et al., 2002). These findings confirmed that the *CYP2C9*2* allele mutation occurred rarely in the East Asian populations, which is in contrast with the higher frequency of the *CYP2C9*2* variant among Caucasians. The *CYP2C9*2* allele occurs at a significantly lower frequency in the African-American population compared to Caucasians. In the case of *CYP2C9*3*, the allele frequency

Table2. Genotype frequencies of *CYP2C9* and *CYP2C19* and comparison with gender and age groups in the Mongolian population.

Gene	Genotype	N = 280	Frequency (%)	Male		Female		18-33 years		34-51 years	
				N = 119	Frequency	N = 161	Frequency	N = 139	Frequency	N = 141	Frequency
CYP2C9	*CYP2C9*1/*1*	260	0.93	101	0.85	159**	0.99	130	0.94	130	0.92
	*CYP2C9*1/*2*	0	-	0	-	0	-	0	-	0	-
	*CYP2C9*1/*3*	20	0.07	18	0.15	2**	0.01	9	0.06	11	0.08
	*CYP2C9*2/*2*	0	-	0	-	0	-	0	-	0	-
	*CYP2C9*2/*3*	0	-	0	-	0	-	0	-	0	-
	*CYP2C9*3/*3*	0	-	0	-	0	-	0	-	0	-
CYP2C19	*CYP2C19*1/*1*	142	0.51	56	0.47	86	0.53	70	0.5	72	0.5
	*CYP2C19*1/*2*	98	0.35	43	0.36	55	0.34	45	0.32	53	0.38
	*CYP2C19*1/*3*	18	0.06	8	0.07	10	0.06	12	0.09	6	0.04
	Poor metabolizer (sum)	22	0.08								
	*CYP2C19*2/*2*	18	0.06	10	0.08	8	0.06	10	0.07	8	0.06
	*CYP2C19*2/*3*	2	0.01	1	0.01	1	0.01	1	0.01	1	0.01
	*CYP2C19*3/*3*	2	0.01	1	0.01	1	0.01	1	0.01	1	0.01

N = number of subjects. **P < 0.01 compared to the male group.

©FUNPEC-RP www.funpecrp.com.br

Table3. Comparison of allele frequencies of *CYP2C9* and *CYP2C19* with other different ethnic populations.

Ethnicity	N	*1	*2	*3	Reference
CYP2C9					
Chinese-Mongolian	560	0.970	0	0.030	Present study
Chinese-Han	196	0.974	0	0.026	Sullivan-Klose et al., 1996
Japanese	436	0.979	0	0.021	Nasu et al., 1997
African-American	200	0.985	0.010***	0.005**	Sullivan-Klose et al., 1996
European-American	200	0.860	0.080***	0.060**	Sullivan-Klose et al., 1996
CYP2C19					
Chinese-Mongolian	560	0.720	0.240	0.040	Present study
Chinese-Han	400	0.697	0.247	0.033	Chen et al., 2008
Japanese	106	0.670	0.230	0.100	Goldstein et al., 1997
African American	216	0.750	0.250	0.000***	Goldstein et al., 1997
European American	210	0.870	0.130***	0.000***	Goldstein et al., 1997

N = total number of alleles; **P < 0.05 compared to the Mongolian population; ***P < 0.001 compared to the Mongolian population.

in the Chinese Mongolian population was similar to that in Han Chinese and Japanese, but was higher than that in Caucasians and Africans. In our study, we identified 7% of the subjects with the *CYP2C9*1*/**3* heterozygote, but no subjects with the *CYP2C9*3*/**3* homozygote were detected. This result is similar to that in other Eastern populations (Xie et al., 2002). However, our results showed that 90% of the *CYP2C9*1/*3* genotype in the Chinese Mongolian were from men ($P < 0.005$). Considering the relatively small sample size, this finding should be further confirmed with a larger sample size. The *CYP2C9*2* or *CYC2P9*3* variant has been associated with an increased risk of serious or life threatening bleeding events in warfarin-treated patients (Sanderson et al., 2005; Goto et al., 2010). Therefore, identifying *CYP2C9* variants could potentially improve clinical management in patients commencing warfarin, especially for male patients in the Chinese Mongolian population, if our finding is confirmed in the future.

