Regular Article

Inter-individual Variability of In Vivo CYP2D6 Activity in Different Genotypes

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Full text of this paper is available at http://www.jstage.jst.go.jp/browse/dmpk

Summary: Cytochrome P450 2D6 (CYP2D6), which has a large number of genetic polymorphisms, is involved in the metabolism of a wide range of substrates. Dextromethorphan (DM) is a well-known probe drug for CYP2D6 and metabolic ratio (MR) is often used to measure the enzyme activity in vivo. Using the literature values of DM MR, we estimated the inter-individual variability of CYP2D6 hepatic intrinsic clearance (CLint,h,2D6) in each genotype by Monte Carlo simulation and found that the homozygote of CYP2D6*1 and the heterozygote of CYP2D6*1 and null alleles had a coefficient of variation (CV) of 43% and 56%, respectively. The variability of homozygotes of CYP2D6*2 and CYP2D6*10 was 63% and 66%, while that of the heterozygotes of CYP2D6*2 and null alleles and CYP2D6*10 and null alleles was 125% and 109%, respectively. Based on the variability and reported frequency of the CYP2D6 genotype in Asians and Caucasians, the inter-individual variability of CLint,h,2D6 of extensive metabolizers was estimated at 60–70%, which provided comparable variability of AUC with the literature values of DM, tolterodine, risperidone and atomoxetine. It is suggested that the produced inter-individual variability of CLint,h,2D6 in each genotype is useful for estimating AUC variability of the CYP2D6 substrates in the regional population.

Keywords: inter-individual variability; CYP2D6; Monte Carlo simulation; physiologically based pharmacokinetics; pharmacogenetics; dextromethorphan; drug discovery

Introduction

During the process of drug development, inter-individual variability is a key factor demanding close attention. For example, variability of exposure is sometimes associated with the variability of pharmacological effects, especially with respect to drug efficacy and safety. SimCYP software, which is commercially available, can predict inter-individual variability using a physiologically based pharmacokinetic (PBPK) model that employs a bottom-up approach. Variability is estimated from in vitro information, including expression in the recombinant cytochrome P450 (CYP) system, evaluation of metabolizing enzyme activity in each genotype, and demographic parameters such as body weight and frequency of genotypes in target populations, with some scaling factors.1,2 Recently, Kato et al. proposed a different method to estimate the inter-individual variability of hepatic intrinsic clearance (CLint,h).3 They collected various coefficients of variation (CV) of CYP3A4 content in human liver microsomes (33–99%) from the literature and extracted 33% CLint,h variability of CYP3A4 using a dispersion model based on the in vivo variability of the area under the plasma concentration curve (AUC) of CYP3A4 substrates. For CYP2D6, Ito et al. reported a CV of 60% for CLint,h of extensive metabolizers (EM) and intermediate metabolizers (IM), using the metabolic ratio (MR) of CYP2D6 probe drugs.4 CYP2D6 genotypes have approximately 80 polymorphisms [http://www.cypalleles.ki.se/cyp2d6.htm], and the number of reported genotypes continues to increase, accounting for a wide range of enzyme activities from deficient to ultra-rapid metabolism. Among the reported polymorphisms, some alleles cause increased activity by gene multiplication (CYP2D6*1×N, CYP2D6*2×N), while others lose their enzyme activity as a result of chromosomal deletion.

Received August 6, 2011; Accepted January 12, 2012
J-STAGE Advance Published Date: January 24, 2012, doi:10.2133/dmpk.DMPK-11-RG-078
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(CYP2D6*5), splice site mutation (CYP2D6*4), or insertion of a stop codon with deletion of a single base pair (CYP2D6*3 and CYP2D6*6). Some polymorphisms lead to decreased enzyme activity (CYP2D6*10, CYP2D6*17). Thus, CYP2D6 genotypes are associated with the inter-individual variability of CYP2D6 activity in vivo. This may explain the larger variability in CLint,h of CYP2D6 (CLint,h,2D6) compared with CYP3A4.

The frequency of the CYP2D6 allele is influenced by regional population differences and is also responsible for ethnic differences in enzyme activity. The frequency of poor metabolizers (PM) in Caucasian populations is approximately 10%, but among Asian populations it is 0.1–1.6%. The frequency of the reduced-type allele CYP2D6*10 is approximately 40% in East Asia, but only 3% in Europe. Consequently, the finding of Caucasian EM having 60% variability by Ito et al. may not apply to other ethnicities or regions.

