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Genetic polymorphisms of drug-metabolizing phase I enzymes CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in Han, Uighur, Hui and Mongolian Chinese populations

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We randomly evaluated 672 unrelated, healthy Chinese volunteers (136 Han, 214 Uighur, 164 Hui and 158 Mongolian) to compare CYP3A4, CYP2C9, CYP2C19 and CYP2D6 allele frequencies. Genomic DNA was extracted from peripheral leukocytes and genotyped for CYP3A4*5, CYP3A4*18, CYP2C9*2, CYP2C9*13, CYP2C19*2, CYP2C19*3 and CYP2D6*10 by PCR-restriction fragment length polymorphism analysis (PCR-RFLP). Our results showed that there is no significant difference in the distribution of CYP2C19*3 and CYP3A4*18 genotypes in the Han, Uighur, Hui and Mongolian Chinese populations. The CYP2C9*13/*13 and CYP3A4*5 genotypes were not observed in any of the four Chinese populations. We found a higher incidence of the CYP2C9*2 allele in Uighur populations, compared to the Han, Hui and Mongolian populations. The incidence of the CYP2C19*2 allele in the Han population was not significantly different from that in the Uighur, Hui or Mongolian populations; however, the Uighur population showed significantly lower rates of this allele than the Hui and Mongolian populations, and the Mongolian population had a significantly lower incidence of this allele than the Hui population. There was no significant difference in the presence of the CYP2D6*10 allele in the Mongolian, Han or Hui populations. However, the Uighur population showed significantly lower rates of this allele than the other three populations. These findings provide basic genetic information for further pharmacogenomic investigations in the Chinese population.

1. Introduction

The cytochrome P450 (CYP) enzymes CYP3A4, CYP2C9, CYP2C19 and CYP2D6 mediate 70–80% of phase I-dependent metabolism of clinically administered drugs. Genetic polymorphisms are known to contribute to inter-individual variations in the metabolism of numerous drugs in humans. Mutations in genes coding for drug metabolizing enzymes can result in enzyme variants with high, low or no activity (Ingelman-Sundberg 2004; Daly et al. 1993; Wrighton et al. 1996; Meyer 1994). Pharmacokinetic polymorphisms divide the population into at least two phenotypes: poor metabolizers (PMs) and extensive metabolizers (EMs). The PM condition can lead to an excessive or prolonged therapeutic effect or drug-related toxicity after a normal dose, conferring a genetic predisposition to drug-induced adverse effects. In comparison, EM populations may not achieve therapeutic levels of the drug administered at a standard dose resulting in the lack of a therapeutic effect. The use of phenotyping or genotyping tests to determine an individual's metabolic capacity may become an important tool for a more rational and safe drug administration, especially for agents with a narrow therapeutic index.

CYP3A4 is involved in the metabolism of more than 50% of clinical therapeutic drugs including antibiotics, nafazodone, and trazodone (Wang and Zhou 2005). CYP3A4*5 and *18 are the predominant mutations affecting the metabolism of certain drugs in Chinese people. CYP2C9, the most abundant human CYP2C isoform, is polymorphically expressed (Miners and Birkett 1998). This enzyme metabolizes a number of therapeutically important drugs, including most nonsteroidal anti-inflammatory drugs, S-warfarin, phenytoin and losartan. CYP2C19 is another member of the cytochrome P450 (CYP) superfamily of enzymes and is involved in the metabolism of a number of clinically important drugs (Goldstein and de Morais 1994; Daly 1995). Among the 21 variants of CYP2C19, two principle alleles, CYP2C19*2 and CYP2C19*3, are associated with the PM phenotype in Caucasians and Asian populations (de Morais et al.1995, 1994). In the case of EM, the activity of CYP2C19 is significantly reduced in Asians compared with Caucasian subjects, likely reflecting the higher frequency of heterozygous subjects in Asian populations (Bertilsson et al. 1992). CYP2D6, although expressed at lower levels compared with other human CYPs, is involved in the biotransformation of approximately 20% of commonly used drugs, such as antipsychotics, antihistamines, antidepressants, β-blockers and antiarrhythmics (Gardiner and Begg 2006). The most common allele associated with the PM phenotype in Chinese people is CYP2D6*10 (Ling et al. 2002). China is a multiracial country, comprising 55 ethnic minorities other than the Han majority, with significant disparities in genetics, physiology, pathology, eating habits and living environments. These variable factors may contribute to interethnic differences in drug responses. Considering the

