**Evaluation of Alteration in Hepatic and Intestinal BCRP Function In Vivo from ABCG2 c.421C>A Polymorphism Based on PBPK Analysis of Rosuvastatin**

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**ABSTRACT**

Polymorphism c.421C>A in the ABCG2 gene is thought to reduce the activity of breast cancer resistance protein (BCRP), a xenobiotic transporter, although it is not clear which organ(s) contributes to the polymorphism-associated pharmacokinetic change. The aim of the present study was to estimate quantitatively the influence of c.421C>A on intestinal and hepatic BCRP activity using a physiologically based pharmacokinetic (PBPK) model of rosuvastatin developed from clinical data and several in vitro studies. Simultaneous fitting of clinical data for orally and intravenously administered rosuvastatin, obtained in human subjects without genotype information, was first performed with the PBPK model to estimate intrinsic clearance for hepatic elementary process. The fraction of BCRP activity in 421CA and 421AA (fca and faa values, respectively) with respect to that in 421CC subjects was then estimated based on extended clearance concepts and simultaneous fitting to oral administration data for the three genotypes (421CC, 421CA, and 421AA). On the assumption that c.421C>A affects both intestinal and hepatic BCRP, clinical data in each genotype were well reproduced by the model, and the estimated terminal half-life was compatible with the observed values. The assumption that c.421C>A affects only either intestinal or hepatic BCRP gave poorer agreement with observed values. The faa values obtained on the former assumption were 0.48-0.54. Thus, PBPK model analysis enabled quantitative evaluation of alteration in BCRP activity owing to c.421C>A, and BCRP activity in 421AA was estimated as half that in 421CC.

**Introduction**

ATP-binding cassette subfamily G2 (ABCG2) encodes breast cancer resistance protein (BCRP), which exports various types of xenobiotics and therapeutic agents by using ATP hydrolysis as a driving force (Hirano et al., 2005; Mao and Unadkat, 2015). BCRP is expressed in various organs, including the small intestine, liver, kidney, and brain (Maliepaard et al., 2001). In humans, BCRP is considered to act as an efflux transport system in small intestinal epithelial cells, based largely on recent observations of pharmacokinetic changes of its substrate drugs due to either drug interaction (Adkison et al., 2010; Kusuhara et al., 2012) or polymorphism of ABCG2 gene (Urquhart et al., 2008; Yamasaki et al., 2008; Gotanda et al., 2015). The major polymorphism c.421C>A in the ABCG2 gene, which leads glutamine to lysine substitution at position 141 (Q141K), is found in various populations (Ieiri, 2012; Tomita et al., 2013) and is associated with pharmacokinetic change of multiple therapeutic agents, including rosuvastatin (Keskitalo et al., 2009b; Wan et al., 2015). The polymorphism has also been reported to affect the pharmacodynamics of rosuvastatin (Bailey et al., 2010; Tomlinson et al., 2010) and the frequency of side effects after administration of other substrate drugs (Mizuno et al., 2012).

Polymorphism c.421C>A is thought to alter the function and/or expression of ABCG2 gene product. For example, Imai et al. (2002) confirmed that c.421C>A decreases the BCRP protein expression level in ABCG2-overexpressing murine PA317 cells. The protein expression level of mutated BCRP was estimated to be 24%–47% that of wild-type BCRP in several cell lines (Kondo et al., 2004; Tamura et al., 2006; Matsuo et al., 2009). Gotanda et al. (2015) and Kobayashi et al. (2005) reported the protein expression level in vivo in human red blood cell and placental samples from homozygous subjects as 15% and 49%, respectively, compared with that in wild-type subjects. On the other hand, no effect of the polymorphism on BCRP activity was found in LLC-PK1 cells (Mizuara et al., 2004) and human intestinal samples (Zambers et al., 2003; Urquhart et al., 2008). Thus, the effect of c.421C>A polymorphism on BCRP activity remains controversial. Although the mechanisms involved are still unknown, it has been proposed that c.421C>A may affect ATPase activity (Mizuara et al., 2004, Morisaki et al., 2005), substrate specificity (Honjo et al., 2002), and proteasomal

**ABBREVIATIONS:** ABCG2, ATP-binding cassette subfamily G2; AUC, area under the blood concentration-time curve; BCRP, breast cancer resistance protein; Clint, intrinsic clearance; FaFg, fraction absorbed; GI, gastrointestinal; PBPK, physiologically based pharmacokinetic model.
degradation (Kondo et al., 2004; Nakagawa et al., 2011), indicating that multiple factors are involved in the pharmacokinetic changes. Therefore, further analyses are needed to examine the pharmacokinetic changes attributable to the ABCG2 polymorphism in individual organs for each substrate drug; however, it is difficult to estimate quantitatively functional change in BCRP activity in vivo in humans.

Tanaka et al. (2015) estimated the BCRP activity in the small intestine of c.421C>A homozygous subjects as 23% of that in wild-type subjects by means of a mathematical model of intestinal absorption that they developed using the changes in the area under the plasma concentration-time curve (AUC) of rosuvastatin and six other drugs owing to the polymorphism. They assumed that c.421C>A polymorphism affects the activity of BCRP only in the small intestine (Tanaka et al., 2015). Nevertheless, BCRP is also expressed on the canalicular membrane of hepatocytes (Maliepaard et al., 2001), and rosuvastatin is primarily taken up by hepatocytes after oral absorption, followed by excretion into the bile (Elshby et al., 2012), with minimal hepatic metabolism (Cooper et al., 2002; Martin et al., 2003b). Therefore, c.421C>A polymorphism of BCRP in the liver may also affect rosuvastatin’s pharmacokinetics.

