Pharmacokinetic impact of *SLCO1A2* polymorphisms on imatinib disposition in patients with chronic myeloid leukemia

Y Yamakawa[1], A Hamada[1,2], T Shuto[3], M Yuki[1], T Uchida[1], H Kai[3], T Kawaguchi[4] and H Saito[1,2]

[1]Department of Clinical Pharmaceutical Sciences, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

[2]Department of Pharmacy, Kumamoto University Hospital, Kumamoto, Japan

[3]Department of Molecular Medicine, Graduate School of Pharmaceutical Sciences, Kumamoto University, Kumamoto, Japan.

[4]Department of Hematology and Infectious Diseases, Kumamoto University Hospital, Kumamoto, Japan.

Correspondence: Hideyuki Saito

Address: Department of Pharmacy, Kumamoto University Hospital, 1-1-1 Honjo, Kumamoto, 860-8556, Japan

Fax number: +81 96 373 5820

Telephone number: + 81 96 373 5820

E-mail address: saitohide@fc.kuh.kumamoto-u.ac.jp

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ABSTRACT

The purpose of this study was to explore the role of the organic-anion transporting polypeptide (OATP) 1A2 that is encoded by *SLCO1A2*, in the cellular uptake of the BCR-ABL tyrosine kinase inhibitor imatinib, and the relationship between *SLCO1A2* polymorphisms and the pharmacokinetics of imatinib in chronic myeloid leukemia (CML) patients. Imatinib uptake was significantly enhanced in OATP1A2-transfected human embryonic kidney (HEK) 293 cells (*P*=0.002). Naringin, an OATP1A2 inhibitor, decreased the transport of imatinib in OATP1A2-transfected HEK293, the human intestinal cell line Caco-2, and the CML cell line K562 cells. Linkage disequilibrium was found between the *SLCO1A2* -1105G>A and -1032G>A genotypes in 34 CML patients and 100 healthy subjects. Imatinib clearance of CML patients was influenced by the *SLCO1A2* -1105G>A/-1032G>A genotype (*P*=0.075) and the *SLCO1A2* -361GG genotype (*P*=0.005). These findings suggest that imatinib is transported into cells by OATP1A2, and that *SLCO1A2* polymorphisms significantly affect imatinib pharmacokinetics.

INTRODUCTION

Organic-anion transporting polypeptides (OATPs; gene name *SLCO*) belong to the superfamily of solute carriers that are expressed in a variety of organs and are important for drug disposition. OATPs mediate the cellular uptake of a wide range of endogenous substrates and drugs.[1] Because variations in OATP sequence appear to affect the pharmacokinetics of drugs, these transporters have been suggested to be partially involved in inter-individual variability of efficacy or in the adverse events of some drugs. Therefore, the association of numerous genetic polymorphisms in the *SLCO* genes with drug disposition has been investigated.[2, 3]

OATP1A2, encoded by *SLCO1A2*, is a member of the OATP superfamily. OATP1A2 has also been shown to transport a wide range of endogenous metabolites and xenobiotics including bile acids and steroid hormones.[4, 5] Since OATP1A2 is primarily expressed in epithelial cells of the intestine, liver and kidney,[6, 7] it may play a pharmacokinetic role in drug disposition.

It has been reported that *SLCO1A2* polymorphisms including 404A>T (rs45502302), 559G>A and 833delA (rs11568555) lead to decreased uptake of substrates *in vitro*.[7, 8] However, little is known about whether *SLCO1A2*

polymorphisms are associated with pharmacokinetics and/or pharmacodynamics of therapeutic drugs in patients.[5] Recently, the association of *SLCO1A2* polymorphisms (rs10841795, 38T>C; rs11568563, 516T>G) with plasma concentrations of lopinavir was evaluated.[9] However, no associations were observed. Further clinical studies are required to evaluate the impact of *SLCO1A2* single nucleotide polymorphisms (SNPs) on the pharmacokinetics of several drugs.

Imatinib mesylate has been approved as a molecular target drug that selectively inhibits Bcr-Abl tyrosine kinase, which causes Philadelphia-positive chronic myeloid leukemia (CML) as well as KIT tyrosine kinase, which causes KIT-positive gastrointestinal stromal tumors (GIST).[10, 11] Considerable inter-individual differences in imatinib pharmacokinetics have been observed.[12] It is therefore important to identify covariates which could provide a predictive marker for imatinib exposure in order to maintain a trough plasma level at an effective concentration (approximately 2μM).[13, 14] The organic cation transporters OCT1 and OCTN2, as well as the organic anion transporting polypeptides OATP1B3 and OATP1A2, have been suggested to mediate the uptake of imatinib into cells overexpressing each transporter.[15-17] OATP1A2

appears to transport a broad spectrum of substrates, and imatinib is recognized as one of the typical substrates of OATP1A2.[5, 16] In the present study, we evaluated the role of OATP1A2 in the transepithelial transport of imatinib in the human intestinal cell line Caco-2 and in the accumulation of imatinib in the CML cell line K562, and examined the relationship between *SLCO1A2* polymorphisms and the pharmacokinetics of imatinib in CML patients.