Dextromethorphan (DM) has been appraised as one of the best probe drugs specifically for CYP2D6, from points of in vivo/in vitro correlation, contribution of CYP2D6 to metabolism, and registration of CYP2D6 as a therapeutic drug. Myrand et al. used DM as a probe drug to investigate ethnic differences in pharmacokinetics and pharmacogenetics among Caucasian, Chinese, Korean, and first- and third-generation Japanese populations, with more than 100 volunteers in each population. They concluded that there were no ethnic differences in MR in each genotype of CYP2D6.

Extensive evidence on the relationship between MR and CYP2D6 phenotypes and genotypes has been accumulated using DM. However, there are no reports for estimation of the variability of CLint,h,2D6 using DM MR. In this study, we introduced a theory to transfer the MR of DM to CLint,h,2D6 with DM in different genotypes were estimated and estimate mean values with variability in each CYP2D6 genotype, which can then be used to estimate the mean population exposure and variability.

Methods

Estimation scheme of mean and standard deviation (SD) of CLint,h,2D6 with DM: The mean and SD of CLint,h,2D6 with DM in different genotypes were estimated from the mean and SD of MR. The estimation method is described in Figure 1. Numerous MR values (mean ± SD) were generated from CLint,h,2D6 (mean ± SD) and CLint,h,2D6 (mean ± SD) were made. Data regarding DM MR (mean ± SD) were extracted from the literature with genotype information, and the CLint,h,2D6 (mean ± SD) values corresponding to MR were found from the combinations as mentioned above.

Theory of estimation of CLint,h,2D6 from DM MR:

MR = \frac{\text{amount of unchanged drug collected in urine}}{\text{amount of metabolite(s) collected in urine}} \quad (1)

where amount of metabolite(s) includes further metabolites from the target metabolite. Under the condition of linear pharmacokinetics, MR is expressed as follows:

MR = \frac{D \cdot F_a \cdot F_g \cdot F_h \cdot \text{CL}_r \cdot \text{CL}_t}{\text{CL}_{m,tot}} \quad (2)

where D represents amount of dose and F_a, F_g, and F_h are the fraction absorbed, intestinal and hepatic availability, respectively. CL_{m,tot} and CL_r are total clearance and renal clearance, respectively. CL_t is the sumation of hepatic clearance (CL_h) and CL_r and f_m2D6 and R_{m,r} are the contribution of CYP2D6 to the metabolism, expressed as Eqs. (3) and (4), respectively.

f_m2D6 = \frac{\text{CL}_{int,h,2D6}}{\text{CL}_{int,h,2D6} + \text{CL}_{int,h,others}} \quad (3)

R_{m,r} = \frac{\text{CL}_{m,r}}{\text{CL}_{m,tot}} \quad (4)

Theory of estimation of CLint,h,2D6 from DM MR:

MR = \frac{1 - \frac{\text{CL}_h}{Q_h} \cdot \text{CL}_r}{f_m2D6 \left( \text{CL}_{m,tot} - \frac{1 - \frac{\text{CL}_h}{Q_h} \cdot \text{CL}_r}{\text{CL}_{m,r}} \right)} \quad (5)

where Q_h represents hepatic blood flow rate.

CL_h was determined by hepatic blood flow rate.

Fig. 1. A scheme for estimation of CYP2D6 intrinsic clearance (CLint,h,2D6) of dextromethorphan in each CYP2D6 genotype using metabolic ratio (MR)
$$CL_h = Q_h \left[ 1 - \frac{4a}{(1 + a)^2 \cdot \exp[(a - 1)/2/D_N] - (1 - a)^2 \cdot \exp[-(a + 1)/2/D_N]} \right]$$

$$a = (1 + 4R_N \cdot D_N)^{1/2}, R_N = f_R \cdot \frac{CL_{int,h}}{Q_h}$$

where $f_R$ and $D_N$ are the unbound fraction in the blood and the dispersion number, respectively. $D_N$ was assumed to be $0.17^{10-12}$.