The aim of the present study was to estimate quantitatively the change in the pharmacokinetics of rosuvastatin attributable to ABCG2 gene polymorphism c.421C>A based on physiologically based pharmacokinetic (PBPK) model analysis of previously reported clinical data. The advantages of PBPK models include incorporation of multiple pharmacokinetic processes, such as gastrointestinal (GI) absorption and hepatic disposition, into a single mathematical model that can quantitatively describe the change in each process resulting from various factors such as drug interaction and pharmacogenetic effects. Therefore, development of a PBPK model may be the best approach to examine the effect of c.421C>A polymorphism on both intestinal and hepatic BCRP activities in humans. To discriminate quantitatively the effect of the polymorphism on the intestinal absorption and biliary excretion processes for rosuvastatin, we used the developed model to test three hypotheses in the present study: 1) c.421C>A affects only the intestinal absorption of rosuvastatin, as proposed previously (Tanaka et al., 2015); 2) c.421C>A affects only biliary excretion of rosuvastatin; 3) c.421C>A affects both processes. By combining the PBPK model analysis with extended clearance concepts, we show that the third hypothesis provides the best fit to the observational data.

Materials and Methods

Construction of PBPK Model. First, a PBPK model of rosuvastatin (Supplemental Fig. S1) was constructed. The basic structure of this PBPK model was originally constructed in the previous study (Yoshikado et al., 2016) but was modified in the present study to include the stomach and three compartments of the small intestine to account for the delay in the time of the peak concentration after oral administration. This PBPK model without the stomach compartment was most recently used in the analysis of rosuvastatin pharmacokinetics (Sugiyma et al., 2017). A more complicated model may be needed to describe the absorption process of rosuvastatin, but it was not constructed in the present study since only plasma concentration profile data were used in the following analyses. Mass-balance equations in the PBPK model were described in supporting information. Several hybrid parameters, such as $CL_{int,bile}$, $R_{diff}$, $b$, and $g$, were defined in eqs. 1–5 as follows (Yoshikado et al., 2016):

$$ CL_{int,all} = PS_{inf}^b \beta = PS_{diff}^\phi + CL_{int,met}^\phi + CL_{int,bile}^\phi $$

$$ f_{bile} = \frac{CL_{bile}}{CL_{int}} = \frac{CL_{int,met} + CL_{int,bile}}{CL_{int,met} + CL_{int,bile}} $$

$$ R_{diff} = \frac{PS_{diff,inf}}{PS_{inf}} $$

$$ \beta = \frac{CL_{int}}{PS_{diff,inf} + CL_{int}} = \frac{CL_{int,met} + CL_{int,bile}}{PS_{diff,inf} + CL_{int,met} + CL_{int,bile}} $$

$$ \gamma = \frac{PS_{diff,inf}}{PS_{diff,inf}} $$

where $PS_{inf}$ is hepatic uptake intrinsic clearance ($= PS_{act} + PS_{diff,inf}$), $PS_{act}$ is active uptake intrinsic clearance on sinusoidal membrane, $PS_{diff,inf}$ is influx intrinsic clearance by passive diffusion through sinusoidal membrane, $PS_{diff,eff}$ is efflux intrinsic clearance by passive diffusion through sinusoidal membrane, $CL_{int,met}$ is hepatic intrinsic clearance of metabolism, and $CL_{int,bile}$ is hepatic intrinsic clearance of biliary excretion. In the present study, transporter-mediated basal efflux was not considered owing to limited evidence of its importance in humans despite the previous reports in rats (Pfeifer et al., 2013). The following equations (eq. 6–11), including the preceding hybrid parameters, were also used to perform the fitting in step 1:

$$ PS_{act} = CL_{int,all} \beta^\phi(1 + R_{diff}) $$

$$ PS_{diff,inf} = R_{diff}^\phi CL_{act,all} \beta^\phi(1 + R_{diff}) $$

$$ PS_{diff,eff} = PS_{eff} = \frac{R_{diff}^\phi CL_{act,all}}{\beta^\phi(1 + R_{diff})} $$

$$ f_{bile} = 1 - \frac{1 - \beta}{\beta^\phi PS_{diff,eff}} $$

$$ CL_{act} = R_{diff}^\phi CL_{act,all} $$

$$ CL_{act,bile} = f_{bile}^\phi CL_{act,eff} $$

where $CL_{act}$ is the hepatic intrinsic clearance ($= CL_{int,bile} + CL_{int,met}$). Several parameters were fixed to literature values (Supplemental Table S1; Table 1A) (Davies and Morris, 1993; Kawai et al., 1998; Kato et al., 2003; Martin et al., 2003a; Rodgers and Rowland, 2006; Watanabe et al., 2010, 2011). $CL_{int,met}$ and $f_{0}$ (unbound fraction in the liver) were fixed to values obtained from in-house studies (Kondo et al., 2004; Nakagawa et al., 2011). $K_{act}$ and $K_{f0}$ were fixed to values obtained from in-house studies (Rodgers and Rowland, 2006; Watanabe et al., 2010, 2011). $K_{act}$ and $K_{f0}$ were fixed to values obtained from in-house studies (Rodgers and Rowland, 2006; Watanabe et al., 2010, 2011).