The genetic polymorphism of *CYP2C19* has been shown to have the most striking interethnic variation of all the *CYPs* so far. Both *CYP2C19*2* and *CYP2C19*3* alleles were reported to produce truncated proteins without enzyme activity (Gardiner and Begg, 2006). The allele frequency of *CYP2C19*2* in our study was in agreement with previous studies in Han Chinese (Chen et al., 2008), Japanese and African populations (Goldstein et al., 1997), but was significantly higher than that observed in Caucasians (Goldstein et al., 1997). Our result showed that the frequency of *CYP2C19*3* was slightly lower than that in other Asian populations such as Taiwanese, Japanese or Korean (Roh et al., 1996; Goldstein et al., 1997; Liou et al., 2006); however, it was significantly higher than that in Caucasians and Africans (Goldstein et al., 1997). The *CYP2C19*1*/**2* and *CYP2C19*1*/**3*, which indicate the intermediate metabolizer classification, accounted for about 41% (95% confidence interval: 0.35 ± 0.064) of *CYP2C19* genotypes in the Mongolian population, which was similar to that in Mainland Han Chinese (Chen et al., 2008), but lower than that in Han Taiwanese (Liou et al., 2006). The frequency (8.0%) of poor metabolizer including *CYP2C19*2/*2*, *CYP2C19*2/*3* and *CYP2C19*3/*3* altogether in Chinese Mongolians was similar to other Asian populations but markedly higher than in Caucasians and Africans (2 to 7%; Bertilsson, 1995). These data suggest that different ethnic groups may exhibit different sensitivities to drugs metabolized by *CYP2C19*.

The individual response to a drug, such as the risk of toxicity, is a complex equation involving multiple variables. However, genetic polymorphisms (usually inactivating) are one

of the major causes of variation in drug responsiveness (Evans and Johnson, 2001). It has been reported that the drug doses used in clinical trials with East Asian participants are lower than those used in trials with Western participants (Yu et al., 1996; Ross et al., 2001). Understanding the genotype and frequency of *CYP450* allelic variations in the Chinese Mongolian population may help in the development of appropriate strategies for drug therapy, clinical safety, and to improve public health care in this community.

CONCLUSION

In summary, our study showed that the *CYP2C9* and *CYP2C19* variants in Chinese Mongolians are similar to other Asian populations but significantly different from Caucasians and African-Americans. Considering the similarity in allele distribution between Chinese Mongolians and other Asian populations, it may be expected that the severity of drug side effects or lack of efficacy due to individual genetic backgrounds in Chinese Mongolians are also similar to that of other Asian groups.

REFERENCES

Bertilsson L (1995). Geographical/interracial differences in polymorphic drug oxidation. Current state of knowledge of cytochromes P450 (CYP) 2D6 and 2C19. *Clin. Pharmacokinet.* 29: 192-209.

Chen L, Qin S, Xie J, Tang J, et al. (2008). Genetic polymorphism analysis of CYP2C19 in Chinese Han populations from different geographic areas of mainland China. *Pharmacogenomics* 9: 691-702.

de Morais SM, Wilkinson GR, Blaisdell J, Nakamura K, et al. (1994a). The major genetic defect responsible for the polymorphism of S-mephenytoin metabolism in humans. *J. Biol. Chem.* 269: 15419-15422.

de Morais SM, Wilkinson GR, Blaisdell J, Meyer UA, et al. (1994b). Identification of a new genetic defect responsible for the polymorphism of (S)-mephenytoin metabolism in Japanese. *Mol. Pharmacol.* 46: 594-598.

Desta Z, Zhao X, Shin JG and Flockhart DA (2002). Clinical significance of the cytochrome P450 2C19 genetic polymorphism. *Clin. Pharmacokinet.* 41: 913-958.

Evans WE and Johnson JA (2001). Pharmacogenomics: the inherited basis for interindividual differences in drug response. *Annu. Rev. Genomics Hum. Genet.* 2: 9-39.

Gardiner SJ and Begg EJ (2006). Pharmacogenetics, drug-metabolizing enzymes, and clinical practice. *Pharmacol. Rev.* 58: 521-590.

Goldstein JA (2001). Clinical relevance of genetic polymorphisms in the human CYP2C subfamily. *Br. J. Clin. Pharmacol.* 52: 349-355.

Goldstein JA, Ishizaki T, Chiba K, de Morais SM, et al. (1997). Frequencies of the defective CYP2C19 alleles responsible for the mephenytoin poor metabolizer phenotype in various Oriental, Caucasian, Saudi Arabian and American black populations. *Pharmacogenetics* 7: 59-64.

