Metabolic capabilities of cytochrome P450 enzymes in Chinese liver microsomes compared with those in Caucasian liver microsomes

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WHAT IS ALREADY KNOWN ABOUT THIS SUBJECT

- The mean catalytic activities of CYP1A2, CYP2A6, CYP2D6, CYP2E1 and CYP3A4 in human liver microsomes (HLM) of Japanese origin were found to be lower than the corresponding mean values of Caucasian HLM, but the distribution patterns of data in these studies were not well addressed.
- Interindividual variations in metabolic activities of Chinese HLM samples were reported for CYP1A2, CYP2C9, CYP2D6 and CYP3A4, but little is known about the difference in CYP activities between Chinese and Caucasian HLM samples.
- The vast majority of the preceding studies were based on the measurement of metabolite formation rates at a certain fixed substrate concentration, rather than enzyme kinetic analyses.

WHAT THIS STUDY ADDS

- Significant differences in metabolic capability were observed for CYP1A2, CYP2C9, CYP2C19 and CYP2E1 with the median values lower for the Chinese HLM samples *vs.* the Caucasian samples, which were associated with differences in Michaelis constant or maximum velocity.
- Despite negligible differences in metabolic capability for CYP2A6, CYP2B6, CYP2C8, CYP2D6 and CYP3A, Michaelis constant of CYP2B6 appeared to have a significant ethnic difference.

AIM

The most common causes of variability in drug response include differences in drug metabolism, especially when the hepatic cytochrome P450 (CYP) enzymes are involved. The current study was conducted to assess the differences in CYP activities in human liver microsomes (HLM) of Chinese or Caucasian origin.

METHODS

The metabolic capabilities of CYP enzymes in 30 Chinese liver microsomal samples were compared with those of 30 Caucasian samples utilizing enzyme kinetics. Phenacetin *O*-deethylation, coumarin 7-hydroxylation, bupropion hydroxylation, amodiaquine *N*-desethylation, diclofenac 4'-hydroxylation (*S*)-mephenytoin 4'-hydroxylation, dextromethorphan *O*-demethylation, chlorzoxazone 6-hydroxylation and midazolam 1'-hydroxylation/testosterone 6β-hydroxylation were used as probes for activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A, respectively. Mann-Whitney U test was used to assess the differences.

RESULTS

The samples of the two ethnic groups were not significantly different in cytochrome-b_5 concentrations but were significantly different in total CYP concentrations and NADPH-P450 reductase activity ($P < 0.05$). Significant ethnic differences in intrinsic clearance were observed for CYP1A2, CYP2C9, CYP2C19 and CYP2E1; the median values of the Chinese group were 54, 58, 26, and 35% of the corresponding values of the Caucasian group, respectively. These differences were associated with differences in Michaelis constant or maximum velocity. Despite negligible difference in intrinsic clearance, the Michaelis constant of CYP2B6 appeared to have a significant ethnic difference. No ethnic difference was observed for CYP2A6, CYP2C8, CYP2D6 and CYP3A.

CONCLUSIONS

These data extend our knowledge on the ethnic differences in CYP enzymes and will have implications for drug discovery and drug therapy for patients from different ethnic origins.

Introduction

Drug efficacy and toxicity can vary among ethnic groups [1–4]. Recognizing the relevance of the interethnic differences in drug response, the International Conference on Harmonization (ICH) has issued a guideline entitled 'Ethnic Factors in the Acceptability of Foreign Clinical Data' (http://www.ich.org/products/guidelines/efficacy/efficacy-single/article/ethnic-factors-in-the-acceptability-of-foreign-clinical-data.html) to facilitate the registration of medicines among ICH regions. Interethnic differences in drug response involve one or more of three mechanisms, i.e. drug metabolism/transport, drug targets and the disease pathways. The variation in drug metabolism is considered to be profoundly important because it affects the systemic exposure and disposition of drugs.

Cytochromes P450 (CYPs), highly concentrated in the liver and also expressed in many other tissues, are the most prominent family of drug metabolizing enzymes in humans. CYPs are located in the smooth endoplasmic reticulum membrane of cells. Thus they can be recovered in microsomal fractions. Knowledge of the human CYPs responsible for drug metabolism, primarily found in CYP families 1–3 (CYP1–3), is fundamental for drug development and drug therapy. The most significant drug metabolizing CYPs in humans include CYP2C9, CYP2C19, CYP2D6 and CYP3A4, whereas CYP1A2, CYP2C8, CYP2A6, CYP2B6 and CYP2E1 are also responsible for metabolism of some drugs and/or metabolic activation of procarcinogens [5]. CYP genes in the families 1–3 are known to be genetically polymorphic and the genetic variants can alter the activity or expression of the drug metabolizing enzymes. Variations associated with CYPs affecting drug concentration and response occurred at varying allele frequencies among populations of different ethnic origin [6]. Poor metabolizer frequencies of several major CYP enzymes are significantly different between Asians and Caucasians [7]. Understanding the interethnic differences in the metabolic capacity of CYPs is a valuable tool for drug development that can potentially lead to improved safety and efficacy of drug therapy for patients of different ethnic backgrounds.

The process of drug discovery and development has become increasingly reliant on the use of human-derived test systems (e.g. human liver microsomes, hepatocytes, etc.) to screen drug candidates for their liability of CYP inhibition or induction and to determine the involvement of drug metabolizing enzymes and transporters. Interethnic differences should be taken into account when performing *in vitro* drug metabolism studies. Recent results have indicated that testing CYP2D6-metabolized candidates or investigational drugs for allele-specific metabolism/interactions may be important [8]. However, to what extent ethnicity can affect the use of human-derived test systems remains largely unknown and requires more studies. The current study aimed to assess and understand the interethnic differences in the activities of major CYPs in human liver microsomes (HLM) of Chinese or Caucasian origin. Therefore the appropriate HLM can be utilized for future *in vitro* drug metabolism studies, and data would allow the prediction of interethnic variability in the clinical studies by applying these results to physiologically based pharmacokinetic modelling. Nine CYP enzymes, including CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A, in HLM derived from 30 Chinese samples were assessed in terms of catalytic activity in comparison with those from 30 Caucasians samples.

Methods

Samples of human liver microsomes

As shown in Table 1, 30 Chinese samples of HLM, including 24 male samples and six female samples, were obtained from the Research Institute for Liver Diseases (Shanghai) Co. Ltd (Shanghai, China) following the donation regulations in China with fully informed consent, and under the approval of an independent Institutional Review Board. Fourteen HLM samples of Caucasian origin (11 male samples and three female samples) were obtained from the Medical College of Wisconsin under a protocol approved by the Committee for the Conduct of Human Research. Twelve Caucasian samples (seven male samples and five female samples) were purchased from Celsis *In Vitro* Inc (Baltimore, MD, USA), and four male Caucasian samples were purchased from BD Gentest (Woburn, MA, USA). The HLM samples were prepared by differential centrifugation. Because detailed histories of some of the donors were not known, the CYP variations with respect to the 60 HLM samples were analyzed without considerations of disease states, drug intake, smoking, alcohol drinking and other dietary habits. Pooled HLM, obtained from Xenotech (Lenexa, KS, USA), were also used for the development of enzyme activity assays.

Incubation conditions for measurement of the metabolic capabilities of CYP enzymes in HLM

Pilot studies were performed with each biotransformation to ensure that the comparison of metabolic capabilities of the test CYP enzymes were determined under linear metabolite formation conditions with respect to time and microsomal protein concentration. Incubations were performed in duplicate in 96-well plates in a total assay volume of 100 µl with each well, which contained HLM [at 0.05 mg protein ml^{-1} for CYP2C8, CYP2C9 and CYP3A (for testosterone 6β-hydroxylation), 0.1 mg protein ml^{-1} for CYP1A2, CYP2A6, CYP2D6, CYP2E1 and CYP3A (for midazolam 1′-hydroxylation), and 0.2 mg protein ml^{-1} for CYP2B6 and CYP2C19], substrate (concentration ranges as indicated), 100 mM potassium phosphate buffer (pH 7.4, for CYP1A2, CYP2B6, CYP2C8, CYP2C19, CYP2D6, CYP2E1 and