Table 1: Genotype frequencies for CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in Han, Uighur, Hui and Mongolian races of Chinese population

Gene	Genotype	Han (n = 136)		Uyghur (n = 214)		Hui (n = 164)		Mongolian (n = 158)	
		n	Observed(predicted[a]) Frequency	n	Observed(predicted[a]) Frequency	n	Observed(predicted[a]) Frequency	n	Observed(predicted[a]) Frequency
CYP3A4	*1/*1	88	0.6471(0.6662)	157	0.7336(0.7393)	105	0.6402(0.6527)	98	0.6203(0.6360)
	*1/*5	0	0(0)	0	0(0)	0	0(0)	0	0(0)
	*5/*5	0	0(0)	0	0(0)	0	0(0)	0	0(0)
	*1/*18	46	0.3382(0.3000)	54	0.2523(0.2411)	55	0.3354(0.3104)	56	0.3544(0.3230)
	*18/*18	2	0.0147(0.0338)	3	0.0140(0.0200)	4	0.0244(0.0369)	4	0.0253(0.0410)
CYP2C9	*1/*1	133	0.9779(0.9781)	175	0.8187(0.8134)	149	0.9085(0.9107)	150	0.9494(0.9438)
	*1/*2	3	0.0221(0.0218)	35	0.1636(0.1728)	15	0.0915(0.0872)	6	0.0380(0.0492)
	*2/*2	0	0(0)	3	0.0140(0.0092)	0	0(0)	1	0.0063(0.0006)
	*1/*13	0	0(0)	1	0.0047(0.0041)	0	0(0)	1	0.0063(0.0062)
	*13/*13	0	0(0)	0	0(0)	0	0(0)	0	0
CYP2C19	*1/*1	48	0.3529(0.3164)	86	0.4019(0.4280)	32	0.1951(0.2064)	42	0.2658(0.2928)
	*1/*2	53	0.3897(0.4343)	103	0.4813(0.4250)	78	0.4756(0.4488)	81	0.5127(0.4484)
	*2/*2	21	0.1544(0.1490)	16	0.0748(0.1055)	38	0.2317(0.2439)	21	0.1329(0.1719)
	*1/*3	4	0.0294(0.0579)	5	0.0234(0.0257)	7	0.0427(0.0471)	6	0.0380(0.0479)
	*3/*3	0	0(0.0027)	0	0(0.0004)	1	0.0061(0.0027)	0	0(0.0020)
	*2/*3	10	0.0735(0.0398)	4	0.0187(0.0136)	8	0.0488(0.0512)	8	0.0506(0.0367)
CYP2D6	*1/*1	25	0.1838(0.1819)	131	0.6121(0.6017)	59	0.3598(0.3755)	45	0.2848(0.2860)
	*1/*10	66	0.4853(0.4892)	70	0.3271(0.3480)	83	0.5061(0.4746)	79	0.5000(0.4976)
	*10/*10	45	0.3309(0.3289)	13	0.0607(0.0503)	22	0.1341(0.1500)	34	0.2152(0.2164)

[a] Predicted frequencies calculated according to the Hardy–Weinberg equation.

pharmacological implications of genetic polymorphisms in the genes encoding CYP3A4, CYP2C9, CYP2C19 and CYP2D6, the determination of allele distribution patterns in different races might be important for the optimization of pharmacological therapies. Therefore, we evaluated the allelic and genotypic pattern of CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in healthy Han (Liaoning Province), Uighur, Hui and Mongolian nationalities of the Chinese population. A meta-analysis was used to compare the allele and genotype frequencies in the four races.