### Table 1

<table>
<thead>
<tr>
<th>Drug Parameters</th>
<th>Values</th>
<th>References</th>
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<tr>
<td>$K_{act}$</td>
<td>—</td>
<td>—</td>
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<tr>
<td>$K_{f0}$</td>
<td>0.0870</td>
<td>Rodgers and Rowland. (2006)</td>
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<tr>
<td>$K_{act}$</td>
<td>0.144</td>
<td>Rodgers and Rowland. (2006)</td>
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<tr>
<td>$K_{f0}$</td>
<td>0.439</td>
<td>Rodgers and Rowland. (2006)</td>
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<td>$CL_{act}$</td>
<td>19.7</td>
<td>Martin et al. (2003a)</td>
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<td>$CL_{act,met}$</td>
<td>1.59</td>
<td>Martin et al. (2003a)</td>
</tr>
<tr>
<td>$b$</td>
<td>0.174</td>
<td>Watanabe et al. (2010)</td>
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<tr>
<td>$f_{0}$</td>
<td>0.179</td>
<td>Watanabe et al. (2010)</td>
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<tr>
<td>$\beta^\phi$</td>
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<td>Fixed to three different values</td>
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<tr>
<td>$\gamma^\phi$</td>
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<td>—</td>
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<tr>
<td>Dose$_{act}$</td>
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<td>Martin et al. (2003a)</td>
</tr>
<tr>
<td>Dose$_{f0}$</td>
<td>2,000</td>
<td>Martin et al. (2003a)</td>
</tr>
<tr>
<td>$F_{P,F_0}$</td>
<td>0.429</td>
<td>Martin et al. (2003a), Kato et al. (2003), Watanabe et al. (2010)</td>
</tr>
</tbody>
</table>
metabolic studies using human liver microsomes and uptake studies using suspended human hepatocytes, respectively (Yoshikado et al., 2016) (Table 1A), whereas $\gamma$ was obtained using the ratio of influx intrinsic clearance by passive diffusion of ionized form to that of unionized form, which was assumed to be the same as that in Caco-2 cells (see Supplemental Information), although such extrapolation may need to be validated by further analyses. The $\beta$ value was fixed to 0.2, 0.31, or 0.5 (0.31 was the value determined from uptake studies using sandwich-cultured human hepatocytes) (Table 1A) throughout the present analysis since this value cannot be finalized by the present study. The following equation (eq. 12) represents the relationship among $k_i$ (absorption rate constant), $k_f$ (fecal rate constant), and $F_{aFa}$ in this PBPK model (Supplemental Fig. S1):

$$1 - F_{aFa} = \left( \frac{k_i}{k_i + k_f} \right)^3$$

(12)

In the present study, the $k_i$ value was calculated from $k_i$ and $F_{aFa}$ according to eq. 12.

**Estimation of Parameters in Mixed Population (Step 1).** The analysis in the present study is schematically illustrated in Supplemental Fig. S2. In step 1, $k_i$, $k_{stomach}$ (transit rate constant from stomach to the GI tract), $R_{diff}$, $k_{ bile}$ (transit rate constant from the bile compartment to the GI tract), and CLint,all were directly estimated by simultaneous fitting to the PBPK model (Supplemental Fig. S1) of clinical data for orally and intravenously administered rosuvastatin, obtained in mixed white subjects without genotype information (Martin et al., 2003a). In this fitting, the initial value of $R_{diff}$ was set to that obtained from uptake studies with suspended human hepatocytes, and the range of $R_{diff}$ was set as within the highest and lowest values obtained in the experiments (Table 1B). The initial value of CLint,all was obtained from literature information, including the intravenous data of rosuvastatin (Martin et al., 2003a). Four parameters of the hepatic elementary process (CLint,bile, PSmet, PSdiff, and Pslint,bile) were not directly estimated by the fitting, but finally were calculated according to eqs. 6-11 using the fixed and estimated parameters after the fitting (Fig. S2). In addition, BCRP-mediated active transport in intestine (PBcrp), which represents the permeability of active transport in the intestine, was defined and calculated from $F_{aFa}$ according to the intestinal absorption model previously reported (Ito et al., 1999), as follows in eq. 13:

$$F_{aFa} = 1 - \exp \left\{ \frac{P_{diff} \times A_r}{LF/S \left( P_{diff} + P_{BCRP} \right)^2} \right\}$$

where $P_{diff}$, $A_r$ and LF/S represent permeability of passive diffusion ($2.6 \times 10^{-5}$ cm/s; Wininwarter et al., 1998; Tanaka et al., 2015), the area ratio between apical side and basolateral side (20; DeSesso and Jacobson, 2001), and hiallual flow rate divided by the basal surface area ($1.7 \times 10^{-5}$ cm/s; Tanaka et al., 2015), respectively. The $F_{aFa}$ value used for the calculation of PBcrp was shown in Table 1A. Both CLint,bile and PBcrp were defined to represent transporter-mediated permeability on canalicular and apical membranes of liver and small intestine in 421CA and 421AA, respectively, relative to that in 421CC (wild-type). Therefore, the CLint,bile and PBcrp values in 421CA can be written as $f_{cc} \times$ CLint,bile,cc and $f_{cc} \times$ PBcrp,cc, respectively, where CLint,bile,cc and PBcrp,cc represent the CLint,bile and PBcrp values in 421CC subjects, respectively. Then, the following equations can be derived from eq. 15 for each genotype:

$$F_{aFa_{cc}} = 1 - \exp \left\{ \frac{P_{diff} \times A_r}{LF/S \left( P_{diff} + f_{cc} \times \text{PBcrp,cc} \right)^2} \right\}$$