Table 1

Information about HLM of test Chinese and Caucasian samples

Sample ID	Age (years)	Gender	Cause of death/drug history/social history
Chinese group (*n* = 30)			
am01	31	Male	Hepatolithiasis
am02	70	Male	Hepatic cyst
am03	31	Male	Trauma/smoker
am04	21	Male	Trauma
am05	24	Male	Trauma
am06	26	Male	Trauma
am07	28	Male	Trauma/smoker
am08	24	Male	Trauma/smoker
am09	29	Male	Trauma/smoker
am10	38	Male	Trauma/smoker
am11	25	Male	Trauma/smoker
am12	36	Male	Trauma
am13	51	Male	Trauma/smoker
am14	48	Male	Trauma/smoker
am15	32	Male	Trauma
am16	36	Male	Trauma/smoker
am17	27	Male	Trauma/smoker
am18	39	Male	Trauma
am19	44	Male	Trauma
am20	46	Male	Trauma/smoker
am21	28	Male	Trauma
am22	33	Male	Trauma
am23	41	Male	Trauma/smoker
am24	33	Male	Trauma/smoker
aw25	53	Female	Hepatic cyst
aw26	51	Female	Hepatolithiasis
aw27	68	Female	Giant hepatic haemangiomas
aw28	65	Female	Cholangiocarcinoma
aw29	53	Female	Hepatic cyst
aw30	78	Female	Primary carcinoma of liver
Caucasian group (*n* = 30)			
cm01	55	Male	Pulmonary fibrosis/ethanol
cm02	50	Male	Unknown
cm03	22	Male	Unspecified drug abuse
cm04	31	Male	Unknown
cm05	25	Male	Unknown
cm06	55	Male	Unknown
cm07	45	Male	Lisinopril
cm08	67	Male	Unknown
cm09	63	Male	Cerebrovascular trauma
cm10	18	Male	0.24% ethanol at time of death
cm11	82	Male	Cerebrovascular trauma
cm12	23	Male	0.06% ethanol at time of death
cm13	59	Male	Unknown
cm14	77	Male	Head trauma/smoker
cm15	65	Male	Cardiac arrest
cm16	54	Male	Anoxia
cm17	53	Male	Cerebrovascular trauma
cm18	41	Male	Motorcycle trauma
cm19	59	Male	Cerebrovascular trauma/smoker
cm20	58	Male	Subarachnoid haemorrhage
cm21	21	Male	Diabetic, 0.08% ethanol at time of death
cm22	47	Male	Anoxia and cardiopulmonary arrest
cw23	58	Female	Poorly controlled hypertension
cw24	72	Female	Cerebrovascular trauma
cw25	28	Female	Cerebrovascular trauma
cw26	77	Female	Chemotherapy for 1 year for colon cancer
cw27	56	Female	Cerebrovascular trauma
cw28	50	Female	Cerebrovascular trauma
cw29	49	Female	Cerebrovascular trauma
cw30	48	Female	Chlorpheniramine

CYP3A)/100 mM tris(hydroxymethyl)aminomethane buffer (pH 7.4, for CYP2A6 and CYP2C9), an NADPH-generating system comprising 3.3 mM magnesium chloride, 3.3 mM of glucose-6-phosphate, 0.5 U ml^{-1} glucose-6-phosphate dehydrogenase and 1.3 mM NADP. Before commencement of the reaction by adding the NADPH-generating system, the incubation mixture was pre-incubated for 3 min at 37°C. The optimal incubation time was 4 min for midazolam 1'-hydroxylation, 5 min for testosterone 6β-hydroxylation, 10 min for amodiaquine N-desethylation and diclofenac 4'-hydroxylation, 20 min for phenacetin O-deethylation, coumarin 7-hydroxylation, bupropion 1-hydroxylation, chlorzoxazone 6-hydroxylation and dextromethorphan O-demethylation and 30 min for (S)-mephenytoin 4'-hydroxylation. For the biotransformations, seven to ten substrate concentrations were examined over the following ranges: 3.91 to 2000 µM for phenacetin, 0.10 to 50 µM for coumarin, 0.20 to 100 µM for amodiaquine, 0.20 to 100 µM for diclofenac, 1.95 to 1000 µM for (S)-mephenytoin, 0.49 to 250 µM for dextromethorphan, 0.98 to 500 µM for chlorzoxazone, 3.91 to 500 µM for bupropion, 0.05 to 50 µM for midazolam and 3.91 to 250 µM for testosterone. The reactions were terminated by adding 100 µl of ice-cold methanol. The resulting samples were centrifuged at 3000 g for 10 min to remove protein and 10 µl of the supernatant were analyzed by LC-MS/MS.

LC-MS/MS analyses of metabolites

Validated LC-MS/MS-based methods were used to determine metabolite formation rates. The LC-MS/MS system was assembled from a Waters Acquity UPLC separation module (Waters, Milford, MA, USA) and an Applied Biosystems-Sciex API 4000 Q Trap mass spectrometer (Applied Biosystems, Toronto, Canada) with Turbo V ESI-source. Methanol-prepared biosamples were separated using a generic pulse gradient elution [9, 10] on a 5 µm Ultimate XB-C18 column (50 mm × 2.1 mm i.d.; Welch Materials, MD, USA) with mobile phases of $CH_3CN : H_2O$ (10:490, v/v, containing 0.5 mM HCOOH) and $CH_3CN : H_2O$ (495:5, v/v, 0.5 mM HCOOH). The retention times of the measured metabolites ranged from 2.0 to 2.5 min. The MS/MS parameters in the positive or negative electrospray ionization mode were optimized to maximize generation of protonated or deprotonated molecules. The precursor-to-product ion transitions were monitored at m/z 152→110, 161→133, 257→238, 328→283, 310→266, 233→133, 258→157, 184→120, 342→324 and 305→91 for acetaminophen, 7-hydroxycoumarin, hydroxybupropion, desethylamodiaquine, 4'-hydroxydiclofenac, 4'-hydroxymephenytoin, dextrorphan, 6-hydroxychlorzoxazone, 1'-hydroxymidazolam and 6β-hydroxytestosterone, respectively. Matrix-matched standard curves of the peak area of a given analyte vs. the nominal concentration in nM were linear and showed correlation coefficients >0.99. The assays were validated according to the US Food and Drug Administration guid-

ance on bioanalytical method validation (http://www.fda.gov/downloads/drugs/guidancecomplianceregulatory information/guidances/ucm070107.pdf). The intra-day and inter-day assay accuracies ranged from 80% to 108% and from 88% to 109%, respectively, whereas the ranges of precision values for the assays were 0.1%–19% and 0.6%–20%, respectively.

Spectral measurement of cytochrome b$_5$ and total CYP concentrations in HLM

Methods by Omura & Sato [11] for measuring concentrations of cytochrome b$_5$ and total CYP in microsomes were slightly modified using 96-well plates. In brief, 200 µl of diluted HLM (1 mg protein ml^{-1}, pH 7.4) were measured for absorbance at 410 and 425 nm using a SpectraMax M2 Multi-detection Reader (Molecular Devices, Sunnyvale, CA, USA) before and after reduction with 5 µl of 10 mM NADH. The cytochrome b$_5$ concentration was calculated from the absorbance difference between 425 and 410 nm, which was based on an extinction coefficient of 185 mM^{-1} cm^{-1}. Following the measurement of cytochrome b$_5$, the samples were further spiked with 5 µl of 540 mM sodium dithionite and saturated with carbon monoxide gas for 2 min. The total CYP concentration in HLM was determined from the absorbance difference between 450 and 490 nm using an extinction coefficient of 91 mM^{-1} cm^{-1}.

Measurement of NADPH-P450 reductase activity

NADPH-P450 reductase assay is based on the use of the electron acceptor surrogate cytochrome c [12], which was conducted in a 96-well plate. The reaction solution contained 0.3 M potassium phosphate buffer (pH 7.7), 0.2 mM cytochrome c, 1 mM potassium cyanide, HLM at 0.025 mg ml^{-1} microsomal protein and 0.5 mM NADPH. Absorbance of the samples at 550 nm was recorded using the SpectraMax M2 Multi-detection Reader in the kinetic mode before and after the addition of NADPH (0–2 min). The rate of reducing cytochrome c was determined as the slope of the initial linear part of the curve describing the time-dependent increase in absorbance, using an extinction coefficient of 0.021 mM^{-1} cm^{-1}.

Data analysis

Michaelis constant (K_m) and maximum velocity (V_{max}) values were determined by nonlinear regression analysis of initial rates of metabolite formation as a function of substrate concentration using GraFit software (Version 5.0; Erithacus Software Ltd, Surrey, UK). In vitro intrinsic clearance (CL_{int}) was calculated from the ratio of V_{max} to K_m.

Shapiro-Wilk test of normality was used to check the distribution shape of CL_{int}, K_m and V_{max} for each individual CYP enzyme, as well as the distribution shape of cytochrome b$_5$ concentration, total CYP content and NADPH-P450 reductase activity in HLM samples. In addition, the

outliers and extreme values were identified according to whether the values were 1.5–3 and >3 box lengths, respectively, from the upper or lower edge of the box of interquartile range (IQR). All the outlying values were confirmed as correct values and there were no data entry or recording errors. Median and IQR were used as measures of central tendency and dispersion for non-normally distributed data. All the outliers and extreme values were included in the calculations. Mann-Whitney U test, a rank based non-parametric test, was used to compare data between the two ethnic groups and also used to estimate the effects of gender and age (18–50 and 51–82 years old) on the cytochrome b_5 concentration, total CYP concentration and CL_{int} of test individual CYP enzymes. A P value <0.05 was considered to be the minimum level of statistical significance (two-tailed). Before the study was initiated, power calculations were performed using the power analysis program G*power 3 [13] with 80% power and a 0.05 significance level. The results indicated that 30 HLM samples per ethnic group were needed to detect an effect size of 0.6 or greater. As to the gender difference, 24 male and six female HLM samples in the Chinese group would detect an effect size of 1.1 or greater, whereas the detectable effect size would be 1.0 or greater for the Caucasian group containing 22 male and eight female samples. Twenty-two samples of 18–50 years old and eight samples of 51–82 years old comprized the Chinese group, whereas the sample sizes were 14 and 16, respectively, for the Caucasian group. The detectable effect sizes would be 1.0 or greater for the Chinese group and 0.9 or greater for the Caucasian group.