$CL_{int,h}$ is the summation of $CL_{int,h,2D6}$ and $CL_{int,h,others}$.

The DM plasma unbound fraction ($f_U$) was calculated from the equation $f_U = 1/(1 + n\cdot P_i/K_d)$ where $n$, $P_i$, and $K_d$ are the number of binding sites, concentration of plasma protein binding to DM, and dissociation constant, respectively. No inter-individual variability of $K_d$ was assumed. Values for $n$ and the blood-plasma concentration ratio of DM were set as unity.

**Simulation of MR from $CL_{int,h,2D6}$:** In the simulation, MR values of 10,000 individuals were generated from $CL_{int,h,2D6}$, according to Eq. (2), using Monte Carlo simulation with SAS (Version 9.2, SAS Institute, Cary, NC, USA). The distribution of $CL_{int,h,2D6}$ was assumed to be log-normal. The mean values of $CL_{int,h,2D6}$ were changed from $1.00 \times 10^{-4}$ to $15.00 \text{mL/min/mL of liver}$, varying the SD ($CL_{int,h,2D6}$) from 0.01 to 2.00. Combinations of $CL_{int,h,2D6}$ (mean $\pm$ SD) and MR (mean $\pm$ SD) were then made. The reported values of DM $CL_r$ (0.57 mL/min/kg), $CL_{int,h,others}$ (0.20 mL/min/mL liver), $Q_h$ (1.22 mL/min/mL liver), liver volume (19.5 mL/kg), and body weight (70 kg/person) were used for the simulation. Liver density was assumed to be unity. Based on the findings in previous reports, the urinary excretion fraction of the metabolite $R_{m,r}$ (0.534) was estimated as the ratio of the sum of dextrophan and its conjugation to the sum of dextrophan and metabolites via dextrophan in urine (72% of dose) after oral administration of DM.\(^{(15)}\)

**Data collection of MR:** The mean and variability (SD, standard error [SE], and confidence interval [CI]) values of DM MR were collected from the literature with respect to the $CYP2D6$ genotype as a log-transformed data set (Supplement 1). The literature was searched via PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) or comprised cited references. As inclusion criteria for DM MR, African American population data were removed because of racial differences in the same genotype reported between African American and Caucasian populations.\(^{(16)}\) Only MR studies in which the urine collection period was $\geq 8$ h were selected, since the inter-individual variability of DM increases if the collection period is less.\(^{(17)}\) Studies in which measurement of $CYP2D6^{*2}$, $CYP2D6^{*3}$, $CYP2D6^{*4}$, and $CYP2D6^{*10}$ alleles were selected because of a frequency $\geq 5\%$ in the worldwide population,\(^{(18)}\) and data with $n$ sizes $\geq 4$ per genotype were used. If an individual value ($n = 1$) was available, the data were included in the MR dataset (Supplement 1) and the mean and SD were calculated using more than three other individual values. Although the frequency of $CYP2D6^{*41}$ was $7\%$,\(^{(18)}\) its measurement was not included as part of the MR data because the $CYP2D6^{*41}$ allele is recognized as $CYP2D6^{*2}$ when $CYP2D6^{*41}$ is not measured, but this did not affect the value of $CYP2D6^{*1}$.

The mean and variability (SD, SE, and CI) values of MR of debrisoquine, sparteine, and mexiletine were collected from the literature as log-transformed data. For debrisoquine, the quantitative analysis method required a $\geq 8$-h urine collection period.\(^{(9)}\)

**Statistical methods:** When there were multiple sources of MR (mean $\pm$ SD) values for the same genotype, the overall mean and SD values were estimated by integration of each mean and SD value with the following calculation, after log transformation of $CL_{int,h,2D6}$:

Weighted mean (WM) was calculated as:

$$WM = \frac{\sum_{i=1}^{n} (N_i \cdot m_i)}{\sum_{i=1}^{n} N_i}$$

Overall SD was calculated as:

$$SD = \sqrt{\sum_{i=1}^{n} \left[ \left( SD_i^2 \cdot (N_i - 1) + N_i \cdot m_i^2 \right) - WM^2 \cdot \sum_{i=1}^{n} N_i \right] / \sum_{i=1}^{n} N_i - 1}$$

where $N_i$ is the number of observations, $m_i$ is the mean value from the $i$th study, and SD, is the SD from the $i$th study.