2. Investigation and results

The genotype frequencies of CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in Han, Uighur, Hui and Mongolian ethnic populations are summarized in Table 1. Allele frequencies for CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in the Han, Uighur, Hui and Mongolian populations are summarized in Table 2. CYP3A4, CYP2C9, CYP2C19 and CYP2D6 allele and genotype frequencies resulted in equilibria according to the Hardy–Weinberg equation. Deviations of allele frequencies for CYP3A4, CYP2C9, CYP2C19 and CYP2D6 between different populations were assessed with a Chi-squared test (Table 3).

Table 2: Allele Frequency of CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in Han, Uighur, Hui and Mongolian Chinese populations

Gene	Allele	Allele Frequency			
		Han (n = 136)	Uyghur (n = 214)	Hui (n = 164)	Mongolian (n = 158)
CYP3A4	*1	0.8162	0.8598	0.8079	0.7975
	*5	0	0	0	0
	*18	0.1838	0.1402	0.1921	0.2025
CYP2C9	*1	0.9890	0.9019	0.9543	0.9715
	*2	0.0110	0.0958	0.0457	0.0253
	*13	0	0.0023	0	0.0032
CYP2C19	*1	0.5625	0.6542	0.4543	0.5411
	*2	0.3860	0.3248	0.4939	0.4146
	*3	0.0515	0.0210	0.0518	0.0443
CYP2D6	*1	0.4265	0.7757	0.6128	0.5348
	*10	0.5735	0.2243	0.3972	0.4652

In our study, we did not find the CYP2C9*13/*13, CYP3A4*1/*5 and CYP3A4*5/*5 genotypes in any of the four Chinese populations tested. Results from a Chi-squared test indicate that there were no significant ethnic differences in the distribution of CYP3A4*18 and CYP2C19*3 allele genotypes among the Han, Uighur, Hui and Mongolian Chinese populations. The CYP2C9*2 allele was not present at significantly different rates among the Han, Hui, and Mongolian populations, but the incidence of this allele in Uighur populations (9.58%) was significantly higher than in the Han (1.1%), Hui (4.57%) or Mongolian (2.53%) populations. The incidence of the CYP2C19*2 allele in the Han population was not significantly different from the incidence observed in the Uighur, Hui and Mongolian populations; however, the incidence of this allele in the Uighur population (32.48%) was significantly lower than that in the Hui (49.39%) and Mongolian (41.46%) populations. The rate of this allele in the Mongolian population (41.46%) was significantly lower than that in the Hui (49.39%) population. The presence of the CYP2D6*10 allele was not found to be significantly different among the Mongolian (46.52%), Han (57.35%) or Hui (39.72%) populations. However, this allele was found at significantly lower rates in the Uighur population (22.43%) compared to the other three ethnic populations.

3. Discussion

Drug metabolizing enzymes (DMEs) show genetic variations that can lead to inter-individual differences in the response to drugs and consequently, differences in adverse side effects. PM individuals may exhibit toxic side effects or a lack of response during drug treatment through the accumulation of a parent drug or its metabolites. In comparison, EM individuals may require higher than normal doses to achieve therapeutic parent drug levels in the blood.

Table 3: Comparisons between two groups

Comparisons between two groups	CYP3A4	CYP2C9	CYP2C19		CYP2D6
	*18	*2	*2	*3	*10
Han and Uyghur	$P = 0.274$	$P = 0.001$*	$P = 0.290$	$P = 0.087$	$P < 0.01$*
Han and Hui	$P = 0.804$	$P = 0.059$	$P = 0.053$	$P = 0.915$	$P = 0.002$*
Han and Mongolian	$P = 0.686$	$P = 0.023$*	$P = 0.537$	$P = 0.774$	$P = 0.072$
Uyghur and Hui	$P = 0.153$	$P = 0.041$*	$P = 0.001$*	$P = 0.098$	$P < 0.01$*
Uyghur and Mongolian	$P = 0.868$	$P = 0.006$*	$P = 0.073$	$P = 0.149$	$P < 0.01$*
Hui and Mongolian	$P = 0.111$	$P = 0.391$	$P = 0.170$	$P = 0.849$	$P = 0.157$