In addition, the CL_{int} values were transformed by dividing the group median for calculation of Kendall's tau-b coefficients to estimate whether a certain CYP enzyme in HLM was positively or negatively related to another coexisting CYP enzyme, as well as the possible influence of the ethnic factor on the association. Correlation was statistically significant at $P < 0.05$ level (two-tailed). PASW statistics 18 software (SPSS Inc., Chicago, IL, USA) was used for data management and statistical analyses.

Chemicals and reagents

Most CYP substrates and metabolites were obtained from Sigma-Aldrich (St Louis, MO, USA), including phenacetin, acetaminophen, coumarin, 7'-hydroxycoumarin, bupropion, hydroxybupropion, amodiaquine, desethylamodiaquine, diclofenac, 4'-hydroxydiclofenac (S)-mephenytoin (S)-4'-hydroxymephenytoin, dextromethorphan, dextrorphan, chlorzoxazone, 6-hydroxychlorzoxazone and 1'-hydroxymidazolam, except for midazolam and testosterone from the National Institutes for Food and Drug Control (Beijing, China). 6β-hydroxytestosterone was synthesized by the Department of Medicinal Chemistry of Shanghai Institute of Materia Medica (Shanghai, China). Reduced β-nicotinamide adenine dinucleotide (NADH), reduced β-nicotinamide adenine dinucleotide phosphate (NADPH), β-nicotinamide adenine dinucleotide phosphate

hydrate, glucose-6-phosphate monosodium salt, glucose-6-phosphate dehydrogenase and cytochrome c were also purchased from Sigma-Aldrich. Organic solvents and other chemicals used were obtained from Sinopharm Chemical Reagent Co. (Shanghai, China). High purity water was prepared with a Millipore Direct-Q 3 u.v. water purifying system (Bedford, MA, USA).

Results

The concentration of cytochrome b_5 in HLM was measured to judge the quality of the test HLM samples before examining their metabolic capabilities of CYP enzymes. The cytochrome b_5 concentrations in HLM of 30 Chinese samples ranged from 175 to 674 pmol mg^{-1} protein with a median of 269 pmol mg^{-1} protein, whereas those for 30 Caucasian samples ranged from 170 to 489 pmol mg^{-1} protein with a median of 264 pmol mg^{-1} protein (Figure 1). No significant difference was found between the ethnic groups for cytochrome b_5 ($P = 0.7$). Meanwhile, the total CYP content in HLM ranged from 100 to 715 pmol mg^{-1} protein for the Chinese
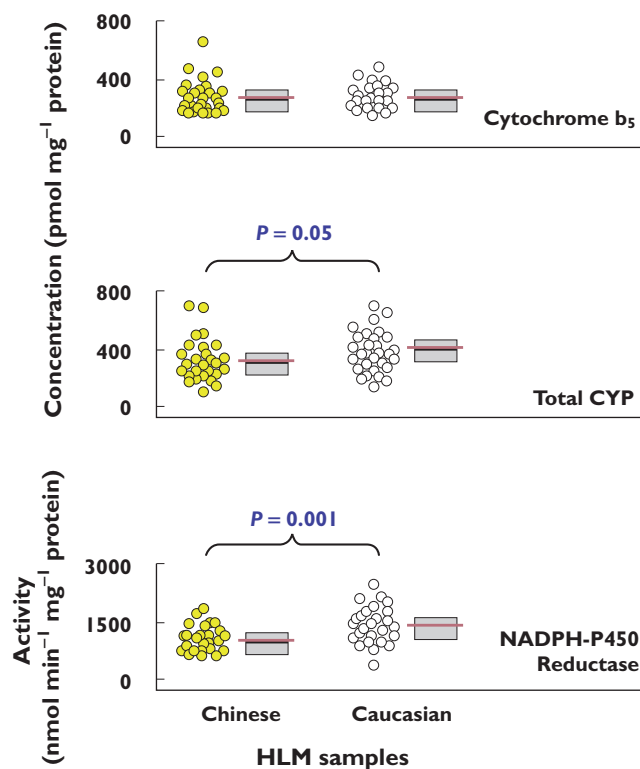
Figure 1

Comparison of the cytochrome b_5, total CYP concentrations and NADPH-P450 reductase activity in HLM between Chinese and Caucasian samples. On the right side of the vertical scatter plot, the associated box plot shows the median as the black horizontal line inside the box and the IQR as length of the box. The associated red horizontal line (longer than the median line) represents the mean value

samples and from 140 to 723 pmol mg^{-1} protein for the Caucasian samples. The total CYP concentration for the Chinese samples was significantly lower than that of the Caucasian samples ($P = 0.05$), but the median values of the two groups were comparable, i.e. the Chinese value being 82% of the Caucasian value. In addition, there was no significant age-related difference in either cytochrome b$_5$ concentration ($P = 0.3$) or total CYP concentration ($P = 0.8$) in HLM for the Chinese samples. The age-related difference was neither significant in total CYP concentration ($P = 1.0$) nor in cytochrome b$_5$ ($P = 0.9$) for the Caucasian samples. When the influence of gender was examined, a statistically significant difference in cytochrome b$_5$ concentration was found for the Chinese samples ($P = 0.01$), while no statistically significant gender difference was found for the Caucasian samples ($P = 0.6$). There was no significant gender-related difference in total CYP concentrations between these two populations ($P = 0.3$ for the Chinese samples and $P = 0.8$ for the Caucasian samples).

Like cytochrome b$_5$, NADPH-P450 reductase plays important role in CYP-mediated drug metabolism via electron transfer [14]. The activity of NADPH-P450 reductase measured by reduction of cytochrome c showed significant difference between Chinese and Caucasian samples with a P value of 0.001 (Figure 1). The NADPH-P450 reductase activity in the Chinese and Caucasian groups ranged from 598 to 1861 and from 411 to 2548 nmol cytochrome c reduced min^{-1} mg^{-1} protein, respectively, and the Chinese median value was 73% of the Caucasian median value. Furthermore, there was neither significant age- nor gender-related differences for NADPH-P450 reductase activity ($P = 0.4$–1) in these populations.

Phenacetin O-deethylation, coumarin 7-hydroxylation, bupropion hydroxylation, amodiaquine N-desethylation, diclofenac 4'-hydroxylation, (S)-mephenytoin 4'-hydroxylation, dextromethorphan O-demethylation and chlorzoxazone 6-hydroxylation were used as indicators of the activities of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP2E1 in HLM (Figure 2), respectively, which are known to be selective for the CYP enzymes [15, 16]. Both midazolam 1'-hydroxylation and testosterone 6β-hydroxylation were used as measures of CYP3A activity in the current study. An earlier study indicated that recombinant CYP3A4 and CYP3A5 have similar CL$_{int}$ towards midazolam metabolism ($\leq 60 \mu M$), whereas CYP3A4 is about 40 times better at forming the 6β-hydroxyl metabolite of testosterone than is CYP3A5 [17].

Figure 3 shows interethnic differences between the Chinese and Caucasian samples in the CL$_{int}$ values in respect of each individual CYP enzyme. The Shapiro-Wilk test showed that most distributions in CL$_{int}$ for both ethnic groups were significantly different from a normal distribution ($P < 0.05$), except for CYP2A6 for the Chinese samples ($P = 0.6$) and the Caucasian samples ($P = 0.2$). Accordingly, non-parametric statistical tests were applied to the data of the current study. Figure 4 depicts comparative profiles of

60 human samples of two ethnic groups in terms of the relative CL$_{int}$ of individual CYP enzymes, which were normalized to the respective median values of the entire sample set.

Within the full data set of both the races, the greatest interindividual difference in CL$_{int}$ was found for the activity of CYP2C19, followed by that of CYP2C8, CYP2B6, CYP1A2, CYP2D6, CYP2E1, CYP2C9 and CYP2A6, demonstrating the percent IQR values (calculated as the IQR as a percentage of the associated median) 321%, 290%, 270%, 204%, 190%, 125%, 105% and 65%, respectively. The interindividual differences for CYP3A towards midazolam 1'-hydroxylation and towards testosterone 6β-hydroxylation were quite comparable, i.e. 91% and 105%, respectively. In addition, there was no significant age-related difference in CL$_{int}$ for the two ethnic groups, except for CYP2C8 ($P = 0.004$, median of 18–50 years old vs. median of 51–82 years old 261 vs. 44 µl min^{-1} mg^{-1} protein), CYP2E1 ($P = 0.000004$, median of 18–50 years old vs. median of 51–82 years old 8.82 vs. 2.58 µl min^{-1} mg^{-1} protein), CYP3A-m ($P = 0.01$, median of 18–50 years old vs. median of 51–82 years old 726 vs. 2121 µl min^{-1} mg^{-1} protein) for the Chinese samples. Meanwhile, gender-related differences in CL$_{int}$ were not significant either, except for CYP1A2 ($P = 0.05$, male median vs. female median, 12.0 vs. 4.2 µl min^{-1} mg^{-1} protein), CYP2C8 ($P = 0.003$, male median vs. female median, 261 vs. 42 µl min^{-1} mg^{-1} protein), CYP2C9 ($P = 0.02$, male median vs. female median, 101 vs. 321 µl min^{-1} mg^{-1} protein), CYP2E1 ($P = 0.001$, male median vs. female median, 7.54 vs. 2.58 µl min^{-1} mg^{-1} protein), and CYP3A towards midazolam metabolism ($P = 0.0008$, male vs. female median 726 vs. 2463 µl min^{-1} mg^{-1} protein) for the Chinese data set.