RESULTS

Uptake of imatinib into HEK293 cells transfected with hOATP1A2

OATP1A2 protein expression was confirmed in hOATP1A2-transfected human embryonic kidney (HEK) 293 cells by Western blotting (Appendix 1(a, b)). Imatinib uptake was significantly enhanced in HEK293 cells expressing OATP1A2 compared with control cells (P=0.002; Fig. 1). Control pCMV6XL5-transfected HEK293 cells appeared relatively high accumulation of imatinib. It has been assumed that high accumulation of imatinib in the control cells was caused at least in part by non-specific permeation or simple diffusion, but not by OATP1A2. The accumulation of imatinib was not enhanced after incubation with the HEK293 cells expressing OATP1A2 in the presence of naringin (Fig. 1).

Transport of imatinib across Caco-2 cell monolayers

Transepithelial transport of imatinib in the apical-to-basal or the basal-to-apical direction was time-dependent for up to 180 min (Fig. 2(a)). Transport of imatinib across Caco-2 cells in the apical-to-basal direction also increased in a substrate concentration-dependent manner (Fig. 2(b)). The estimated *Km* and *Vmax* for

imatinib transport by Caco-2 cells were 3,556 μM and 44.1 nmol/cm^2/30 min, respectively. We also evaluated the effects of naringin on the transepithelial transport of imatinib in the apical-to-basal direction. When imatinib was incubated in the presence of 10 mM naringin, imatinib transport was decreased by 57% (Fig. 2(c)).

Uptake of imatinib into K562 cells

The intracellular accumulation of imatinib in K562 cells was linear for the first 3 min (Fig. 3(a)). Accumulation of imatinib in K562 cells increased in a concentration-dependent manner (Fig. 3(b)). The estimated *Km* and *Vmax* for imatinib uptake by K562 cells were 57 μM and 79.1 nmol/3 min/mg protein, respectively. Subsequently, the uptake of imatinib was evaluated in the presence of naringin. When 50 μM imatinib was incubated in the presence of 2 mM naringin, imatinib uptake was decreased by 43% (Fig. 3(c)).

Analysis of *SLCO1A2* polymorphisms

Three SNPs in the coding region of *SLCO1A2* (404A>T, 559G>A, and 833delA) and five SNPs in the promoter region of *SLCO1A2* (-1105G>A, -1032G>A,

-715T>C, -361G>A, and -189_-188insA) were analyzed in 34 CML patients (Table 1). No patients with heterozygous or homozygous variants of 404A>T, 559G>A, 833delA or -715T>C were observed. Five SNPs in the promoter region of *SLCO1A2* (-1105G>A, -1032G>A, -715T>C, -361G>A, and -189_-188insA) were analyzed in 100 healthy subjects (Table 1). Subjects with heterozygous or homozygous variants of -715T>C were not observed. The allele frequencies of these SNPs in healthy subjects were approximately consistent with those in 34 CML patients. Linkage disequilibrium was found between -1105G>A and -1032G>A genotypes among CML patients and healthy subjects. We found that the genotype -1105G>A was completely consistent with the genotype -1032G>A in 34 CML patients and 100 healthy subjects. Furthermore, all CML patients and healthy subjects with -1105AA/-1032AA genotypes had the -189_-188insA genotype. Neither patients nor healthy volunteers with heterozygous variants of -189_-188 insA were observed, and the genotypes in this population were not in Hardy-Weinberg Equilibrium (P<0.01; the $\chi 2$ test to compare the observed and expected genotype frequencies). The reason for this non-equilibrium is unclear. Further studies with larger sample sizes are required to confirm the allele frequencies.

Impact of *SLCO1A2* polymorphisms on imatinib clearance

The association of *SLCO1A2* polymorphisms with imatinib clearance is depicted in Fig. 4. Patients with the *SLCO1A2* -1105GG/-1032GG genotype (*n*=18) tended to have higher imatinib clearance than those with the *SLCO1A2* -1105GA/-1032GA (*n*=12) or the -1105AA/-1032GA/-189_-188insA genotype (*n*=4) (median ± SD, 9.4 ± 2.6 vs. 7.0 ± 3.3 L/hr; *P*=0.075). Patients with the *SLCO1A2* -361GG genotype (*n*=21) had significantly lower imatinib clearance than those with the GA (*n*=12) or the AA (*n*=1) genotype (7.2 ± 2.8 vs. 10.0 ± 2.5 L/hr; *P*=0.005), suggesting that imatinib clearance in patients carrying the *SLCO1A2* -361GG genotype was 28% lower than that of patients carrying the GA or the AA genotype. Imatinib clearance of patient with *SLCO1A2* -361AA was 7.5 L/hr, i.e., the lowest clearance among patients with the -361GA (*n*=12) or AA (*n*=1) genotype.