Table 4: Primers for polymerase chain reaction (PCR)

Enzyme	Allele	primer	DNA size for PCR
CYP3A4	*5	F:TGTTGCATGCATAGAGGAAGGATGG R:GATGACAGGGTTTGTGACAGGGG	450 bp
	*18	F: 5'-CAC CCT GAT GTC CAG AAA CT-3' R: 5'-AAT TGA AAG CAG ATG AAC CAG AGCC-3'	287 bp
CYP2C9	*2	F:ATGGAAAACAGAGACTTACAGAGGT R:CCAGTAAGGTCAGTGATATGGAGTAG	309 bp
	*13	F:AATATCATGCTAAATCAGGCTTAGC R:CTGCCCGAGGAGCTCTGTAAGTC	323 bp
CYP2C19	*2	F: 5'-AAT TAC AAC CAG AGC TTG GC-3' R: 5'-TAT CAC TTT CCA TAA AAG CAAG-3'	169 bp
	*3	F: 5'-AAA TTG TTT CCA ATC ATT TAG CT-3' R: 5'-ACT TCA GGG CTT GGT CAA TA-3'	271 bp
CYP2D6	*10	F: 5' -TCG GTG TGC TGA GAG TGT CCT- 3' R: 5' -TGG TTT CAC CCA CCA TCC AT- 3'	355 bp

This is the first report comparing the frequencies of CYP3A4, CYP2C9, CYP2C19 and CYP2D6 allelic variants in Han (Liaoning), Uighur, Hui and Mongolian people in the Chinese population. It has been reported that the CYP2C9*13 and CYP3A4*5 mutations might decrease the enzymatic activity of CYP2C9 and CYP3A4, affecting the metabolism of clinical drugs such as CsA and lornoxicam. The results of our study show that no significant ethnic differences exist in the distribution of CYP3A4*18 and CYP2C19*3 alleles among the four Chinese populations. The CYP2C9*1/*13 allele was found in 2 of 158 Chinese subjects, while the CYP3A4*5 allele was found in 2 of 102 Chinese subjects in a previous study. The present study, which is based on a different population of individuals, demonstrated the presence of the CYP2C9*1/*13 allele in 1 of 214 Uighur subjects and in 1 Mongolian subject. We did not observe the CYP2C9*13/*13, CYP3A4*1/*5 or CYP3A4*5/*5 alleles in any of the four Chinese populations. However, we may not have had a large enough sample size to detect these genotypes.

The CYP2C9*2 allele has been associated with lower warfarin dosage requirements (Furuya et al. 1995; Aithal et al. 2005) and a more pronounced response to anticoagulation therapy (Lindh et al. 2005). We found that for CYP2C9*2, there was no significant difference among the Han, Hui and Mongolian populations, but the incidence of this allele in the Uighur population (9.58%) is significantly higher than that in the Han (1.1%, $P = 0.001$), Hui (4.57%, $P = 0.041$) and Mongolian (2.53%, $P = 0.006$) populations.

Our study shows that the allele and genotype frequencies of CYP2C19 in the Han (Liaoning), Uighur, Hui, and Mongolian populations are similar to those of other races in the Chinese population based on previous reports (Ghodke et al. 2007; Alimu et al. 2007; Zhan et al. 2001; Zhang et al. 2002; Ding et al. 2004). However, the CYP2C19*2 allele is highly polymorphic in the Uighur and Hui populations with an allele frequency of 32.5% in the Uighur population and 49.4% in the Hui population. The variability of CYP2C19*2 among different ethnic populations may contribute to drug efficiency and toxicity. Individuals with CYP2C19 (*1/*1, *1/*2, or *1/*3) have sufficient amounts of enzyme to metabolize CYP2C19 substrates and are EMs, whereas individuals with CYP2C19 (*2/*2, *2/*3, or *3/*3) are PMs. In our study, the PM genotype frequency in the Uighur (9.35%) population is lower than in the Han (22.8%, $P = 0.001$), Mongolian (18.4%, $P = 0.011$) or Hui (28.7%, $P < 0.01$) population. The PM genotype frequency in the Mongolian (18.4%) population is lower than in the Hui (28.7%, $P = 0.029$). One can speculate that the PMs are more likely to develop side effects to drugs that are substrates of the enzyme if administered at normal doses. PMs may also experience decreased therapeutic benefits from drugs that are bio-activated to form therapeutically active metabolites, for example proguanil, which is metabolized into cycloguanil (Ward et al. 1991).