Table 2 shows the value ranges of CL$_{int}$, K_m and V_{max} for each individual CYP enzyme of the Chinese and the Caucasian groups, as well as their associated medians and IQRs. The individual kinetic data for the test CYP enzymes are shown in Tables S1–S10. Similar to CL$_{int}$, most values of K_m and V_{max} were also found to be not normally distributed in the Chinese and the Caucasian groups ($P < 0.05$).

CYP1A2

The CL$_{int}$ values for phenacetin O-deethylation by CYP1A2 in HLM from the Chinese samples ranged from 0.6 to 51.4 µl min^{-1} mg^{-1} protein, whereas those from the Caucasian samples were from 2.9 to 92.7 µl min^{-1} mg^{-1} protein. For CL$_{int}$ of the Chinese samples, am16 (51.4 µl min^{-1} mg^{-1} protein) was identified as an outlier. For the Caucasian samples, cm20 was also identified as an outlier (92.7 µl min^{-1} mg^{-1} protein). The Mann-Whitney U test suggested the difference between the two groups was significant ($P = 0.04$) with the median CL$_{int}$ for the Caucasian samples 1.8 times greater than that for the Chinese samples. The observed difference in the CL$_{int}$ between Chinese and Caucasian samples tended to be mainly associated with differences in V_{max}, rather than in K_m. There was
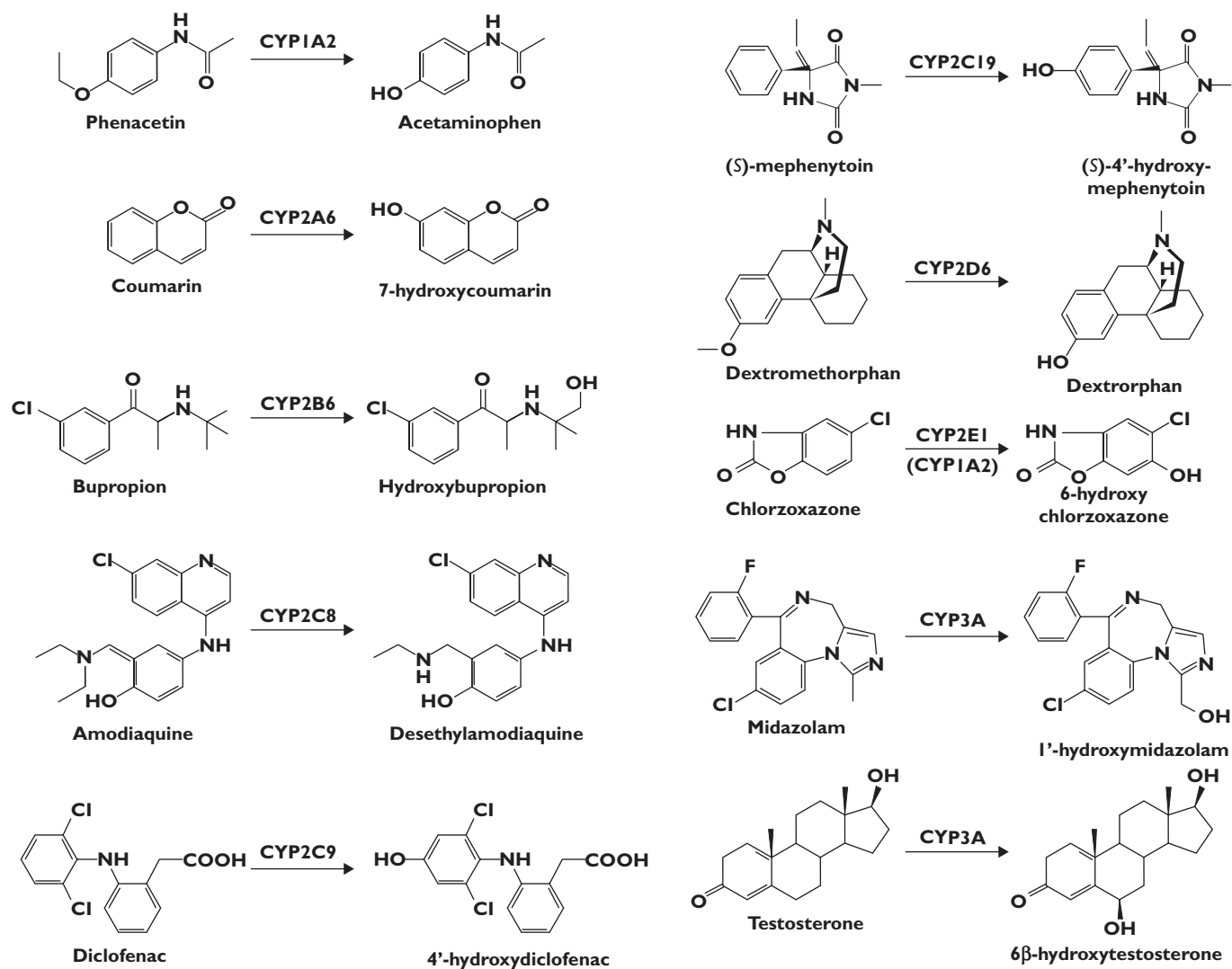
Figure 2

Probe reactions catalyzed by the test human CYP enzymes

a significant difference between the two ethnic groups in V_{max} ($P = 0.02$), whereas the difference in K_m was not significant ($P = 0.3$).

CYP2A6

The CL_{int} values for CYP2A6 towards coumarin 7-hydroxylation ranged from 150 to 1776 µl min^{-1} mg^{-1} protein for Chinese samples and 140 to 1315 µl min^{-1} mg^{-1} protein for Caucasian samples. For CL_{int} of the Chinese samples, am12 (1776 µl min^{-1} mg^{-1} protein) was an outlier. There were no outlying values for the Caucasian samples. Although the two ethnic groups were not considered to be significantly different in CL_{int} ($P = 0.07$), the median value of the Chinese group (933 µl min^{-1} mg^{-1} protein) tended to be slightly greater than that of the Caucasian group (663 µl min^{-1} mg^{-1} protein). The V_{max} was not significantly different between the two ethnic groups ($P = 0.8$). Mean-

while, the median K_m of the Chinese samples (1.12 µM) tended to be lower than that of the Caucasian samples (1.58 µM) with a P value of 0.1.

CYP2B6

The CL_{int} values for bupropion hydroxylation by CYP2B6 for the Chinese samples ranged from 0.1 to 12.6 µl min^{-1} mg^{-1} protein, whereas those for the Caucasian samples were from 0.1 to 17.4 µl min^{-1} mg^{-1} protein. There were three extreme values (am19, am24 and am23 with CL_{int} values of 12.6, 9.21 and 7.74 µl min^{-1} mg^{-1} protein, respectively) in the Chinese data set. The CL_{int} value of cw24 (17.4 µl min^{-1} mg^{-1} protein) was an outlier for the Caucasian samples. Although the median CL_{int} for the Chinese samples (0.5 µl min^{-1} mg^{-1} protein) was lower than that for the Caucasian samples (0.9 µl min^{-1} mg^{-1} protein), the difference between the two ethnic groups was not
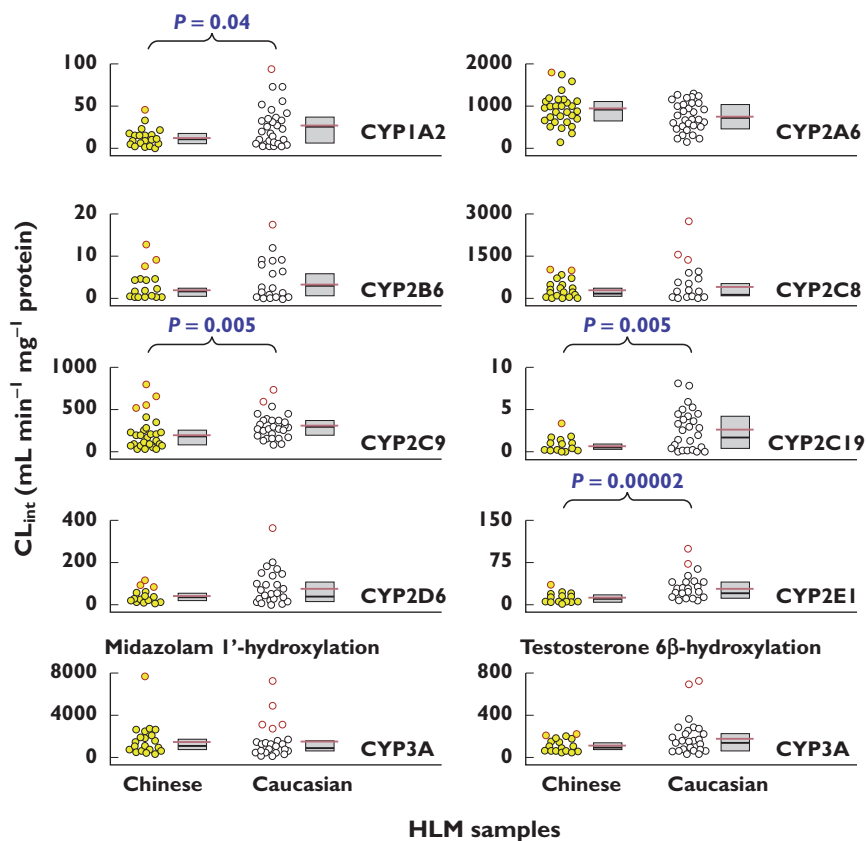
Figure 3