(14)

$$F_{aFa_{aa}} = 1 - \exp \left\{ \frac{P_{diff} \times A_r}{LF/S \left( P_{diff} + f_{aa} \times \text{PBcrp,aa} \right)^2} \right\}$$

(15)

Similarly, according to eqs. 1 and 2, the CLint,all and fbc values in 421CA and 421AA can be written as:

$$\text{CLint,all}_{cc} = \frac{PS_{met} \times \text{CLint,met} + f_{cc} \times \text{CLint,bile,cc}}{PS_{met} + f_{cc} \times \text{CLint,bile,cc} + \text{PS}_{eff}}$$

(16)

$$\text{CLint,all}_{aa} = \frac{PS_{met} \times \text{CLint,met} + f_{aa} \times \text{CLint,bile,aa}}{PS_{met} + f_{aa} \times \text{CLint,bile,aa} + \text{PS}_{eff}}$$

(17)

$$f_{bile_{cc}} = \frac{f_{bc} \times \text{CLint,bile,cc}}{\text{CLint,met} + f_{bc} \times \text{CLint,bile,cc}}$$

(18)

$$f_{bile_{aa}} = \frac{f_{ba} \times \text{CLint,bile,aa}}{\text{CLint,met} + f_{ba} \times \text{CLint,bile,aa}}$$

(19)

where CLint,all,cc and fbc,cc represent CLint,all and fbc values in 421CC subjects, respectively. Based on eqs. 13–19, the ratios of AUC in 421CA (AUC_{CC}) and 421AA (AUC_{AA}) to that in 421CC (AUC_{CC}) and the CLint,bile and PBcrp values in mixed white subjects (CLint,bile,mix and PBcrp,mix, respectively) can be written as follows:

$$\frac{\text{AUC}_{CC}}{\text{AUC}_{CA}} = \frac{\text{CL}_{int,all} + f_{cc} \times \text{CLint,bile,cc}}{\text{CL}_{int,all} + f_{bc} \times \text{CLint,bile,cc}}$$

(20)

$$\frac{\text{AUC}_{CC}}{\text{AUC}_{AA}} = \frac{\text{CL}_{int,all} + f_{aa} \times \text{CLint,bile,aa}}{\text{CL}_{int,all} + f_{ba} \times \text{CLint,bile,aa}}$$

(21)

$$\text{CL}_{int,bile,mix} = \text{CL}_{int,bile,cc} \times f_{freq_{cc}} + f_{bc} \times \text{CL}_{int,bile,cc} \times f_{freq_{cc}}$$

(22)

$$\text{PBcrp,mix} = \text{PBcrp,cc} \times f_{freq_{cc}} + f_{cc} \times \text{PBcrp,cc} \times f_{freq_{cc}}$$

(23)

where $N$ represents the number of liver compartments (five) in the PBPK model (Supplemental Fig. S1). $f_{freq_{cc}}$, $f_{freq_{cc}}$, and $f_{freq_{cc}}$ are the allele frequencies of 421CC, 421CA, and 421AA subjects among white subjects, respectively. These $f_{freq_{cc}}$ should be those found in the original data source in step 1, but no