The inter-study variability was assumed to be included in the inter-individual variability.

The 95% confidence intervals for the standard deviation ($\sigma$) of the overall SD were calculated as:

$$\sqrt{\frac{n - 1}{X_{H}^2}} \cdot SD \leq \sigma \leq \sqrt{\frac{n - 1}{X_{L}^2}} \cdot SD$$

where $X_{H}^2$ and $X_{L}^2$ are the 2.5 and 97.5 percentiles of the chi-square distribution limits.

**Estimation of mean and SD of $CL_{int,h,2D6}$ for each CYP2D6 genotype:** To estimate the mean and SD of $CL_{int,h,2D6}$ from MR literature values for each $CYP2D6$ genotype, the literature MR values (mean $\pm$ SD) were extracted from the combination data set between $CL_{int,h,2D6}$ (mean $\pm$ SD) and MR (mean $\pm$ SD) (see the section “Simulation of MR from $CL_{int,h,2D6}$”).
Estimation of mean and SD of $CL_{int,h,2D6}$ for EM populations: MR of 100,000 individuals was generated by simulation using the $CL_{int,h,2D6}$ and frequency of each CYP2D6 genotype (Supplement 2) for EM populations of Caucasians and Asians (Chinese, Japanese and Korean). To separate the EM population from the IM or PM population, MR of individuals with more than the antimode of 0.03 (non-IM-PM) or 0.3 (non-PM) was extracted to estimate the mean and SD of $CL_{int,h,2D6}$ in all EM populations, respectively.

Simulation of AUC of CYP2D6 substrates: AUC data of CYP2D6 substrates were selected from the literature based on the following conditions: healthy subjects, subject numbers $\geq 10$; availability of EM and PM AUCs; the urinary excretion fraction of the intact drug was $\leq 5$%, and $f_{in2D6}$ was $\geq 90$%. If CYP2D6 substrates have small $f_{in2D6}$ and/or large $f_i$, the simulated AUC variability may be influenced by the variability of metabolic pathways other than CYP2D6, making it difficult to evaluate the variability of the target enzyme of CYP2D6. In this approximate selection, the intrinsic clearance of EM ($CL_{int,h,EM}$) and PM ($CL_{int,h,PM}$) was estimated using $Q_h (1.665 \text{ mL/min})$, plus an assumption that the fraction absorbed and intestinal availability was unity ($F_iF_e = 1$). The value of $f_{in2D6}$ was calculated as $CL_{int,h,EM}/(CL_{int,h,EM} + CL_{int,h,PM})$.

AUC (mean $\pm$ SD) values were generated according to Eq. (5), with mean and SD values of $Q_h (1.22 \pm 0.17 \text{ mL/min/mL liver})^1,14$ liver volume ($19.5 \pm 2.1 \text{ mL/kg}$),$^3,14$ and body weight ($70 \pm 10 \text{ kg/person}$)$^3$, employing Monte Carlo simulation as proposed by Kato et al.$^3$ The mean and SD values of body weight were consistent with the available demographic data$^9,21$ ($69.9 \pm 10.3 \text{ kg/person, } n = 690$) in the clinical studies used in the present MR analyses. AUC divided by dose (AUC/D) was calculated by the following equation:

$$\text{AUC/D} = \frac{F_A \cdot F_e (1 - CL_h/Q_h)}{CL_h} \quad (8)$$

where $F_A$ and $F_e$ were assumed to be 1.0.

The percentile confidence intervals of CV of AUC were estimated by Monte Carlo simulation using Eqs. (6) and (8) with various $CL_{int,h,2D6}$ CV, after estimation of $CL_{int,h}$ corresponding to the literature AUC mean. Since $f_{in2D6}$ was set at more than 90% in the inclusion criteria for compound data, $CL_{int,h}$ was assumed equal to $CL_{int,h,2D6}$ in this AUC confidence interval estimation. The sample size was set as the number of volunteers in the study in the literature and mean, SD and CV values of AUC for 1,000 virtual studies were generated.