Comparison of the metabolic capabilities (CL_{int}) of various CYP enzymes in HLM between Chinese and Caucasian samples. On the right side of the vertical scatter plot, the associated box plot shows the median as the black horizontal line inside the box and the IQR as length of the box. The associated red horizontal line (longer than the median line) represents the mean value. The outliers and extreme values in each ethnic data set are shown as red circles and are mentioned in the Results section

statistically significant ($P = 0.4$). Interethnic difference in V_{max} was also negligible ($P = 0.5$), whereas the median K_m of the Chinese samples (76 μM) was significantly higher than that of the Caucasian samples (32 μM) with a P value of 0.02.

CYP2C8

The CL_{int} values for CYP2C8 for amodiaquine N-desethylation varied from 14 to 1003 μl min^{-1} mg^{-1} protein for the Chinese samples and from 14 to 2745 μl min^{-1} mg^{-1} protein for the Caucasian samples. There were two outliers (am18 and am20, with CL_{int} values of 1003 and 997 μl min^{-1} mg^{-1} protein, respectively) in the Chinese data set, whereas an extreme value (cw29, 2745 μl min^{-1} mg^{-1} protein) and two outliers (cm14 and cm18, 1532 and 1364 μl min^{-1} mg^{-1} protein, respectively) were observed in the Caucasian data set. Although the median CL_{int} for the Chinese data set (194 μl min^{-1} mg^{-1} protein) was higher than that for the Caucasian data set (104 μl min^{-1} mg^{-1} protein), no statistically significant difference was found between the two ethnic groups ($P = 0.6$). There was no significant interethnic difference found

in K_m ($P = 0.6$) or in V_{max} ($P = 0.5$), which was consistent with the observation of negligible difference in CL_{int}.

CYP2C9

The CL_{int} values for diclofenac 4'-hydroxylation by CYP2C9 ranged from 15 to 790 μl min^{-1} mg^{-1} protein for the Chinese samples and 77 to 715 μl min^{-1} mg^{-1} protein for the Caucasian samples. There was an extreme value (am16 with CL_{int} values of 790 μl min^{-1} mg^{-1} protein) and three outliers (am15, aw26 and aw29, with CL_{int} values of 655, 535 and 516 μl min^{-1} mg^{-1} protein, respectively) in the Chinese data set and two outliers (cm04 and cm16 with CL_{int} values of 715 and 587 μl min^{-1} mg^{-1} protein, respectively) in the Caucasian data set. The difference between the two ethnic groups was highly significant with a P value of 0.005 and the median CL_{int} for the Caucasian group was 1.7 times greater than that for the Chinese group. In contrast to CYP1A2, the interethnic difference in CL_{int} for CYP2C9 appeared to be mainly attributed to the difference in K_m, rather than that of V_{max}. There was highly significant difference between the two ethnic groups in K_m
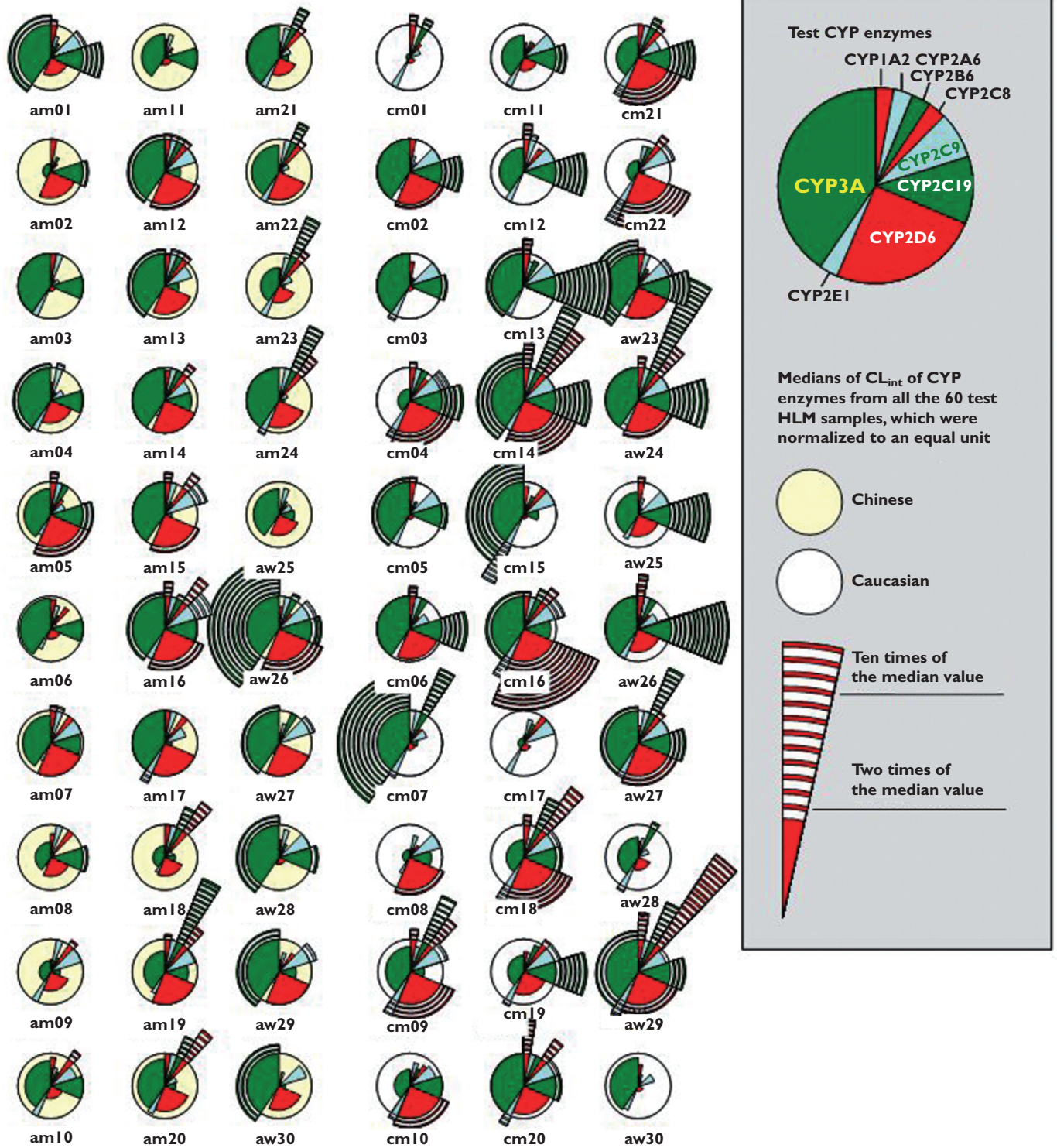
Figure 4

Profiles of multiple CYP enzymes in HLM of the test Chinese or Caucasian samples with respect to metabolic capacities. The data of CYP3A were derived from the CL$_{int}$ towards midazolam 1′-hydroxylation

Table 2

CL$_{int}$, K_m and V_{max} for the individual CYP enzymes in HLM

CYP		Chinese group (n = 30) CL$_{int}$ (µl min^{-1} mg^{-1} protein)	K_m (µM)	V_{max} (pmol min^{-1} mg^{-1} protein)	Caucasian group (n = 30) CL$_{int}$ (µl min^{-1} mg^{-1} protein)	K_m (µM)	V_{max} (pmol min^{-1} mg^{-1} protein)
1A2	Range	0.6–51.4*	31–379	214–2245*	2.9–92.7	30–142	182–2772
	Median	10.9	85	819	20.1	62	1314
	IQR	12.7	80	656	31.1	64	1135
2A6	Range	150–1776	0.29–2.64	125–3143	140–1315	0.49–3.75	134–4372
	Median	933	1.12	939	663	1.58	882
	IQR	468	0.61	1072	610	1.01	1353
2B6	Range	0.1–12.6	13–175*	3–953	0.1–17.4	11–297	2–765
	Median	0.5	76	38	0.9	32	51
	IQR	2.2	80	175	3.1	57	291
2C8	Range	14–1003	0.8–81.0	372–2042	14–2745	0.8–53.2	100–2235
	Median	194	3.9	791	104	7.3	861
	IQR	340	11.6	582	333	12.6	683
2C9	Range	15–790*	4.4–28.5*	385–4704	77–715	3.2–14.8	823–4067
	Median	166	11.6	1565	287	7.0	1916
	IQR	180	12.8	1271	166	4.5	943
2C19	Range	0.03–3.36*	25–288	9–118*	0.02–8.10	9–630	7–302
	Median	0.43	93	29	1.64	60	72
	IQR	0.70	65	42	3.84	90	110
2D6	Range	3–119	2.0–18.2	41–637	0.2–358	1.9–47.0	8–1272
	Median	25	8.9	215	28	6.1	196
	IQR	20	5.5	175	99	7.7	351
2E1	Range	0.5–34.1*	12–117*	32–945	4.1–96.3	6.4–28.4	62–1383
	Median	5.8	34	215	16.8	14.4	230
	IQR	9.0	40	300	19.5	6.5	196
3A-m	Range	191–7505	0.62–6.83	495–5892	90–7089	0.8–12.7	231–12926
	Median	906	2.65	2211	748	2.6	2003
	IQR	902	1.42	1294	847	2.4	3639
3A-t	Range	35–210	17.8–62.2	883–7735*	32–728	15–181	699–31566
	Median	90	24.2	2498	123	30	3236
	IQR	63	16.2	2311	158	31	6728

'3A-m' and '3A-t' represent CYP3A towards midazolam 1'-hydroxylation and towards testosterone 6β-hydroxylation, respectively. *Ethnic differences were statistically significant with a P value of <0.05 (two-tailed).