### TABLE 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Initial Values</th>
<th>Range</th>
<th>Fitted Values</th>
<th>β = 0.2</th>
<th>β = 0.31</th>
<th>β = 0.5</th>
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<tr>
<td>k_i (h⁻¹)</td>
<td>0.1</td>
<td>0.01-6</td>
<td>0.125 ± 0.085</td>
<td>0.130 ± 0.089</td>
<td>0.125 ± 0.083</td>
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<tr>
<td>k_{stomach} (h⁻¹)</td>
<td>0.1</td>
<td>0.01-6</td>
<td>0.413 ± 0.297</td>
<td>0.346 ± 0.210</td>
<td>0.291 ± 0.154</td>
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<tr>
<td>R_{diff}</td>
<td>0.0192</td>
<td>0.00502-0.0408b</td>
<td>0.00502 ± 0.00246</td>
<td>0.00502 ± 0.00350</td>
<td>0.00502 ± 0.00685</td>
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<tr>
<td>k_{ bile}</td>
<td>1</td>
<td>Plus only</td>
<td>2.07 ± 2.56</td>
<td>2.17 ± 2.70</td>
<td>2.73 ± 4.47</td>
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<tr>
<td>CL_{int,all} (Liters/h)</td>
<td>550³</td>
<td>Plus only</td>
<td>680 ± 34</td>
<td>687 ± 38</td>
<td>706 ± 48</td>
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<td>WSS</td>
<td>10.8</td>
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<td>AIC</td>
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<td>93.6</td>
<td>103</td>
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parameters were set to be 1/3- and 3-fold of the initial values, respectively. The lower and upper limits of these six free parameters were set to be those estimated in step 1. The estimated parameters in this step were listed in Supplemental Table S3A, which were also used or estimated in subsequent steps. The estimated faa values under assumption 1 were 0.174 under assumption 2 and 0.208 under assumption 3, whereas the canalicular membrane parameter (CLint,bile) was increased (Table 1C). PBCRP,cc, and PBCRP,ca were fixed to PBCRP,mix, and eqs. 20–23 were simultaneously solved. All the four equations (eqs. 20–23) were simultaneously solved under assumption 3. The ratios of AUC in eqs. 20 and 21 were taken from Tomita et al., 2013). Equations 20 and 21 were obtained by solving the four hepatic intrinsic clearances (CLint,bile,cc, PSact,PSdif,eff, and PSdif,inf) were calculated at the three β values (Table 1C). As the fixed β value was increased, basolateral membrane parameters (PSact,PSdif,eff, and PSdif,inf) were decreased, whereas the canalicular membrane parameter (CLint,bile) was increased (Table 1C). PBCRP,cc, and PBCRP,ca were fixed to PBCRP,mix, and eqs. 20–23 were simultaneously solved. Under assumption 2, on the other hand, all of CLint,bile,cc, CLint,bile,ca, and CLint,bile,aa were fixed to CLint,bile,mix, and eqs. 20, 21, and 23 were solved as simultaneous equations. Under assumption 2, on the other hand, all of CLint,bile,cc, CLint,bile,ca, and CLint,bile,aa were fixed to CLint,bile,mix, and eqs. 20–23 were simultaneously solved. The estimated CLint,bile,cc values were 15.4–26.6, 16.4–29.0, and 16.0–27.6 under assumptions 1, 2, and 3, respectively, and the estimated PBCRP,cc values were 4.72–7.43 × 10−5, 4.37 × 10−5, and 4.54–4.55 × 10−5, respectively (Table 2A). The estimated faa values under assumption 1 were 0.174–0.194 (Table 2A), which are close to that in a previous report (0.23; Tanaka et al., 2015), in which it was similarly assumed that c.421C>A polymorphism only affects intestinal BCRP. Moreover, the faa values were 0.208–0.286 and 0.479–0.529 under assumptions 2 and 3, respectively (Table 2A). Thus, to match the clinically observed AUC ratio in white subjects (AUCwar/AUCw = 2.44; Supplemental Table S2), a higher degree of variability of parameters was required.

### Calculation of Parameters Associated with BCRP Activity in 421CC, 421CA, and 421AA Subjects (CLint,bile,cc, PBCRP,cc, faa, and faa) Based on the Extended Clearance Concept (Step 2)

The CLint,bile,cc, PBCRP,cc, faa, and faa values (Table 2A) were calculated based on the three different assumptions. The estimated CLint,bile,cc values were 15.4–26.6, 16.4–29.0, and 16.0–27.6 under assumptions 1, 2, and 3, respectively, and the estimated PBCRP,cc values were 4.72–7.43 × 10−5, 4.37 × 10−5, and 4.54–4.55 × 10−5, respectively (Table 2A). The estimated faa values under assumption 1 were 0.174–0.194 (Table 2A), which are close to that in a previous report (0.23; Tanaka et al., 2015), in which it was similarly assumed that c.421C>A polymorphism only affects intestinal BCRP. Moreover, the faa values were 0.208–0.286 and 0.479–0.529 under assumptions 2 and 3, respectively (Table 2A). Thus, to match the clinically observed AUC ratio in white subjects (AUCwar/AUCw = 2.44; Supplemental Table S2), a higher degree of variability of parameters was required.

### Results

**Estimation of Pharmacokinetic Parameters in Mixed Caucasian Subjects (Step 1)**

The estimated parameters in this step were listed in Table 1B. Because of the difficulty in optimization of β values, the fitting was performed with three different fixed β values (0.2, 0.31, or 0.5). As shown in Fig. 1, clinical data in mixed Caucasian subjects reported by Martin et al. (2003a) were reproduced by the fitted lines, supporting the validity of the PBPK model constructed in this step. The fitted lines obtained at any β value examined can almost explain the observed data (Fig. 1). Among the five parameters directly estimated by the fitting, the Rdif values reached the lower limit of the initial range, whereas the S.D. values of kbile were higher than the mean values (Table 1B), suggesting relatively lower reliability of these parameters. After the fitting, four hepatic intrinsic clearances (CLint,bile,cc, PSact,PSdif,eff, and PSdif,inf) were calculated at the three β values (Table 1C). As the fixed β value was increased, basolateral membrane parameters (PSact,PSdif,eff, and PSdif,inf) were decreased, whereas the canalicular membrane parameter (CLint,bile) was increased (Table 1C). PBCRP,cc, and PBCRP,ca were fixed to PBCRP,mix, and eqs. 20–23 were simultaneously solved. Under assumption 2, on the other hand, all of CLint,bile,cc, CLint,bile,ca, and CLint,bile,aa were fixed to CLint,bile,mix, and eqs. 20, 21, and 23 were solved as simultaneous equations. Under assumption 2, on the other hand, all of CLint,bile,cc, CLint,bile,ca, and CLint,bile,aa were fixed to CLint,bile,mix, and eqs. 20–23 were simultaneously solved. All the four equations (eqs. 20–23) were simultaneously solved under assumption 3. The ratios of AUC in eqs. 20 and 21 were taken from previously reported clinical data (Keskitalo et al., 2009b; Supplemental Table S2), whereas both CLint,bile,mix and PBCRP,mix in eqs. 22 and 23 were fixed to those obtained in step 1 as CLint,bile and PBCRP, respectively. The Fg values in each genotype were also calculated under these three assumptions by using eqs. 13–15.