Goto T, Miura M, Murata A, Terata K, et al. (2010). Standard warfarin dose in a patient with the CYP2C9*3/*3 genotype leads to hematuria. *Clin. Chim. Acta* 411: 1375-1377.

Hitchen L (2006). Adverse drug reactions result in 250,000 UK admissions a year. *BMJ* 332: 1109.

Kesavan R, Narayan SK and Adithan C (2010). Influence of CYP2C9 and CYP2C19 genetic polymorphisms on phenytoin-induced neurological toxicity in Indian epileptic patients. *Eur. J. Clin. Pharmacol.* 66: 689-696.

Kimura S, Pastewka J, Gelboin HV and Gonzalez FJ (1987). cDNA and amino acid sequences of two members of the human P450IIC gene subfamily. *Nucleic Acids Res.* 15: 10053-10054.

Lee CR, Goldstein JA and Pieper JA (2002). Cytochrome P450 2C9 polymorphisms: a comprehensive review of the *in-vitro* and human data. *Pharmacogenetics* 12: 251-263.

Leung AY, Chow HC, Kwong YL, Lie AK, et al. (2001). Genetic polymorphism in exon 4 of cytochrome P450 CYP2C9 may be associated with warfarin sensitivity in Chinese patients. *Blood* 98: 2584-2587.

Liou YH, Lin CT, Wu YJ and Wu LS (2006). The high prevalence of the poor and ultrarapid metabolite alleles of CYP2D6, CYP2C9, CYP2C19, CYP3A4, and CYP3A5 in Taiwanese population. *J. Hum. Genet.* 51: 857-863.

Miners JO and Birkett DJ (1998). Cytochrome P4502C9: an enzyme of major importance in human drug metabolism. *Br.*

J. Clin. Pharmacol. 45: 525-538.
Nasu K, Kubota T and Ishizaki T (1997). Genetic analysis of CYP2C9 polymorphism in a Japanese population. *Pharmacogenetics* 7: 405-409.
Niu CY, Luo JY and Hao ZM (2004). Genetic polymorphism analysis of cytochrome P4502C19 in Chinese Uigur and Han populations. *Chin. J. Dig. Dis.* 5: 76-80.
Roh HK, Dahl ML, Tybring G, Yamada H, et al. (1996). CYP2C19 genotype and phenotype determined by omeprazole in a Korean population. *Pharmacogenetics* 6: 547-551.
Ross AM, Gao R, Coyne KS, Chen J, et al. (2001). A randomized trial confirming the efficacy of reduced dose recombinant tissue plasminogen activator in a Chinese myocardial infarction population and demonstrating superiority to usual dose urokinase: the TUCC trial. *Am. Heart J.* 142: 244-247.
Sanderson S, Emery J and Higgins J (2005). CYP2C9 gene variants, drug dose, and bleeding risk in warfarin-treated patients: a HuGEnet systematic review and meta-analysis. *Genet. Med.* 7: 97-104.
Sullivan-Klose TH, Ghanayem BI, Bell DA, Zhang ZY, et al. (1996). The role of the CYP2C9-Leu359 allelic variant in the tolbutamide polymorphism. *Pharmacogenetics* 6: 341-349.
Xie HG, Prasad HC, Kim RB and Stein CM (2002). CYP2C9 allelic variants: ethnic distribution and functional significance. *Adv. Drug Deliv. Rev.* 54: 1257-1270.
Yu HC, Chan TY, Critchley JA and Woo KS (1996). Factors determining the maintenance dose of warfarin in Chinese patients. *QJM* 89: 127-135.
Zhang S, Dong Z, Tang L, Zhou Q, et al. (2002). Cytochrome P450 2C19 gene polymorphism in four Chinese nationality populations. *Zhonghua Yi Xue Yi Chuan Xue Za Zhi* 19: 52-54.
Zhou SF, Liu JP and Chowbay B (2009a). Polymorphism of human cytochrome P450 enzymes and its clinical impact. *Drug Metab. Rev.* 41: 89-295.
Zhou SF, Zhou ZW, Yang LP and Cai JP (2009b). Substrates, inducers, inhibitors and structure-activity relationships of human Cytochrome P450 2C9 and implications in drug development. *Curr. Med. Chem.* 16: 3480-3675.

 ©FUNPEC-RP www.funpecrp.com.br