DISCUSSION

The present study suggests that OATP1A2 is involved in imatinib transport by intestinal and CML cells and that pharmacokinetics of imatinib are significantly influenced by *SLCO1A2* polymorphisms. These data suggest that OATP1A2 is important for imatinib pharmacokinetics. To our knowledge, this is the first report indicating the association of *SLCO1A2* polymorphisms with pharmacokinetics of therapeutic drugs in patients.

Hu *et al*. reported OATP1A2-mediated transport of imatinib in *Xenopus leavis* oocytes overexpressing OATP1A2,[16] which is consistent with the present study (Fig. 1). Naringin, which is found in grapefruit juice,[18, 19] inhibited the accumulation of imatinib in HEK293 cells expressing OATP1A2. Consequently, we attempted to clarify the role of OATP1A2 in the transepithelial transport of imatinib in intestinal cells using naringin as a typical OATP1A2 inhibitor. We found that naringin decreased the transport of imatinib across Caco-2 cells in the apical-to-basal direction (Fig. 2(c)), indicating that OATP1A2 may facilitate intestinal absorption of imatinib, at least in part. The relative mRNA expression of OATP1A2 in the intestine suggested to be extremely lower than that of other OATPs,[20] whereas this observation doesn't imply that OATP1A2 possesses

lower transport activity because the expression of OATP1A2 mRNA may not directly reflect the amount of OATP1A2 protein or its functional activity. For example, it was suggested that OATP1A2 but no other OATPs was responsible for fexofenadine absorption.[21] In this study, imatinib absorption is also suggested to be facilitated by OATP1A2. Area under the concentration-time curve (AUC) but not clearance is suitable parameter if *SLCO1A2* polymorphisms affect intestinal absorption of imatinib. In this study, sampling time after dose was predominant at 24hr. Further clinical studies with appropriate data for the estimation of time-dependent change in the concentration of imatinib are required to evaluate the impact of *SLCO1A2* polymorphisms on imatinib AUC.

OATP1A2 has been reported to be expressed in the intestine, liver, kidney, lung, testis and brain.[6, 22] The expression of OATP1A2 in various organs, especially in the intestine, liver and kidney, organs which are known to be important determinants of pharmacokinetics, may imply a role for OATP1A2 in drug distribution and/or in the disposition of several substrate drugs including imatinib. In human liver, OATP1A2 is localized to cholangiocytes[7] and to the basolateral (sinusoidal) membrane of hepatocytes.[6] In the human kidney, OATP1A2 is localized to the apical domain of distal nephrons.[7] It is possible that

OATP1A2 mediates hepatic uptake of imatinib from the blood and/or reabsorption of imatinib in the distal tubules of the nephrons.

Little is known about the role of OATP1A2 in CML cells. The expression of OATP1A2 in K562 cells was confirmed by Western blotting (Appendix 1(a, b)). We evaluated uptake of imatinib into K562 cells. An inhibition study indicated that naringin decreased imatinib uptake (Fig. 3(c)). These results indicate that OATP1A2 may be involved in imatinib influx transport in target tumor cells. The organic cation transporter 1 (OCT1) has been reported to be the major influx transporter for imatinib in CML cells,[15, 23] and the expression of OCT1 correlated to the clinical response to imatinib.[24] Therefore, the present study raises the possibility that OATP1A2 may be one of the determinants of the clinical response to imatinib.

For *SLCO1A2* genotyping assays, we focused on the 404A>T, 559G>A, and 833delA polymorphisms which have been reported to lead to a decreased uptake of substrates *in vitro*.[7, 8] However, patients with heterozygous or homozygous variants of *SLCO1A2* 404A>T, 559G>A, and 833A>del were not observed in the present study. Since some SNPs in the promoter region of *SLCO1A2* have already been described,[25] we analyzed these SNPs in the

promoter region. Although no patients with heterozygous or homozygous variants of -715T>C were observed, SNPs, including -1105G>A, -1032G>A, -361G>A, and -189_-188insA, were identified in CML patients. Interestingly, linkage disequilibrium was found between -1105G>A and -1032G>A among CML patients, and this linkage was then confirmed in 100 healthy subjects. Furthermore, all CML patients and healthy subjects with -1105AA/-1032AA genotypes had the -189_-188insA genotype.