**Simultaneous PBPK Model Fitting of Blood Concentration-Time Profiles in 421CC, 421CA, and 421AA Subjects (CLint,bile,cc, PBCRP,cc, faa, and faa)**

Based on the Extended Clearance Concept (Step 2). The CLint,bile,cc, PBCRP,cc, faa, and faa values (Table 2A) were calculated based on the three different assumptions. The estimated CLint,bile,cc values were 15.4–26.6, 16.4–29.0, and 16.0–27.6 under assumptions 1, 2, and 3, respectively, and the estimated PBCRP,cc values were 4.72–7.43 × 10−5, 4.37 × 10−5, and 4.54–4.55 × 10−5, respectively (Table 2A). The estimated faa values under assumption 1 were 0.174–0.194 (Table 2A), which are close to that in a previous report (0.23; Tanaka et al., 2015), in which it was similarly assumed that c.421C>A polymorphism only affects intestinal BCRP. Moreover, the faa values were 0.208–0.286 and 0.479–0.529 under assumptions 2 and 3, respectively (Table 2A).

## Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Fitted values</th>
</tr>
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<tbody>
<tr>
<td>CLint,bile,cc (L/min)</td>
<td>15.4 ± 7.6&lt;sup&gt;a&lt;/sup&gt;, 18.3 ± 12.8&lt;sup&gt;a&lt;/sup&gt;, 26.6 ± 36.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CLint,bile,ca (L/min)</td>
<td>3383 ± 1658&lt;sup&gt;a&lt;/sup&gt;, 2205 ± 1538&lt;sup&gt;a&lt;/sup&gt;, 1405 ± 1918&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>CLint,faa (L/min)</td>
<td>67.9 ± 33.5&lt;sup&gt;a&lt;/sup&gt;, 44.3 ± 31.0&lt;sup&gt;b&lt;/sup&gt;, 28.2 ± 38.5&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>CLint,full (L/min)</td>
<td>17.0 ± 8.4&lt;sup&gt;a&lt;/sup&gt;, 11.1 ± 7.7&lt;sup&gt;b&lt;/sup&gt;, 7.05 ± 9.64&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>a</sup>In-house data obtained in metabolic study using human liver microsomes.

<sup>b</sup>In-house data obtained in uptake study using sandwich-cultured human hepatocytes.

### Materials and Methods

**Determination of γ** was shown in Supplemental Information.

Values are shown as the mean ± standard deviation.

Determined based on the clearance concept using reported intravenous data (Martin et al., 2003a).

<sup>a</sup>WSS was calculated using eq. 13.

<sup>b</sup>ABC was calculated using eq. 14.

All these parameters were mathematically calculated using parameters shown in Table 1A and 1B.

<sup>a</sup>Standard deviation values were calculated by applying the propagation of error assuming independent variables.
Rosuvastatin is one of the best available clinical substrates for hepatic and/or intestinal BCRP (Lee et al., 2015). In the present study, the $f_{aa}$ and $f_{ca}$ values were defined to represent possible change in BCRP activity due to ABCG2 gene polymorphism. The $f_{aa}$ values estimated based on extended clearance concepts using the AUC ratio for each polymorphism (step 2) were 0.17–0.19, 0.21–0.29, and 0.48–0.53 on the assumptions that the polymorphism affects only intestinal BCRP (assumption 1), only hepatic BCRP (assumption 2), and both intestinal and hepatic BCRP (assumption 3), respectively (Table 2A). On the other hand, the BCRP activities estimated based on the fitting approach using extended clearance concepts using the AUC ratio for each polymorphism (step 2) were 0.42–0.43 in white subjects (Keskitalo et al., 2009b; Supplementary Table S2), respectively. In the present study, the $f_{ca}$ and $f_{aa}$ values were thus obtained between the extended clearance concept-based approach and the fitting approach. This result showed that no
parameter set could reproduce both the AUC and the concentration-time profile of rosuvastatin in the 421CC, 421CA, and 421AA subjects at the same time under assumption 1. The WSS and AIC values in assumptions 1 and 3 were largest and lowest, respectively, regardless of the used β (Table 3), which further suggests that assumption 1 showed less validity, whereas assumption 3 is more appropriate than the other assumptions.

Tanaka et al. (2015) calculated BCRP activity of the 421AA subject under assumption 1. The effect of BCRP activity on biliary excretion was thought to be little than that on intestinal absorption because of clinical observed unchanged \( t_{1/2} \) by c.421C>A polymorphism (Zhang et al., 2006; Keskitalo et al., 2009b; Wan et al., 2015). In the present study, however, the \( t_{1/2} \) in 421AA was greater than that in 421CC under assumption 1 but almost the same as that in 421CC under assumptions 2 and 3 (Table 4), whereas clinically observed \( t_{1/2} \) in 421AA was almost the same as that in 421CC (Supplemental Table S2). Thus, similar \( t_{1/2} \) values between 421CC and 421AA cannot be explained by the assumption 1 but can be explained by assumptions 2 and 3, in which c.421C>A polymorphism is assumed to affect BCRP activity in the liver, probably because of extensive enterohepatic circulation of rosuvastatin: The decrease in hepatic elimination resulting from c.421C>A polymorphism leads to an increase in GI absorption, resulting in compensation for the impact on systemic elimination.