Our results show significant ethnic differences in the distribution of the CYP2D6*10 allele in the Han, Uighur, Hui and Mongolian Chinese populations ($P < 0.01$). The frequency of this allele in the Mongolian population was not significantly different from that in the Han or Hui populations. However, the frequency of this allele in the Uighur population (22.43%) was significantly lower than that in the Han (57.35%, $P < 0.01$), Hui (39.72%, $P < 0.01$) and Mongolian (46.52%, $P < 0.01$) pop-

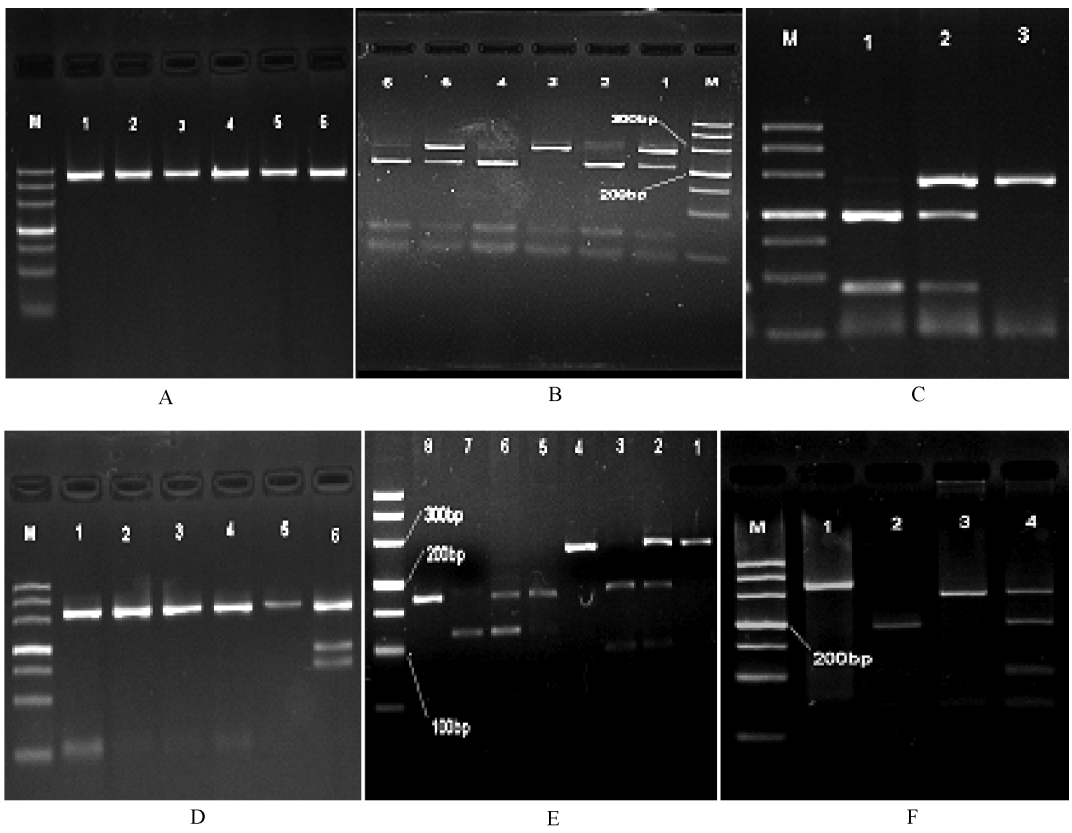
Table 5: Annealing temperature

Enzyme	CYP3A4		CYP2C9		CYP2C19		CYP2D6
Allele	*5	*18	*2	*13	*2	*3	*10
Temperature(°C)	62	62	55	58	55	58	60