When we analyzed the association of polymorphisms in these SNPs with imatinib pharmacokinetics, we found that patients with the *SLCO1A2* -1105GG/-1032GG genotype tended to have higher imatinib clearance than those with the *SLCO1A2* -1105GA/-1032GA or -1105AA/-1032AA/-189_-188insA genotype (Fig. 4(a)) but no significant different was observed (P=0.075). Next, we evaluated the association of *SLCO1A2* -1105G>A/-1032G>A/-189_-188insA genotypes with luciferase activity (Appendix 2). A luciferase reporter gene assay in the human hepatoma cell line HepG2 transfected with the *SLCO1A2* promoter fragment indicated that the luciferase activity of the -1105GG/-1032GG-containing (Wild Type) promoter was not significantly different from that of the -1105AA/-1032AA/-189_-188insA-containing (Mutant)

promoter. These SNPs may have no effect on the gene expression of *SLCO1A2*.

Conversely, significant differences in imatinib clearance between patients with the *SLCO1A2* -361GG, GA or AA genotypes were observed (Fig. 4(b)). Because OATP1A2 may facilitate intestinal absorption of imatinib, *SLCO1A2* polymorphism is assumed to affect imatinib absorption, thereby causing changes in the imatinib pharmacokinetics. Further investigation is needed to reveal the mechanisms of the effect of *SLCO1A2* polymorphism on the imatinib pharmacokinetics.

We had carefully monitored clinical response of CML patients as described previously.[26] Thirty-three of 34 patients achieved a favorable response; major molecular response in 15 patients, complete cytogenetic response in 15 patients, and complete hematologic response in 3 patients. Only one patient appeared the resistance to imatinib therapy. Because beneficial efficacy was confirmed in most patients, no correlation was found between the clinical response and *SLCO1A2* polymorphisms.

OATP1A2 has been shown to transport many drugs including fexofenadine,[27-29] methotrexate,[8] lopinavir,[9] and pravastatin.[30] It is important to determine the impact of genetic variability at the *SLCO1A2* -361G>A region on

the pharmacokinetics, therapeutic effects or side effects of OATP1A2 substrate drugs. It is of interest to evaluate whether OATP1A2 is responsible for transport of nilotinib and dasatinib, as the plasma membrane transporter mediating these tyrosine kinase inhibitors remains unclear. We have recently evaluated cellular uptake of nilotinib in HEK293 cells expressing OATP1A2, resulting in no significant accumulation compared with control cells (data not shown). Further in vitro study is required to reveal the proposed transporters and their substrate affinities for imatinib, nilotinib and dasatinib.

To date, significant association of genetic polymorphisms in *ABCB1*,[31-33] *ABCG2*[34] and *SLCO1B3*[33] with imatinib pharmacokinetics has been reported. Each contribution of genetic factors including *ABCB1*, *ABCG2*, *SLCO1B3* and *SLCO1A2* to imatinib clearance remains unclear. Since the individual variability of imatinib pharmacokinetics may be affected by multiple SNPs of transporters, further studies based on genotypic characterization including that of *SLCO1A2* are required to construct therapeutic guidelines for imatinib treatment of CML. In addition, the relative mRNA expression of OATP2B1 in the intestine is suggested to be higher compared with OATP1A2.[20] Further investigation is required to reveal whether OATP2B1 and its genetic polymorphisms are involved in imatinib

disposition.

In conclusion, OATP1A2 appears to be involved in imatinib transport in intestinal cells and in target CML cells, and *SLCO1A2* polymorphisms do correlate with imatinib pharmacokinetics. Since these data suggest that OATP1A2 is an important factor for imatinib pharmacokinetics, further clinical studies are required to confirm the correlation between polymorphisms in the *SLCO1A2* gene including that of -361G>A, and the therapeutic effects or side effects of imatinib in patients.

METHODS

Cellular uptake study in HEK293 cells

To confirm the uptake of imatinib into cells overexpressing OATP1A2, the pCMV6XL5/OATP1A2 vector (Origene Technologies, Rockville, MD) was used to transfect HEK293 cells. HEK293 cells were cultured in DMEM (Sigma, St. Louis, MO) supplemented with 10% fetal bovine serum (FBS) at 37 °C under 5% CO_2. After 24-hr cultivation in Poly-D-Lysine-Coated 6-well plates (Iwaki, Tokyo, Japan), 4 μg pCMV6XL5/OATP1A2 or control pCMV6XL5 vector was transfected by adding 10 μL/well of Lipofectamine 2000 (Invitrogen, Carlsbad, CA). At 48 hr after transfection, cells were washed twice in phosphate-buffered saline (PBS) at 37 °C. Cells were incubated in 1 mL of incubation medium (145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose and 5 mM *N*-(2-hydroxyethyl)piperazine-*N*'-2-ethanesulfonic acid (HEPES) (pH 7.4)) containing 2 μM imatinib (Selleck chemicals, Houston, TX) with or without 1 mM naringin (Sigma), an OATP1A2 inhibitor.[19] After an incubation time of 1 min at 37 °C, cells were washed once in 5 mL ice-cold PBS containing 1% bovine serum albumin (BSA) and twice in 5 mL BSA-free ice-cold PBS. The cells were then lysed by incubation in 500 μL of 50% methanol/high performance liquid