($P = 0.002$), whereas the difference in V_{max} was not significant ($P = 0.3$).

CYP2C19

The CL$_{int}$ values for CYP2C19 for (S)-mephenytoin 4'-hydroxylation varied from 0.03 to 3.36 µl min^{-1} mg^{-1} protein for the Chinese data set and 0.02 to 8.10 µl min^{-1} mg^{-1} protein for the Caucasian data set. There was an outlier (am01, 3.36 µl min^{-1} mg^{-1} protein) in the Chinese data set. No outlying values were found in the Caucasian data set. The interethnic difference in CL$_{int}$ was highly significant between Chinese and Caucasian groups with a P value of 0.005. The median CL$_{int}$ for the Caucasian group was 3.8 times greater than that for the Chinese group. Similar to CYP1A2, the CL$_{int}$ difference between the Chinese and Caucasian groups was mainly associated with the difference in V_{max}, vs. a difference in K_m. The difference in V_{max} was significant between the two ethnic groups ($P = 0.006$), whereas the difference in K_m was not considered to be significant ($P = 0.1$).

CYP2D6

The CL$_{int}$ values for dextromethorphan O-demethylation by CYP2D6 were from 3 to 119 µl min^{-1} mg^{-1} protein for the Chinese group and 0.2 to 358 µl min^{-1} mg^{-1} protein for the Caucasian group. There were two extreme values (aw27 and am16 with CL$_{int}$ values of 119 and 97 µl min^{-1} mg^{-1} protein, respectively) and an outlier (am05, 82 µl min^{-1} mg^{-1} protein) observed in the Chinese data set of CL$_{int}$ and an extreme value (cm16, 358 µl min^{-1} mg^{-1} protein) in the Caucasian data set. The median CL$_{int}$ for the Chinese data set was comparable with that of the Caucasian group and there was no statistically significant difference between the two ethnic groups ($P = 0.6$). Neither K_m nor V_{max} were found to have a significant difference between the two ethnic groups with P values of 0.3 and 0.9, respectively.

CYP2E1

The CL$_{int}$ for CYP2E1 towards chlorzoxazone 6-hydroxylation varied from 0.5 to 34.1 µl min^{-1} mg^{-1} protein for the Chinese group and 4.1 to 96.3 µl min^{-1} mg^{-1}

protein for the Caucasian group. There was an outlier (am17, 34.1 µl min^{-1} mg^{-1} protein) in Chinese data set and one extreme value (cm01, 96.3 µl min^{-1} mg^{-1} protein) and an outlier (cm22, 58.8 µl min^{-1} mg^{-1} protein) in the Caucasian data set. The difference in CL_{int} between the two ethnic groups was highly significant ($P = 0.00002$) with the median CL_{int} for the Caucasian group 2.9 times greater than that for the Chinese group. It is worth noting that there is no ideal probe reaction for CYP2E1 activity. Although chlorzoxazone 6-hydroxylation was used in the current studies as an indicator of the activity of CYP2E1 in HLM, the concomitant expression of CYP1A2 in HLM would also considerably contribute to the reaction for some samples. The difference in CL_{int} for chlorzoxazone 6-hydroxylation between Chinese and Caucasian groups tended to be mainly attributed to the difference in K_m, rather than that of V_{max}. The difference between the two ethnic groups in K_m was highly significant ($P = 0.000000003$), whereas the difference in V_{max} was not significant ($P = 1$).

CYP3A

The ranges of CL_{int} for midazolam 1'-hydroxylation for the Chinese and Caucasian groups were 191 to 7505 and 90 to 7089 µl min^{-1} mg^{-1} protein, respectively, whereas the ranges for testosterone 6β-hydroxylation were 35 to 210 and 32 to 728 µl min^{-1} mg^{-1} protein, respectively. In the Chinese data set, the sample aw27 (7505 µl min^{-1}·mg protein^{-1}) was characterized as an extreme value for midazolam 1'-hydroxylation, while am19 (210 µl min^{-1} mg^{-1} protein) and am05 (206 µl min^{-1} mg^{-1} protein) were charac-

terized as outliers for testosterone metabolism. Among the Caucasian samples, cm07 exhibited an extreme value of 7089 µl min^{-1} mg^{-1} protein, cm01, cm14, cw30 and cw29 gave outliers of 4790, 2945, 2904, and 2611 µl min^{-1} mg^{-1} protein, respectively, for midazolam metabolism, whereas cw29 gave an extreme value of 728 µL min^{-1} mg^{-1} protein and cm14 gave an outlier of 700 µl min^{-1} mg^{-1}·protein for testosterone metabolism. No statistically significant difference was found between the two ethnic groups in CYP3A metabolic capability for midazolam 1'-hydroxylation ($P = 0.5$). Although the median CL_{int} for testosterone 6β-hydroxylation for the Caucasian group was 1.4 times greater than that for the Chinese group, the difference between the two ethnic groups was not considered to be significant ($P = 0.2$). Neither K_m nor V_{max} for testosterone 6β-hydroxylation were found to have significant difference between the two ethnic groups with P values of 0.4 and 0.06, respectively.

Correlations among CL_{int} of individual CYP enzymes and those between cytochrome b_5 concentration or NADPH-P450 reductase activity and CYP enzyme activity

A comparison of the Kendall's tau-b correlations in Table 3 showed that there were statistically significant associations of the activity of CYP3A with the activities associated with CYP1A2, CYP2A6, CYP2B6 or CYP2C9. These associations were both CYP3A activity indicator dependent and ethnically dependent. The correlations of CYP3A for testosterone 6β-hydroxylation with CYP2B6 activity were statistically significant for both the Chinese ($P = 0.02$) and the

Table 3

Associations between individual CYP enzymes in transformed CL_{int}

Kendall's tau-b coefficient of CYP enzyme									
1A2	2A6	2B6	2C8	2C9	2C19	2D6	2E1	3A-m	3A-t
1A2	0.22 (0.21)	0.07 (0.20)	0.24 (0.20)	0.08 (0.09)	0.25 (0.38*)	0.21 (0.02)	0.03 (0.02)	−0.09 (0.27*)	0.21 (0.23)
	2A6	0.19 (0.34*)	0.30* (0.30*)	0.08 (0.09)	−0.06 (0.27*)	0.27* (0.26*)	0.27* (0.10)	0.03 (0.25)	0.03 (0.39*)
		2B6	0.44* (0.52*)	0.02 (0.02)	−0.26* (−0.01)	0.17 (0.32*)	0.28* (0.33*)	−0.13 (0.17)	0.30* (0.36*)
			2C8	0.01 (0.30*)	−0.26* (0.05)	0.12 (0.46*)	0.30* (0.45*)	−0.26* (0.06)	0.14 (0.31*)
				2C9	−0.04 (0.10)	0.38* (0.27*)	−0.11 (0.16)	0.42* (0.02)	0.17 (−0.10)
					2C19	−0.12 (0.02)	−0.22 (−0.20)	−0.04 (0.20)	0.15 (0.25*)
						2D6	−0.01 (0.27*)	0.15 (−0.07)	0.04 (0.14)
							2E1	−0.16 (0.02)	0.13 (0.07)
								3A-m	−0.01 (0.32*)

CL_{int} values were transformed by dividing by the group median for calculation of Kendall's tau-b coefficients that were used to measure the associations. In addition to the data for the Chinese samples, the data for the Caucasian samples were also exhibited in parentheses. '3A-m' and '3A-t' represent the CYP3A towards midazolam metabolism and testosterone metabolism, respectively. * Correlations were statistically significant with a P value of <0.05 (two-tailed).

Caucasian ($P = 0.006$) groups, while the correlations with CYP2A6 activity, CYP2C8 activity, CYP2C19 activity and CYP3A for midazolam 1′-hydroxylation were significant for only the Caucasian groups, with P values of 0.002, 0.02, 0.05 and 0.01, respectively. Meanwhile, the correlations of CYP3A for midazolam 1′-hydroxylation and CYP1A2 activity was statistically significant in only the Caucasian data set ($P = 0.04$). The correlation of CYP3A for midazolam 1′-hydroxylation and CYP2C8, CYP2C9 activities was significant for only the Chinese group, with P values of 0.04 and 0.001, respectively.