Table 6: FastDigest restriction endonuclease

Enzyme	Allele	FastDigest restriction endonuclease	DNA fragments digested		
			wild	heterozygous	homozygous
CYP3A4	*5	Cla	450 bp	450, 250, 200 bp	250, 200 bp
	*18	Rsal I	217, 70 bp	287, 217, 70 bp	287 bp
CYP2C9	*2	Ava I	195, 91 bp	286, 195, 91 bp	286 bp
	*13	Mva I	323 bp	323, 178, 145 bp	178, 145 bp
CYP2C19	*2	Sma I	120, 49 bp	169, 120, 49 bp	169 bp
	*3	BamH I	175, 96 bp	271, 175, 96 bp	271 bp
CYP2D6	*10	Hph I	287 bp	287, 187, 100 bp	187, 100 bp

ulations. Furthermore, the Hui (39.72%) population exhibited this allele at significantly lower rates than the Han (57.35%, $P = 0.002$) population. Individuals carrying the CYP2D6*10 allele are intermediate metabolizers (IMs) (Cai et al. 1997). IMs are a result of the *10/*10 genotype. The IM frequency in the Uighur population (6.07%) is lower than that in the



A: Lane M: Marker (50 bp-500 bp); Lane 1-6: CYP3A4*5 (wild).
B: Lane M: Marker (50 bp-500 bp); Lane 2, 4, 6: CYP3A4*18 (wild,); Lane 1, 5: CYP3A4*18 (heterozygous); Lane3: CYP3A4*18 (homozygous).
C: Lane M: Marker (50 bp-500 bp); Lane1: CYP2C9*2 (wild); Lane2: CYP2C9*2 (heterozygous); Lane3: CYP2C9*2 (homozygous).
D: Lane M: Marker (50 bp-500 bp); Lane 1-5: CYP2C9*13 (wild); Lane 6: CYP2C9*13 (heterozygous).
E: Lane M: Marker (50 bp-500 bp); Lane7: CYP2C19*2 (wild); Lane6: CYP2C19*2 (heterozygous); Lane 5: CYP2C19*2 (homozygous); Lane 3: CYP2C19*3 (wild); Lane 2: CYP2C19*3 (heterozygous); Lane 1: CYP2C19*3 (homozygous).
F: Lane M: Marker (50 bp-500 bp); Lane 3: CYP2D6*10 (wild); Lane 4: CYP2D6*10 (heterozygous); Lane 2: CYP2D6*10 (homozygous)

Fig. 1: PCR products digested with FastDigest restriction endonuclease A: Lane M: Marker (50 bp-500 bp); Lane 1–6: CYP3A4*5 (wild). B: Lane M: Marker (50 bp-500 bp); Lane 2, 4, 6: CYP3A4*18 (wild,); Lane 1, 5: CYP3A4*18 (heterozygous); Lane3: CYP3A4*18 (homozygous). C: Lane M: Marker (50 bp-500 bp); Lane M: Marker (50 bp-500 bp); Lane1: CYP2C9*2 (wild); Lane2: CYP2C9*2 (heterozygous); Lane3: CYP2C9*2 (homozygous). D: Lane M: Marker (50 bp-500 bp); Lane 1–5: CYP2C9*13 (wild); Lane 6: CYP2C9*13 (heterozygous). E: Lane M: Marker (50 bp-500 bp); Lane7: CYP2C19*2 (wild); Lane6: CYP2C19*2 (heterozygous); Lane 5: CYP2C19*2 (homozygous); Lane 3: CYP2C19*3 (wild); Lane 2: CYP2C19*3 (heterozygous); Lane 1: CYP2C19*3 (homozygous). F: Lane M: Marker (50 bp-500 bp); Lane 3: CYP2D6*10 (wild); Lane 4: CYP2D6*10 (heterozygous); Lane 2: CYP2D6*10 (homozygous)

Han (33.1%, $P < 0.0001$), Hui (13.4%, $P = 0.008$) or Mongolian (21.5%, $P < 0.01$) populations. The IM frequency in the Mongolian (21.5%, $P = 0.026$) and Hui (13.4%, $P < 0.01$) populations is less than that in the Han population (33.1%).