chromatography (HPLC) mobile phase (4:6:0.1 mixture of acetonitrile, water, and phosphoric acid) for 24 hr. The resultant solution was centrifuged at 12,000 x *g* and 4 °C for 10 min. The supernatant was used for quantification of imatinib using HPLC.[35] The pellet was mixed with 1N NaOH to solubilize the cells. The protein content of the solubilized cells was determined using a BioRad Protein Assay kit (Bio-Rad, Hercules, CA).

Inhibitory effects of naringin on imatinib transport in Caco-2 cell monolayers.

Transcellular transport of imatinib by Caco-2 cells was determined using cell monolayers in Transwell chambers (Corning, Lowell, MA) in order to evaluate whether OATP1A2 facilitates intestinal absorption. Caco-2 cells were seeded on chambers at a cell density of 3 x 10^5 cells/well. The medium consisted of DMEM supplemented with 10% FBS and 1% nonessential amino acids (Invitrogen). The cells were grown in an atmosphere of 5% CO_2 at 37 °C. Cell confluency was confirmed using the Millicell-ERS (Millipore, Billerica, MA). After washing cells twice with 37 °C PBS, cells were pre-incubated in incubation medium (145 mM NaCl, 3 mM KCl, 1 mM $CaCl_2$, 0.5 mM $MgCl_2$, 5 mM D-glucose and 5 mM

HEPES (pH 7.4 on the basal side of the monolayers) or MES (pH 6.5 at the apical side of the monolayers)) at 37 °C for 30 min. To examine the linearity of imatinib transport, incubation medium containing 4 mM imatinib was added to either the apical or the basal side, and 200 μL aliquots were taken over 10-180 min from the relevant side for quantification of transported imatinib. For evaluation of imatinib transport from the apical to the basal direction, incubation medium containing imatinib (0.1-5 mM) was added to the apical side, and 200 μL aliquots at the basal side were taken 30 min later. The *Km* and *Vmax* for imatinib transport were estimated according to the Eadie-Hofstee equation:

$$V = -Km \cdot V / [S] + Vmax$$

where *V* is imatinib that is transported from the apical to the basal direction; *Km* is the Michaelis-Menten constant; [*S*] is the substrate concentration; *Vmax* is the maximum imatinib transport. For the inhibition study, incubation medium containing 3.5 mM imatinib with or without naringin (1-10 mM) was added to the apical side and 200 μL aliquots at the basal side were taken 30 min later for imatinib quantification.

Inhibitory effects of naringin on imatinib uptake by K562 cells.

K562 cells were cultured in RPMI 1640 medium (Nissui, Tokyo, Japan) supplemented with 10% FBS at 37 °C under 5% CO_2. Cells were washed with 37 °C incubation medium (pH 7.4), and 2.5 x 10^6 cells were pre-incubated in incubation medium (pH 7.4) at 37 °C for 30 min. To examine the linearity of imatinib uptake, incubation medium containing 2 μM imatinib was added. After incubation for 0.5-20 min, cells were washed once in 3 mL ice-cold PBS containing 1% BSA and twice in 3 mL BSA-free ice-cold PBS. Cells were then lysed by incubation in 300 μL of 50% methanol/HPLC mobile phase for 24 hr. The resultant solution was centrifuged at 12,000 x *g* and 4 °C for 10 min. Imatinib and protein content in the supernatant of K562 were quantified using the same methods used for the HEK293 cell experiments. For evaluation of imatinib uptake, incubation medium containing imatinib (10-100 μM) was added, and the cells were incubated for 3 min at 37 °C. The *Km* and *Vmax* for imatinib uptake were estimated according to the Eadie-Hofstee equation. For the inhibition study, incubation medium containing 50 μM imatinib with or without naringin (50-2,000 μM) was added, and cells were incubated for 3 min at 37 °C.

Pharmacokinetic analysis of imatinib clearance in CML patients.