The protein expression level of BCRP c.421C>A variant in transfected cell lines was reported to be 24%–47% that of wild-type BCRP (Kondo et al., 2004; Tamura et al., 2006; Matsuo et al., 2009). It was also reported that BCRP protein expression in human placenta of homozygous subjects is about half that in the case of wild-type BCRP (Kobayashi et al., 2005). In the present study, if we assume that the transport of rosuvastatin in apical membranes of small intestine and liver of humans is primarily mediated by BCRP and is directly affected by the BCRP expression level, the \( f_{aa} \) value should represent the fraction of BCRP expression level in homozygous subjects relative to that in wild-type, and we can conclude that assumption 3, which proposed the \( f_{aa} \) value of 0.48–0.54 (Table 2), gives the best agreement with those reported values for the decrease in BCRP expression level; however, some of previous reports indicated a minimal effect of the polymorphism on the BCRP expression level (Mizuarai et al., 2004; Zamber et al., 2003; Urquhart et al., 2008), and the validity of assumption 3 cannot be fully supported by such previous reports alone. In addition, only the mean values of plasma concentration data without tissue or biliary ones were used for the present analysis, resulting in the limitation of the parameter estimates. The PBPK model (Supplemental Fig. S1) relies on the large number of assumptions and sole use of plasma data, which may not be informative enough but provide limitation in the evaluation of the
reduced BCRP activity. Population PBPK approach, as suggested by Tsamandouras et al. (2015), may provide another better estimation. Confirmation of the modeling using either pharmacodynamic or positron emission tomography data may also be necessary to obtain a final conclusion.

The present findings quantitatively support the importance of BCRP in the absorption and the biliary excretion of rosuvastatin; however, rosuvastatin is also a substrate of other ABC transporters, such as multidrug resistance-associated protein (MRP) 2 and P-glycoprotein (Li et al., 2013; Zhou et al., 2013), both of which are considered to contribute to the biliary excretion of various drugs. Nevertheless, in the present study, it was assumed that BCRP is the only contributor to the CL_int,bile, and this assumption may overestimate the role of BCRP. The $f_{ua}$ values obtained in step 3 was not close to zero under any assumptions (Table 2B), and this result may be explained by the contribution of transporters other than BCRP. Thus, more information about the roles of these transporters in humans is needed to explain more accurately the clinically observed data.

Previous information about the effect of c.421C>T polymorphism on rosuvastatin pharmacokinetics is comprehensively summarized in Supplemental Table S2. It is noteworthy that the influence of c.421C>T polymorphism in homozygous subjects is much more marked than would be expected from the change in heterozygous subjects: The AUC of rosuvastatin in heterozygous subjects was only 1.2 times greater than that in wild-type subjects, whereas that in homozygous subjects was 2.4 times higher (Keskitalo et al., 2009b; Supplemental Table S2). A similar tendency is observed in all the reports listed in Supplemental Table S2, except only the report by Zhou et al. (2013).

In addition, similar phenomena have also been reported for atorvastatin (Keskitalo et al., 2009b; Birmingham et al., 2015b), fluvastatin (Keskitalo et al., 2009a), and sulfasalazine (Yamasaki et al., 2008) owing to c.421C>T polymorphism. In the present study, BCRP activity in heterozygous and homozygous subjects was individually estimated by assessing $f_{ca}$ and $f_{ua}$ values, respectively, in steps 2 and 3, and a similar tendency was reproduced under all the assumptions: The $f_{ua}$ values were relatively close to unity, whereas the $f_{ca}$ values were much lower than the unity (Table 2). Although the reason for the apparent inconsistency between heterozygous and homozygous subjects is unclear, a possible explanation would be the difference of the stability and activity in the different combinations of BCRP dimerization. Three combinations of BCRP dimer can be considered for the subjects with the heterozygous polymorphism of c.421C>T: the homodimer of wild-type, the homodimer of variant, and the heterodimer of wild-type and variant. If the stability and activity of the heterodimer are similar to those of the homodimer of wild-type, the BCRP activity in the heterozygous subject might also be similar to that in wild-type subject. Further study at the molecular level of wild-type and mutated gene products of BCRP is required for the estimation of the BCRP activity in heterozygous subjects.

Much lower levels of BCRP expression and transport activity have been reported for other polymorphisms, such as c.376C>T (Matsuo et al., 2009), and for multi heterozygous (c.376C>T and c.421C>A, and c.34G>A and c.421C>A) subjects (Kobayashi et al., 2005; Gotanda et al., 2015; Wan et al., 2015). The allele frequency of c.376C>T polymorphism is only 0.028 in Japanese subjects (Maekawa et al., 2006), and limited information is available for other racial groups, Table 3

### Table 3

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Values</th>
<th>β = 0.2</th>
<th>β = 0.31</th>
<th>β = 0.5</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.421C&gt;A polymorphism affects only intestinal BCRP (assumption 1)</td>
<td>AUC (ng*h/ml)</td>
<td>2.21</td>
<td>2.16</td>
<td>2.10</td>
</tr>
<tr>
<td>WSS</td>
<td></td>
<td>42.5</td>
<td>41.7</td>
<td>40.7</td>
</tr>
<tr>
<td>AIC</td>
<td></td>
<td>2.07</td>
<td>1.91</td>
<td>1.72</td>
</tr>
<tr>
<td>c.421C&gt;A polymorphism affects only hepatic BCRP (assumption 2)</td>
<td>AUC (ng*h/ml)</td>
<td>40.4</td>
<td>37.2</td>
<td>33.5</td>
</tr>
<tr>
<td>WSS</td>
<td></td>
<td>1.71</td>
<td>1.64</td>
<td>1.54</td>
</tr>
<tr>
<td>AIC</td>
<td></td>
<td>33.4</td>
<td>31.9</td>
<td>29.6</td>
</tr>
</tbody>
</table>