Results

$CL_{int,h,2D6}$ in each genotype: Using the relationship between $CL_{int,h,2D6}$ and MR devised by Eq. (2) with the dispersion model, the combination of $CL_{int,h,2D6}$ (mean $\pm$ SD) and MR (mean $\pm$ SD) was determined by Monte Carlo simulation with various mean and SD values of $CL_{int,h,2D6}$. We drew the relationship curve between $CL_{int,h,2D6}$ and MR and estimated the values of mean and SD of $\log (CL_{int,h,2D6})$ corresponding to those of $\log (MR)$ cited in various literature reports (Fig. 2 and Supplement 1). The relationship between both log-transformed parameters ($\log (CL_{int,h,2D6})$ and $\log (MR)$) was determined to be non-linear. MR gradually decreased with an increase in $CL_{int,h,2D6}$. When $CL_{int,h,2D6}$ was less than $Q_h/f_1$, which was approximately 0.2 of $\log (CL_{int,h,2D6})$, MR rapidly decreased. With the assumption of a log-normal distribution of $CL_{int,h,2D6}$, MR was also likely to have a log-normal distribution when $CL_{int,h,2D6}$ was lower. However, MR distribution was skewed with higher values of $CL_{int,h,2D6}$ leading to larger variability.

Almost all reported values, except for cases of homozygotes of null alleles, could be plotted around a curved region. For the homozygotes of null alleles, the SD values of $\log (MR)$ were comparable with those of $\log (CL_{int,h,2D6})$, while the other SD values of $\log (MR)$ were larger than those of $\log (CL_{int,h,2D6})$ (Fig. 2 and Supplement 1).

As is well known, $CL_{int,h,2D6}$ showed higher values in gene duplicates including the CYP2D6*1 allele than in CYP2D6*1/1 (Table 1). The activity of homozygotes decreased in the following order: CYP2D6*1>CYP2D6*2>CYP2D6*10, and heterozygotes showed activity that was intermediate between those of homozygotes of each allele. $CL_{int,h,2D6}$ of CYP2D6*2/*2 × 2 was comparable with that of CYP2D6*2/*2, showing no effect of duplication. All combinations of heterozygotes with null alleles indicated reduced activity compared to the homozygote of the functional allele.

The variability of $CL_{int,h,2D6}$ in a genotype group of more than 10 subjects was used for accurate estimation. Some differences in variability were found among geno-
Null allele represents nonfunctional CYP2D6 types. The CV of homozygotes of a reduced-type allele (CYP2D6*10/*10, 66%) was comparatively larger than that of CYP2D6*1/*1 (43%) but similar to CYP2D6*2/*2 (63%). The variability of CYP2D6*1/*10 (53%) was intermediate between CYP2D6*1/*1 and CYP2D6*10/*10, while that of CYP2D6*2/*10 (49%) was smaller than the variability of CYP2D6*10/*10 and CYP2D6*2/*2. Changes in variability for heterozygotes with null alleles were unique. The variability of CYP2D6*1/*null (56%) was slightly larger than that of CYP2D6*1/*1, while CYP2D6*2/*null and CYP2D6*10/*null showed approximately 2-fold larger CV values than those of CYP2D6*2/*2 and CYP2D6*10/*10, respectively.

**Estimation of CYP2D6 EM:** CYP2D6 activity in the Caucasian EM population (CL_{int,h,2D6} EM) was simulated using the mean (SD) (Table 1) and frequency values reported in the literature (Supplement 1). CL_{int,h,2D6} EM values of non-IM-PM and non-PM were estimated as 6.37 and 5.94 mL/min/mL liver, respectively. The simulated CV values were 57% and 65% for non-IM-PM and non-PM, respectively.

Similarly, CYP2D6 EM activity was also estimated separately for Chinese, Japanese and Korean populations. The values were 4.65 (CV: 59%), 5.00 (57%), and 4.74 mL/min/mL liver (60%) for non-IM-PM and 3.57 (CV: 72%), 4.11 (66%), and 3.54 mL/min/mL liver (74%) for non-PM, respectively. Thus, the Asian population showed consistent mean (4–5 mL/min/mL liver) and CV values (60–70%) comparable to the Caucasian population.