34 Japanese patients with CML who were receiving imatinib were analyzed. The detailed clinical background of the patients, the analytical methods used and the clinical effects have been described in a previous report.[26] The study was approved by the Ethics Committee of Kumamoto University. All patients provided informed consent prior to participation in this study. The population pharmacokinetic (PPK) analysis was performed as described previously.[33] The concentration-time profile and parameter estimates of the PPK model were described in the previous report. In brief, PPK parameters were obtained using the NONMEM program (Version 6 2.0, GloboMax, ICON Development Solutions, Ellicott City, MD). The first order conditional estimation method (FOCE) was used throughout the model building procedure. PPK analysis was performed using 622 imatinib concentrations with the one-compartment with first-order absorption and elimination model, because imatinib pharmacokinetics was best characterized by this model. We collected blood samples at steady-state (on day 30 of imatinib treatment or later), and sampling time after dose was predominant at 24hr. The detail of sampling time was 4 samples at 0-1hr, 55 samples at 1-8hr, 97 samples at 8-16hr, and 466 samples at 16-30.5h. Population means of clearance, volume of distribution, and absorption rate constant were 8.7 L/hr,

430 L and 2.06, respectively. It was suggested that variability in the clearance of imatinib was about 73 %.[36] In this study, approximately 4.6-fold variability in individual clearance was observed (range, 3.4-15.5 L/hr). Because the individual variability in imatinib pharmacokinetics often leads to an insufficient clinical outcome among patients with CML,[13] it should be important to identify covariates predicting variability in imatinib clearance. Therefore, we used clearance as an index of pharmacokinetic profiles of imatinib.

Genotyping analysis.

DNA was obtained from whole blood of 34 CML patients using the MagNA Pure LC DNA Isolation Kit I TISSUE (Roche Diagnostics, Mannheim, Germany). DNA sets of 100 Japanese healthy subjects were provided by the Health Science Research Resources Bank (Tokyo, Japan). Subsequently, PCR reactions were performed as described previously.[33] PCR amplifications were performed using the primers listed in Table 2. After cleanup using Microcon-100 (Millipore), direct nucleotide sequencing was performed.

Statistical analyses.

Statistical analyses of transport experiments were adjusted for multiple comparisons using Dunnet's method. The relationship between *SLCO1A2* genotypes and their individual clearance of imatinib was analyzed using the Mann-Whitney U test. Two-tailed *P* values < 0.05 were considered statistically significant. Statistical analyses were performed using the R program v.2.7.1 (http://cran.r-project.org).

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CONFLICT OF INTEREST

The authors declared no conflict of interest.

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FIGURE LEGENDS

Figure 1

Uptake of imatinib (2 μM) into HEK293 cells transfected with OATP1A2 and the inhibitory effect of naringin. Transfected cells were treated with or without 1 mM of the OATP1A2 inhibitor naringin for 1 min.

*, $P < 0.01$ versus control pCMV6XL5 vector. Data are expressed as mean values ± SD (*n*=3).

Figure 2

Transport of imatinib across Caco-2 cell monolayers and the inhibitory effect of naringin. (a) Transport of imatinib from the apical to the basal (○), and the basal to the apical (□), side of the cell over time. Imatinib (4 mM) was added to either the apical or basal side. Samples aliquots (200 μL) were taken over 10-180 min from the relevant side. (b) Eadie-Hofstee plot analysis of imatinib transport. Imatinib (0.1-5 mM) was added to the apical side. Samples aliquots (200 μL) at the basal side were taken 30 min later. (c) Inhibitory effect of naringin on imatinib transport. Imatinib (3.5 mM) with or without the OATP1A2 inhibitor naringin (1-10 mM) was added to the apical side. Samples aliquots (200 μL) at the basal side

were taken 30 min later. Data are expressed as mean values ± SD (*n*=3).

Figure 3

Uptake of imatinib into K562 cells and the inhibitory effect of naringin. (a) Uptake of imatinib (2 μM) over time. Samples were taken over 0.5-20 min. (b) Eadie-Hofstee plot analysis of imatinib uptake. Imatinib (10-100 μM) was added, and the cells were incubated for 3 min. (c) Inhibitory effect of naringin on imatinib uptake. Imatinib (50 μM) with or without the OATP1A2 inhibitor naringin (50-2,000 μM) was added, and the cells were incubated for 3 min. Data are expressed as mean values ± SD (*n*=3).

Figure 4

Association of *SLCO1A2* (a) -1105G>A/-1032G>A and (b) -361G>A polymorphisms with imatinib clearance. Imatinib clearance in patients was estimated using population pharmacokinetic (PPK) analysis. The line inside each box indicates the median; the bottom edge is the first quartile and the top edge is the third quartile; the error bars represent minimal and maximal values.

Appendix 1

Western blot analysis of OATP1A2 in transfected HEK293, Caco-2 and K562 cells. Western blot analysis of OATP1A2 in pCMV6XL5 vector-transfected HEK293 (lane 1), hOATP1A2-transfected HEK293 (lane 2), Caco-2 (lane 4), and K562 (lane 5) cells.

(a) OATP1A2 protein in total lysates. (b) The expression of OATP1A2 in a crude membrane fraction. (c) The expression of β-actin in total lysates, used as a loading control.