Table 4

### Table 4

AUC and $t_{1/2}$ values obtained by the moment analysis of the simulated blood concentration-time profiles

<table>
<thead>
<tr>
<th>c.421C&gt;A Genotype</th>
<th>$\beta = 0.2$</th>
<th>$\beta = 0.31$</th>
<th>$\beta = 0.5$</th>
<th>Reported Values</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AUC (ng*h/ml)</td>
<td>$t_{1/2}$ (h)</td>
<td>AUC (ng*h/ml)</td>
<td>$t_{1/2}$ (h)</td>
</tr>
<tr>
<td>CC</td>
<td>42.6</td>
<td>1</td>
<td>10.0</td>
<td>2.16</td>
</tr>
<tr>
<td>CA</td>
<td>54.9</td>
<td>1</td>
<td>11.0</td>
<td>55.0</td>
</tr>
<tr>
<td>AA</td>
<td>96.7</td>
<td>1</td>
<td>14.2</td>
<td>96.5</td>
</tr>
<tr>
<td>CC</td>
<td>41.1</td>
<td>1</td>
<td>11.8</td>
<td>40.7</td>
</tr>
<tr>
<td>CA</td>
<td>53.2</td>
<td>1</td>
<td>11.3</td>
<td>53.3</td>
</tr>
<tr>
<td>AA</td>
<td>109</td>
<td>1</td>
<td>11.8</td>
<td>109</td>
</tr>
<tr>
<td>CC</td>
<td>40.8</td>
<td>1</td>
<td>10.9</td>
<td>41.0</td>
</tr>
<tr>
<td>CA</td>
<td>54.1</td>
<td>1</td>
<td>11.3</td>
<td>54.3</td>
</tr>
<tr>
<td>AA</td>
<td>110</td>
<td>1</td>
<td>12.5</td>
<td>110</td>
</tr>
</tbody>
</table>

In addition, similar phenomena have also been reported for c.34G>A polymorphism (Keskitalo et al., 2009b; Birmingham et al., 2015b), fluvastatin (Keskitalo et al., 2009a), and sulfasalazine (Yamasaki et al., 2008) owing to c.421C>T polymorphism. In the present study, BCRP activity in heterozygous and homozygous subjects was individually estimated by assessing $f_{ca}$ and $f_{ua}$ values, respectively, in steps 2 and 3, and a similar tendency was reproduced under all the assumptions: The $f_{ua}$ values were relatively close to unity, whereas the $f_{ca}$ values were much lower than the unity (Table 2). Although the reason for the apparent inconsistency between heterozygous and homozygous subjects is unclear, a possible explanation would be the difference of the stability and activity in the different combinations of BCRP dimerization. Three combinations of BCRP dimer can be considered for the subjects with the heterozygous polymorphism of c.421C>T: the homodimer of wild-type, the homodimer of variant, and the heterodimer of wild-type and variant. If the stability and activity of the heterodimer are similar to those of the homodimer of wild-type, the BCRP activity in the heterozygous subject might also be similar to that in wild-type subject. Further study at the molecular level of wild-type and mutated gene products of BCRP is required for the estimation of the BCRP activity in heterozygous subjects.

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whereas such variants might lead to significant side effects or reduced drug efficacy. The PBPK model analysis and simulation used here should be improved to predict the potential risks in patients with such rare polymorphisms of BCRP.

The strategy in the present study for quantitative estimation of the influence of c.421C>A polymorphism on BCRP activity requires pharmacokinetic profiles in a mixed population after oral and intravenous administration (step 1) and after oral administration in each genotype and allele frequency (steps 2 and 3). Tomita et al. (2013) have proposed that the ethnic difference in allele frequency of polymorphisms in OATP1B1 and ABCG2 genes cannot fully explain differences in pharmacokinetics of rosuvastatin between white and Asian populations, suggesting the existence of unknown factors besides the allele frequency responsible for ethnic difference in OATP1B1 activity. Since similar unknown factors also may be present in BCRP activity, the present studies used the pharmacokinetic information only in white subjects. From this point of view, it can be reasonably speculated that the present evaluation of alteration in BCRP activity (Table 2) could be valid only in white populations and that all the literature information should be obtained from Asian populations if we attempt to evaluate BCRP activity in Asian populations. On the other hand, Wu et al. (2017) have recently found no ethnic difference in pharmacokinetics of rosuvastatin after oral administration once a day. All the subjects are wild-type for both genes (OATP1B1 and BCRP). Based on their proposal, the findings obtained in the present study may also be applicable to Asian populations if we assume no ethnic difference in the overall pharmacokinetics of rosuvastatin other than pharmacogenetics. Further studies are needed to clarify the relevance to ethnic difference of the present evaluation of the BCRP activity in each ABCG2 genotype.

Authorship Contributions
Participated in research design: Tomishimo, Sugiyama.
Performed data analyses: Futatsugi, Tomishimo.
Wrote or contributed to the writing of the manuscript: Futatsugi, Tomishimo, Yoshikado, Sugiyama, Kato.

References


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