Our comparison of the genetic variation in the genes encoding CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in Han, Uighur, Hui and Mongolian Chinese populations shows significant differences and significant similarities in genotype distribution. The differences could be attributed to the ethnic origin and geographical distribution of these populations, their cultural and dietary habits, or other environmental factors. It is of potential clinical importance to identify individuals from different areas of China who have altered pharmacokinetics for CYP3A4, CYP2C9, CYP2C19 and CYP2D6 substrates so that appropriate dosage strategies for these drugs can be adopted and adverse drug reactions can be avoided.

In summary, we determined allele and genotype frequencies of CYP3A4, CYP2C9, CYP2C19 and CYP2D6 in Han (Liaoning), Uighur, Hui and Mongolian Chinese populations. Our study provides fundamental and useful information for further phenotypic studies in the Chinese population. Our findings confirm the existence of interethnic differences in the CYP2C9, CYP2C19 and CYP2D6 allele and genotype frequencies in Han (Liaoning), Uighur, Hui and Mongolian Chinese populations. Further studies are required to assess the clinical significance of those differences in treatment outcome and to determine optimal dosage of drugs metabolized by these polymorphic enzymes.

4. Experimental

4.1. Chemicals and reagents

A PCR Master Mix (2X) was purchased from Fermentas (USA). Agarose G-10 was purchased from Gene Company (Spain). FastDigest restriction endonucleases were purchased from Fermentas (USA) and New England Biolabs (USA).

4.2. Study population

Venous blood samples (5 ml) were obtained from volunteers of four different nationalities in China [Han ($n = 136$), Uighur ($n = 214$), Hui ($n = 164$) and Mongolian ($n = 158$)]. The Han volunteers were from Liaoning Province, the Uighur volunteers were from Xinjiang Province, the Hui volunteers were from Ningxia Province and the Mongolia volunteers were from Inner Mongolia Province. The volunteers from each group were between 19 and 25 years of age. Volunteers who were descendants by at least three generations of a single heritage were assigned to that ethnic group. All volunteers were in good health as determined by history, physical and blood examination, which included a complete blood count as well as renal and liver function tests. Each volunteer was informed of the details of the study, including the risks and benefits, and each volunteer provided written informed consent before participating in the study. The study protocol was performed in accordance with the revised Declaration of Helsinki and approved by the independent Ethics Committee of the General Hospital of Shenyang Military Region.

4.3. DNA extraction

Genomic DNA was isolated from whole blood using TIANamp Whole Blood DNA Extraction Kit (TIANGEN BIOTECH BEIJING CO, LTD). DNA concentrations were determined by UV spectrophotometry. The DNA purity was 99.5%.

4.4. PCR amplification and digestion

Briefly, PCR reactions were conducted in a reaction volume of 50 μL with 20 ng of genomic DNA, 1.25 U Taq DNA polymerase, 100 mM $MgCl_2$, 10 mM dNTPs and 20 pM of each primer. The DNA sequence for each primer (Chu et al. 2003; Qiu et al. 2008; He et al. 2008; Hu et al. 2007; Zhen et al. 2007) is given in Table 4.

The initial denaturation step was performed at 94 °C for 5 min, followed by 35 cycles of denaturation at 94 °C for 40 s, annealing at the annealing temperature (Table 5) for 40 s with extension at 72 °C for 30 s and final extension at 72 °C for 7 min. All of these steps were performed using a GeneAmp PCR system 2700 Thermal Cycler (Applied Biosystems). The PCR products were digested with 10 U FastDigest restriction endonuclease Table 6. The digested products were analyzed on 3% agarose gels and stained with ethidium bromide (Fig.).

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