Using those mean and CV values of CL_{int,h,2D6}, AUC and its variability in EM were simulated and compared with the previously reported values for DM (Fig. 3). The simulated values were plotted around literature values for both Caucasian and Asian populations. CYP2D6 substrates other than DM, risperidone, atomoxetine and tolterodine were selected to compare simulated and literature values, because they had \( f_{m2D6} \geq 0.9 \) and \( f_e \leq 0.05 \). The AUCs of 4 CYP2D6 substrates were plotted around the predicted curves of 60% or 70% of the CV of CL_{int,h,2D6}. All of the literature AUC values were within 95 percentile confidence intervals of simulated AUC CV using 60% and 70% of CL_{int,h,2D6} CV. Including DM, at least 10 out of 11 plots were clearly different from the simulated curve with 33% CV, which was the estimated variability of CYP3A4. Actually, the values, except for one plot of atomoxetine (the lowest plot in Fig. 3), were outside of 95 percentiles of simulated AUC CV using 33% of CL_{int,h,2D6} CV.
tolterodine for CYP2D6*10/*10. The \( f_{\text{m2D6}} \) was estimated as 0.92 using PM AUC and \( f_e \) was ≤5%. The AUC/D was plotted on the simulated curve of CL\(_{\text{int,h,2D6}}\) with 60% CV (Fig. 3).

Comparison of MR variability among CYP2D6 probe drugs: For sparteine and debrisoquine, the relationship between CL\(_{\text{int,h,2D6}}\) and MR is inversely proportional and the variability of MR is reflected in the variability of CL\(_{\text{int,h,2D6}}\). Although few studies have reported sparteine or debrisoquine MR values with variability for CYP2D6 in each genotype, it was possible to extract those for the wild type (CYP2D6*1/*1). We then compared the presented variability of CL\(_{\text{int,h,2D6}}\) estimated from DM with the variability of MR of other probe drugs (Fig. 4). Debrisoquine MR provided approximately 10% larger CV compared to the variability of CL\(_{\text{int,h,2D6}}\) for DM, as pooled values. Sparteine and mexiletine MR CV values obtained from a single report of each were within the range of DM CV values. For comparison, it is necessary to consider that in the present study, almost all genomic analyses for plots, except those for DM, did not separate the alleles of CYP2D6*2.

Discussion

The present study provides mean and CV values for CL\(_{\text{int,h,2D6}}\) of DM in each genotype. If the frequency of all genotypes is known for one group, using the CV values, it is possible to estimate the variability of CL\(_{\text{int,h,2D6}}\) in the population. Moreover, it is possible to estimate the variability of AUC if the contributions of elimination pathways other than CYP2D6 are negligible. We estimated the variability of CL\(_{\text{int,h,2D6}}\) of EM in Caucasian and Asian populations. Using these values, AUC variability was simulated and determined to be comparable to the values reported in the literature. MR histograms of debrisoquine and sparteine in Caucasians revealed the variability of CL\(_{\text{int,h,2D6}}\) in EM to be 45–70%, which is consistent with the estimation in our study (~60%).

Neafsey et al. compared the debrisoquine MR in Caucasian and Asian groups and suggested that the ethnic difference was caused by a lesser frequency of CYP2D6*1 and CYP2D6*2 and a greater frequency of CYP2D6*10 in Asians. To date, many studies have provided information with regard to the frequency of CYP2D6 genetic polymorphisms. Using the accumulated information of genotype frequency and the present results of variability of CL\(_{\text{int,h,2D6}}\) in each genotype, it has been possible to estimate the variability of AUC of CYP2D6 substrates.

Though DM is a good CYP2D6 probe drug, the relationship between log-transformed MR and CL\(_{\text{int,h,2D6}}\) is non-linear because of the Qh limited pharmacokinetics.
of DM in EM; therefore, MR variability is not equal to \( CL_{int,h,2D6} \) variability. In the present study, we developed a method of transferring the variability of DM MR to that of \( CL_{int,h,2D6} \) using Monte Carlo simulation. To our knowledge, no study has estimated DM \( CL_{int,h,2D6} \) from DM MR and no study has conducted meta-analysis of \( CL_{int,h,2D6} \) in each genotype using DM MR. A unique finding in the present study is that the heterozygote of \( CYP2D6*2 \) and null alleles and \( CYP2D6*10 \) and null alleles showed larger variability (125% and 109%, respectively) than the homozygote of \( CYP2D6*2 \) and \( CYP2D6*10 \) alleles (63% and 66%, respectively). The contribution of epigenetic factors, e.g., gene methylation and microRNA expression, may be responsible for this difference in variability.