Appendix 2

Association of *SLCO1A2* -1105G>A/-1032G>A/-189_188insA genotypes with luciferase activity. The luciferase vector alone (pGL3 basic) was used as a negative control. A luciferase reporter gene assay in HepG2 cells transfected with the -1137 to +15 *SLCO1A2* promoter fragment revealed no significant difference in luciferase activity between the promoter containing -1105GG/-1032GG (Wild Type) and the promoter containing -1105AA/-1032AA/-189_188insA (Mutant). Data are expressed as mean values ± SD (*n*=3). Statistical significance was calculated using the Mann-Whitney test.

Table 1. Analysis of polymorphisms in the promoter region of *SLCO1A2*

Position	Base pair change	dbSNP ID	CML patients (*n*=34)			Healthy volunteers (*n*=100)		
			WT	Het	Var	WT	Het	Var
-1105	G>A	rs4148977	18	12	4	47	43	10
-1032	G>A	rs4148978	18	12	4	47	43	10
-715	T>C	rs4148979	34	0	0	100	0	0
-361	G>A	rs3764043	21	12	1	75	22	3
-189_-188	insA	rs3834939	30	0	4	90	0	10

WT, homozygous reference sequence; Het, heterozygous variant; Var, homozygous variant

Table 2. Primer sequences for PCR reactions

Position	Forward	Reverse	Product size (bp)
404A>T	5’-CATCCACAGGCAGATTATTGAA-3’	5’-CGGCCCTTTGACTCATTTT-3’	600
559G>A	5’-ACTAGGGGTGCCCTGAGAAG-3’	5’-ACCTCCAGGGGCACTAGACT-3’	549
833delA	5’-TAGTTGGTTGGGACCCGATA-3’	5’-AGTACCATAGGAAGAATCGGACT-3’	486
-1105G>A, -1032G>A and -715T>C	5’-GGCACGTACTTGGCTTTCTT-3’	5’-TGGAAAATAATCCCACGATTG-3’	594
-361G>A and -189_-188insA	5’-GATTTTTGAGCCTTGGTAGGG-3’	5’-GCTCTTCAGGGTGTTCCAAG-3’	482