Notable interethnic differences were found for the activity of CYP2Cs associated with other CYP enzyme activities. CYP2C8 activity was significantly correlated with those of CYP2A6 ($P = 0.02$), CYP2B6 ($P = 0.0007$) and CYP2E1 ($P = 0.02$) in the Chinese data set. Similar associations in activity were also found for the Caucasian group with P values of 0.03, 0.00006, and 0.0005, respectively. In addition, CYP2C8 activity was significantly correlated with that of CYP2C19 for the Chinese samples ($P = 0.05$) and with that of CYP2C9 and CYP2D6 for the Caucasian samples with P values of 0.02 and 0.0003, respectively. The enzyme activity of CYP2C9 was also associated with that of CYP2D6 for both the Chinese samples ($P = 0.03$) and the Caucasian samples ($P = 0.04$). With regard to CYP2C19, the association in activity with CYP2B6 was statistically significant in only the Chinese data set ($P = 0.05$) and the association with CYP1A2 and CYP2A6 was significant in only the Caucasian data set, with P values of 0.003 and 0.04, respectively. In addition, the enzyme activity of CYP2E1 was associated with CYP2A6 ($P = 0.04$) or CYP2B6 ($P = 0.03$) for the Chinese samples, and the enzyme activity of CYP2E1 was associated with CYP2D6 activity ($P = 0.04$) and that of CYP2B6 was associated with the activities of CYP2A6 ($P = 0.01$), CYP2D6 ($P = 0.01$) or CYP2E1 ($P = 0.01$) for the Caucasian samples. The correlations of enzyme activity of CYP2D6 with that of CYP2A6 were statistically significant for both the Chinese ($P = 0.04$) and the Caucasian ($P = 0.04$) groups.

There was a significant correlation between cytochrome b_5 concentration and CYP enzyme activity for CYP2C9 and CYP3A in the Chinese data set and CYP2E1 in the Caucasian data set, but no significant correlation was observed for other CYPs. Similarly, only CYP2C9 and CYP3A correlated significantly with NADPH-P450 reductase activity in Chinese data set, while there was no significant correlation found for the other CYP enzymes.

Discussion

Statistical tests can be either parametric or non-parametric. In the current study, utilizing a relatively large population of 60 HLM samples, most of the data for CL_{int}, K_m, V_{max}, cytochrome b_5 concentrations, total CYP concentrations and NADPH-P450 reductase activity were found to be non-normally distributed. Thus, the non-parametric Mann-Whitney U test and Kendall's tau-b correlation analysis were used in the analyses of the data. It is worth pointing out that the use of non-parametric tests would avoid the outliers biasing the summary statistics and P values [18]. Several earlier studies compared the CYP activities and concentrations in HLM between Japanese and Caucasian samples [19–23]. However, the distribution shapes of the results in these studies were not well addressed.

CYP1A2 is highly inducible by dietary and environmental factors, which may mask genetic variation in enzyme activity [24]. The current work found that CYP1A2 activity exhibited large interindividual variation in CL_{int}. The results suggested lower CYP1A2 activity in HLM from the Chinese samples than that from Caucasians. This is consistent with the results of an earlier clinical study in Swedes and Koreans using the plasma paraxanthine : caffeine ratio to indicate CYP1A2 activity [25]. In addition, *in vitro* CYP1A2 activity towards phenacetin O-deethylation was observed here to be lower within the Chinese female samples than the Chinese male samples, but such a gender difference was not significant with the Caucasian samples. In earlier human studies, CYP1A2 activity towards caffeine metabolism appeared to be lower for women than men in Chinese [26] and Caucasians [27].

Interethnic differences in CYP2A6-mediated metabolism tend to be mainly attributed to genetic polymorphism [28]. The defective *CYP2A6* variants in Asians include *CYP2A6*4*, *CYP2A6*7* and *CYP2A6*9*, whereas in Caucasians there is only *CYP2A6*9*. In the current study, no significant interethnic difference between Chinese and Caucasian samples was observed with CL_{int} for CYP2A6. However, Shimada *et al.* [20] reported that both the CYP2A6 protein concentration in HLM and the coumarin 7-hydroxylase activity were lower in Japanese HLM samples than Caucasian samples. In addition, the difference between Japanese subjects and other Asians populations was also reported [29]. Japanese subjects had a significantly lower metabolic ratio of cotinine to nicotine than Korean subjects, but there was no difference between Caucasian and Korean subjects. Japanese had a higher frequency of *CYP2A6*4* (~20%) than Koreans (~10%). Consistent with the Korean population, Chinese have been reported to have a lower frequency of *CYP2A6*4* (~5%) than Japanese (~20%), but a higher frequency of *CYP2A6*1* (~80%) than Japanese (~45%) [29, 30]. These results are consistent with the insignificant difference in the activity of CYP2A6 between Chinese and Caucasian HLM samples. Further, the results reported here and those of the previous studies suggest differences between the Chinese, Japanese and Korean populations that requires additional studies to be confirmed.

Genetic polymorphisms with CYP2B6 are common and the frequencies of the various alleles of *CYP2B6* for Asians appear to be comparable with those in Caucasians, albeit

with some differences [31, 32]. In the current study, no significant difference in CL_{int} for CYP2B6 towards bupropion hydroxylation was found between the Chinese and the Caucasian samples. However, the associated K_m values were observed to be of statistically significant difference between the ethnic groups with the median K_m of Chinese samples 2.4 times greater than that of Caucasian samples. Jinno *et al.* [33] compared the metabolic capabilities of several cDNA recombinant CYP2B6 allelic variants towards 7-ethoxy-4-trifluoromethylcoumarin *O*-deethylation, including CYP2B6.1, CYP2B6.2, CYP2B6.3, CYP2B6.4, CYP2B6.5, CYP2B6.6 and CYP2B6.7, and found that the tested CYP2B6 allelic variants possessed comparable K_m values ranging from 5.5 to 7.7 µM. The reason for our observed ethnic difference in K_m is unknown.

CYP2C8 is a slightly inducible CYP enzyme [34]. Little is known about the effects of *CYP2C8* variant alleles on drug metabolism. *CYP2C8*3* and **4* are primarily found in Caucasians at frequencies of 10–17% and 5–8%, whereas they are extremely rare in Asians [35]. However, neither clinical nor *in vitro* data show substantial effect of *CYP2C8*3* or *CYP2C8*4* alleles on paclitaxel 6α-hydroxylation [36, 37]. In the current report, no significant interethnic differences were observed in K_m, V_{max} or CL_{int} values for CYP2C8 toward amodiaquine metabolism. It is worth noting that the outlying values of CL_{int} in the Chinese and Caucasian data sets appeared to be mainly the result of low K_m values.

CYP2C9 is a quantitatively significant CYP enzyme in human liver and metabolizes about 15% of clinically important drugs. Most studies on *CYP2C9* genetic variations have been associated with the alleles *CYP2C9*2* and *CYP2C9*3*, which are found in Caucasians with frequencies 8–19% and 3–10%, respectively, but virtually absent in Asians [35]. The enzyme encoded by *CYP2C9*2* for diclofenac 4'-hydroxylation has a 59% CL_{int}, a 76% K_m and a 44% V_{max} of the corresponding values of *CYP2C9*1*, while the enzyme of *CYP2C9*3* possesses a 31% CL_{int}, a three times larger K_m and an equal V_{max} as compared with the wild type enzyme [38]. Such genotype specific enzyme kinetic data appear to be substrate-dependent, i.e. the CL_{int} ranging from 7% to 111% (the median CL_{int} 60%, excluding the outlier values), K_m from 17% to 157% (the median K_m 94%) and V_{max} from 8% to 100% (the median V_{max} 53%) for CYP2C9.2 and the CL_{int} from 2% to 57% (the median CL_{int} 10%), K_m from 18% to 825% (the median K_m 325%) and V_{max} from 4% to 130% (the median V_{max} 30%) for CYP2C9.3 as compared with the respective data of CYP2C9.1 [39]. Contrary to the expectation from these *in vitro* enzyme kinetic data, however, the results of the current study indicate a significantly lower diclofenac 4'-hydroxylase activity in Chinese HLM than Caucasian HLM, which was mainly associated with a difference in K_m. Consistent with the results reported here, Chinese and Japanese patients take lower warfarin doses than Caucasians to maintain the same therapeutic efficacy [40, 41]. The lower warfarin dose in Asians suggests that future

studies on additional defective *CYP2C9* alleles in Asians or on other factors affecting the activity of *CYP2C9* are needed. Recently Gu *et al.* [42] reported that Chinese patients with the *CYP2C9 IVS3-65 GC* or *CC* genotype received lower doses of warfarin (0.9 ± 0.2 mg day^{-1}) than those with the *CYP2C9*1/*1* genotype (2.6 ± 1.4 mg day^{-1}). Still, the plasma total and unbound warfarin concentrations were significantly lower in the patients with the *CYP2C9*1/*1* genotype. The frequencies and intrinsic enzyme activities of *CYP2C9 IVS3-65 GC* and *CC* in Chinese and Caucasian populations remain to be better understood.