To evaluate variability of \( CL_{int,h,2D6} \) separately from variability of other parameters, Eq. (5) was used. For our analysis, we assumed that the variability of DM \( CL_{int,h,2D6} \) accounts for variability of DM MR and contribution to variability by other parameters is negligible. Then, the variability for only \( CL_{int,h,2D6} \) was finally set. It is obvious that the other parameters, i.e., \( CL_{r}, CL_{int,h,other}, h_{m,r}, h_{b}, Q_b \), body weight, and liver volume, will show variability too; therefore, the influence of other parameters on \( CL_{int,h,2D6} \) and its variability was tested. When all the possible variability of parameters was incorporated in the simulation, the CV for genotypes was a maximum of 13% lower than when using only the variability of \( CL_{int,h,2D6} \) (data not shown). Hence, the influence of other parameters on \( CL_{int,h,2D6} \) CV was essentially negligible.

In the present process of data collection, inclusion criteria for literature concerning DM MR were measurements of alleles \( CYP2D6^1 \), \( CYP2D6^2 \), \( CYP2D6^3 \), \( CYP2D6^4 \), \( CYP2D6^5 \), and \( CYP2D6^10 \) but not \( CYP2D6^41 \) (frequency: 7%\(^3\)) because of less evidence; instead, \( CYP2D6^41 \) was included in \( CYP2D6^2 \). Lee et al. reported the relationship for \( CYP2D6^41 \) between DM and MR.\(^2\) From the values, using the present method, we estimated \( CL_{int,h,2D6} \) of \( CYP2D6^41/41 \) to be 1.4 mL/min/mL liver with a CV of 62% (\( n = 8 \), data not shown), which was approximately 1/4th of the presented \( CL_{int,h,2D6} \) for \( CYP2D6^2/2 \), with comparable variability. If there is no contamination of \( CYP2D6^41 \) to \( CYP2D6^2 \), \( CL_{int,h,2D6} \) of \( CYP2D6^2/2 \) should be higher with smaller variability. The \( CYP2D6^2/2 \) \( CL_{int,h,2D6} \) estimated from the reported value was slightly higher at 5.9 mL/min/mL liver with slightly larger variability (CV: 75%). The influence of non-measurement of \( CYP2D6^41 \), therefore, is likely to be negligible.

We estimated \( CL_{int,h,2D6} \) of EM as approximately 4–6 mL/min/mL liver (from the MR values reported in the literature) and the variability of EM to be 60–70% in Caucasian and Asian populations. With the values, mean and CV values of DM AUC in EM were predicted using the dispersion model, which proved consistent with literature values of EM (Fig. 3). The CV values of DM AUC were 100–150%. The variability of AUC was previously reported to show 77% CV,\(^10\) using the \textit{in vitro}–\textit{in vivo} extrapolation scaling process by means of a well-stirred model, but the estimation was lower than the observed value (110%). This difference could be explained by differences in the hepatic pharmacokinetic model and the fact that \textit{in vitro} data were used in the previous study. We provided our estimates from MR \textit{in vivo} data using the dispersion model.

Regarding AUC variability of CYP2D6 substrates, Dorne et al. reported it as 67–71% CV of oral CL for non-phenotyped population and EM.\(^3\) However, those values were simply estimated as the mean of CV values of various CYP2D6 substrates. As seen in Figure 3, the variability of AUC/D decreased with increases in the value of CV values of various CYP2D6 substrates. As seen in Figure 3, the variability of AUC/D decreased with increases in the value of CV values of various CYP2D6 substrates. As seen in Figure 3, the variability of AUC/D decreased with increases in the value of CV values of various CYP2D6 substrates.

In conclusion, \( CL_{int,h,2D6} \) of DM and its variability were determined in each genotype. Using estimations, the variability of AUC in EM was predicted, which proved to be consistent with previous clinical findings. Thus, it is suggested that the produced inter-individual variability of \( CL_{int,h,2D6} \) in each genotype is useful for estimating AUC variability of the CYP2D6 substrates in the regional population.

Acknowledgments: We thank Dr. Masayo Oishi from Keio University for helpful advice regarding SAS programming.

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