Genetic differences are a major reason for the large interindividual and interethnic variations in CYP2C19 activity. *CYP2C19*2* and *CYP2C19*3*, two null variants, are responsible for 99% of the poor metabolizer phenotype [43]. The *CYP2C19*2* and *CYP2C19*3* allele frequencies are higher in Asians (30 and 10%, respectively) than in Caucasians (14 and 0%, respectively) [44]. In addition, *CYP2C19*17* is a recently identified allele causing ultrarapid or extensive metabolism of CYP2C19 substrates and the frequency in the Caucasian population is four times higher than that of the Asians (<5%) [45, 46]. Herein and consistent with the known genetic differences, significantly decreased metabolic capability of CYP2C19 in HLM was observed with the Chinese samples compared with the Caucasian samples, which was mainly attributed to the difference in V_{max}.

Despite its relatively low abundance in human liver, CYP2D6 is responsible for the metabolism of 25–30% of clinically important drugs. CYP2D6 is not known to be significantly inducible and the genetic variation contributes largely to the interindividual and interethnic variations in metabolic activity [47]. The appearance of poor metabolizers is lower in Asians (~1%) than Caucasians (5–10%), whereas enzyme activity is lower in Asian extensive metabolizers than in Caucasian counterparts. *CYP2D6*3*, *CYP2D6*4*, *CYP2D6*5* and *CYP2D6*6* account for over 95% of Caucasian poor metabolizers. *CYP2D6*10* is specific variant in Asians at a frequency of 40–60%. Compared with the wild-type, the enzyme encoded by *CYP2D6*10* shows decreased metabolic capacity in a substrate dependent fashion [8] and Japanese HLM homozygous for *CYP2D6*10* tend to have a lower CYP2D6 expression level [21]. In the current study, dextromethorphan *O*-demethylase activity in HLM showed a large interindividual difference, which appeared to mask the interethnic difference.

Unlike CYP2B6, CYP2C9, CYP2C19 and CYP2D6 that have significant polymorphisms, CYP2E1 is relatively conserved. This CYP enzyme is also inducible [47]. In the current work, a significantly lower chlorzoxazone 6-hydroxylase activity (CL_{int}) was observed in Chinese HLM than Caucasian HLM. The interethnic difference in CL_{int} appeared to be mainly associated with the different K_m. Consistent with the current results, the data by Kim *et al.*

[22] suggested that chlorzoxazone 6-hydroxylase activity (measured at one substrate concentration) in Japanese HLM samples was lower than that of Caucasian samples. In addition, the Japanese samples had lower protein concentration of CYP2E1 than the Caucasian samples.

The CYP3A subfamily, mainly CYP3A4 and CYP3A5, represents the most abundant CYP enzymes in human liver and intestine and is associated with the metabolism of ~50% of drugs. The clinical relevance of *CYP3A4* genotypes appears to be relatively limited due to the strong environmental impact via induction or inhibition. In addition, the effect of *CYP3A5* polymorphisms on the total CYP3A activity appears to depend on the concomitant expression of CYP3A4. Testosterone and midazolam show some selectivity for CYP3A4 and CYP3A5 [17]. In the current study, no difference for CL_{int}, as well as that of K_m or V_{max}, of CYP3A was observed between the Chinese and the Caucasian samples.

Recent data have shown co-induction of CYP2C and CYP2B with CYP3A [48, 49]. The nuclear receptors pregnane X receptor and constitutive androstane receptor mediate the induction of CYP3A4, CYP2B and CYP2C enzymes. Wortham *et al.* found significant correlations among mRNA levels of CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19 and NADPH-P450 reductase, suggesting that these xenobiotic metabolism genes are coregulated at the transcriptional level [50]. In addition, they also observed correlations among CYP enzyme activities (the metabolite formation rate at a single fixed substrate concentration) with 20 HLM samples (11 male and six female Caucasian samples, two male Hispanic samples and one male African American sample), i.e. four correlations for CYP3A (with CYP2A6, CYP2B6, CYP2C8 and CYP2C9), three additional for CYP2C8 (with CYP2A6, CYP2B6 and CYP2C9), four additional for CYP2C9 (with CYP2A6, CYP2B6, CYP2D6 and CYP2E1), two additional for CYP2A6 (with CYP2B6 and CYP2E1), and one additional for CYP2B6 (with CYP2D6). In the current study, significant correlations were found among CL_{int} of CYPs, including six correlations for CYP3A (with CYP2B6 and CYP2C8 in both the ethnic groups, CYP1A2, CYP2A6 and CYP2C19 in the Caucasian group and CYP2C9 in the Chinese group), six additional for CYP2C8 (with CYP2A6, CYP2B6 and CYP2E1 in both the ethnic groups, CYP2C19 in the Chinese group, CYP2C9 and CYP2D6 in the Caucasian groups), one additional for CYP2C9 (with CYP2D6 in both the ethnic groups), three additional for CYP2C19 (with CYP1A2 and CYP2A6 in the Caucasian group and CYP2B6 in the Chinese group), three additional for CYP2A6 (with CYP2D6 in both the ethnic groups, CYP2B6 in the Caucasian group and CYP2E1 in the Chinese group), and two additional for CYP2B6 (with CYP2E1 in both the ethnic groups and CYP2D6 in the Caucasian group) and one additional for CYP2D6 (with CYP2E1 in the Caucasian group). According to these data, the correlations appeared to be ethnically dependent. The data presented here with the Caucasian group are largely con-

Table 4

Summary of interethnic differences in CL_{int}, K_m and V_{max} of CYP enzymes in HLM samples of Chinese origin ($n = 30$) compared with those of Caucasian samples ($n = 30$)

CYP	CL_{int}	K_m	V_{max}
1A2	↓	↔	↓
2A6	↔	↔	↔
2B6	↔	↑	↔
2C8	↔	↔	↔
2C9	↓	↑	↔
2C19	↓	↔	↓
2D6	↔	↔	↔
2E1	↓	↑	↔
3A-m	↔	↔	↔
3A-t	↔	↔	↔

The marks '↑' or '↓' denotes a significantly higher or lower value for the Chinese group ($P < 0.05$), whereas '↔' implies no statistically significant difference.

sistent with the data by Wortham *et al.* with respect to the various correlations. The inconsistent results might be due to the difference in HLM samples assessed. In addition, differences in CYP enzyme activity substrates might also have some influence on the results.

Gaining an understanding of interethnic differences in CYP activities is important in order to select the right doses for clinical studies and to avoid unnecessary toxicity due to over-exposure or absence of efficacy due to under-exposure to the drug candidates. In today's environment, multinational pharmaceutical companies are developing drugs that can be used in equally safe and efficient ways in the different regions where they are marketed. Detailed knowledge about interindividual and interethnic variations of the CYP enzymes will facilitate the use of tailored therapy for drugs predominantly eliminated by CYP enzymes and having narrow therapeutic indices. For example, the detailed enzyme kinetics and ranges of values generated in the current study should be useful in improving physiologically based pharmacokinetic models predicting the pharmacokinetics of compounds in development by incorporating ethnic differences and variability. Accordingly, the information about hepatic CYP activities (Figure 3) will be particularly useful for rational choices of drug candidates and predicted doses.

In summary, the metabolic capabilities of nine drug metabolizing CYP enzymes were compared between Chinese HLM and Caucasian HLM. As shown in Table 4, the notable findings include that the Chinese samples had significantly lower CL_{int} for substrates of CYP1A2, CYP2C9, CYP2C19 and CYP2E1 than the Caucasian samples (the median values of the Chinese group being 54, 58, 26, and 35% of the corresponding values of the Caucasian group, respectively), which were mainly attributed to the different K_m or V_{max} values. However, no significant ethnic difference in CL_{int} was observed for CYP2A6, CYP2B6, CYP2C8, CYP2D6, CYP3A for midazolam 1'-hydroxylation, or CYP3A

for testosterone 6β-hydroxylation. The median values of the Chinese group were 141, 56, 187, 89, 121 or 73% of the corresponding values of the Caucasian group, respectively. Despite insignificant interethnic differences in CL_{int}, CYP2B6 had significantly different K_m values. The data of the current study significantly extend the knowledge on the ethnic differences in CYP enzymes and have implications for drug discovery and drug therapy (especially for drugs with a narrow therapeutic window) for patients of different ethnic origins. However, since the Chinese and Caucasian samples were not matched for genotype, medication/disease history and diet, the causes or sources of the observed differences are not well understood. Additional studies are planned to look at the genetic differences between Chinese and Caucasian liver samples and match them with microsomal CYP activities.

Competing Interests

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1
Individual kinetic data for CYP1A2
Table S2
Individual kinetic data for CYP2A6
Table S3
Individual kinetic data for CYP2B6

Table S4
Individual kinetic data for CYP2C8
Table S5
Individual kinetic data for CYP2C9
Table S6
Individual kinetic data for CYP2C19
Table S7
Individual kinetic data for CYP2D6
Table S8
Individual kinetic data for CYP2E1
Table S9
Individual kinetic data for CYP3A-m
Table S10
Individual kinetic data for CYP3A-t

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