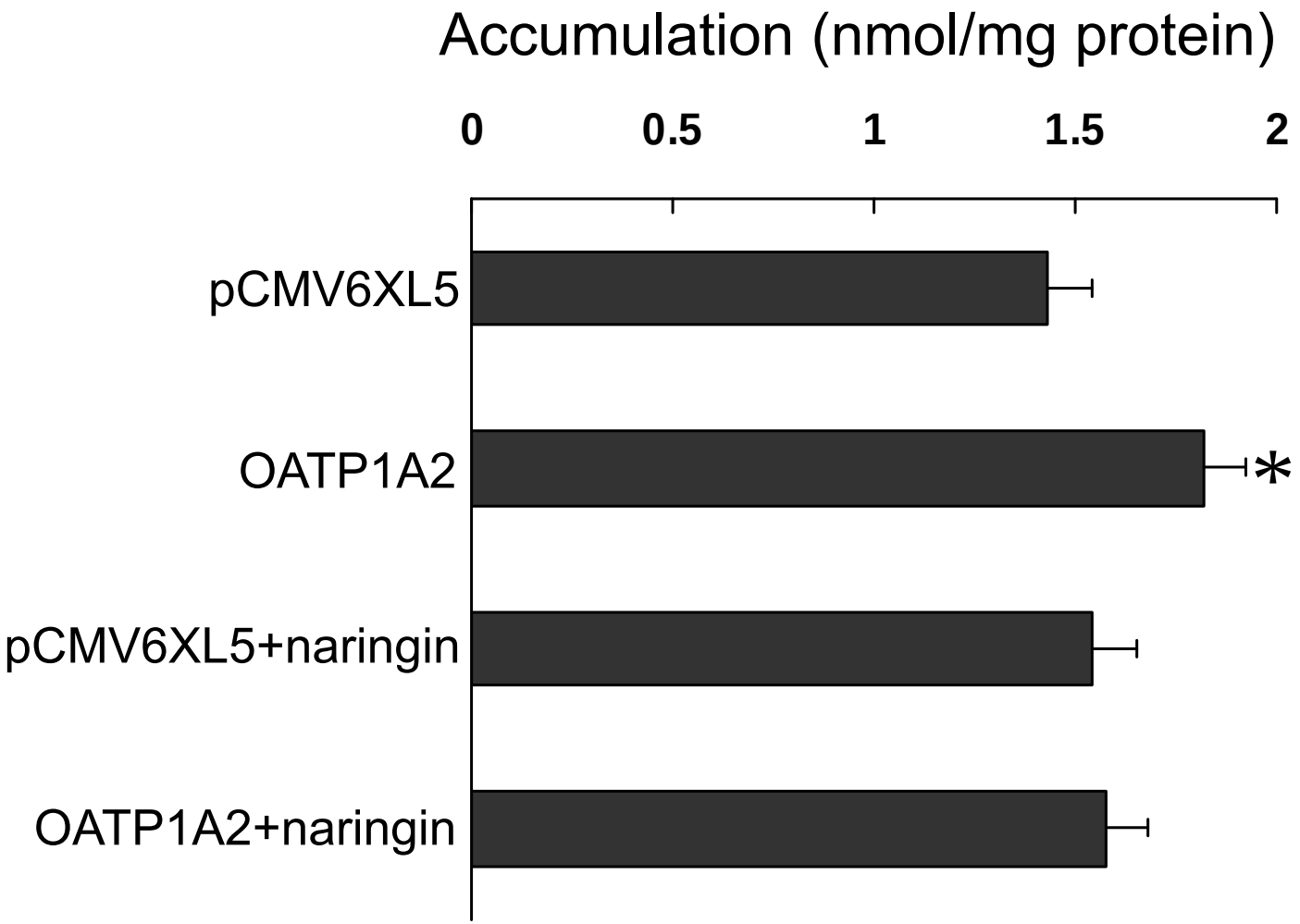

Accumulation (nmol/mg protein)
0
0.5
1
1.5
2
pCMV6XL5
OATP1A2
*
pCMV6XL5+naringin
OATP1A2+naringin

Figure 1

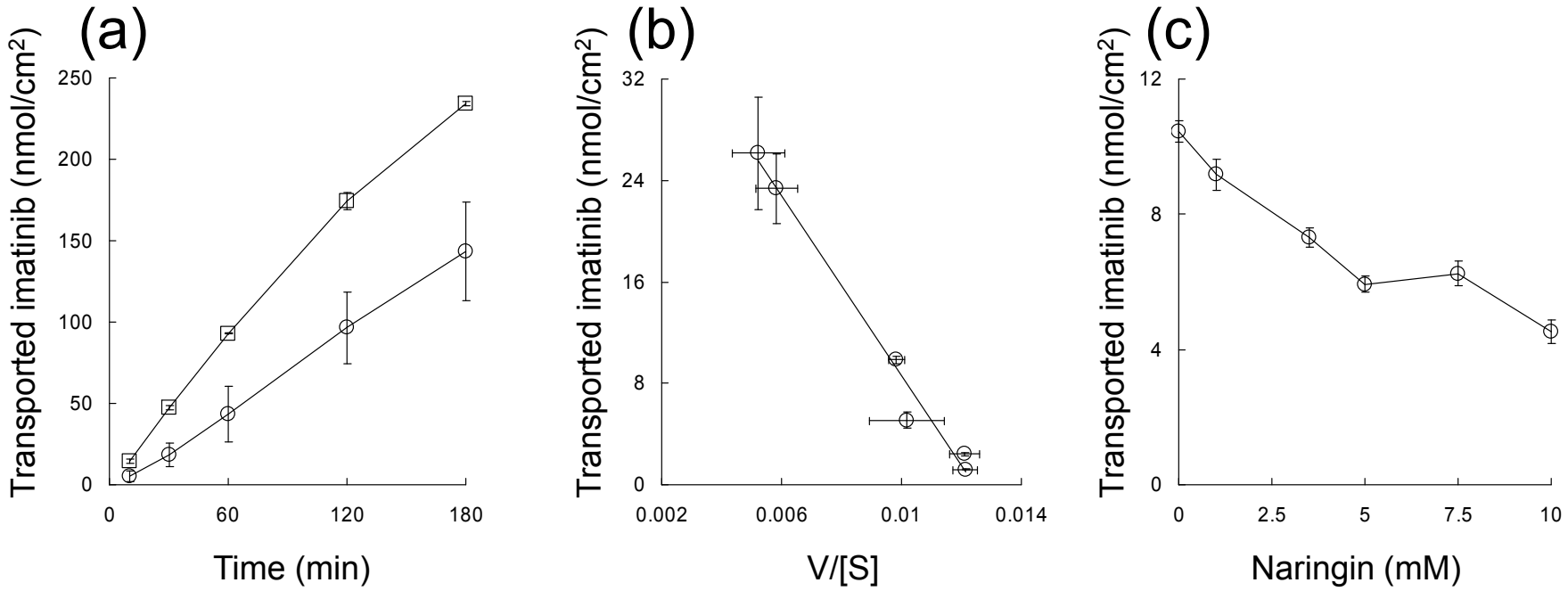

(a)
Transported imatinib (nmol/cm2)
250
200
150
100
50
0
0
60
120
180
Time (min)
(b)
Transported imatinib (nmol/cm2)
32
24
16
8
0
0.002
0.006
0.01
0.014
V/[S]
(c)
Transported imatinib (nmol/cm2)
12
8
4
0
0
2.5
5
7.5
10
Naringin (mM)

Figure 2

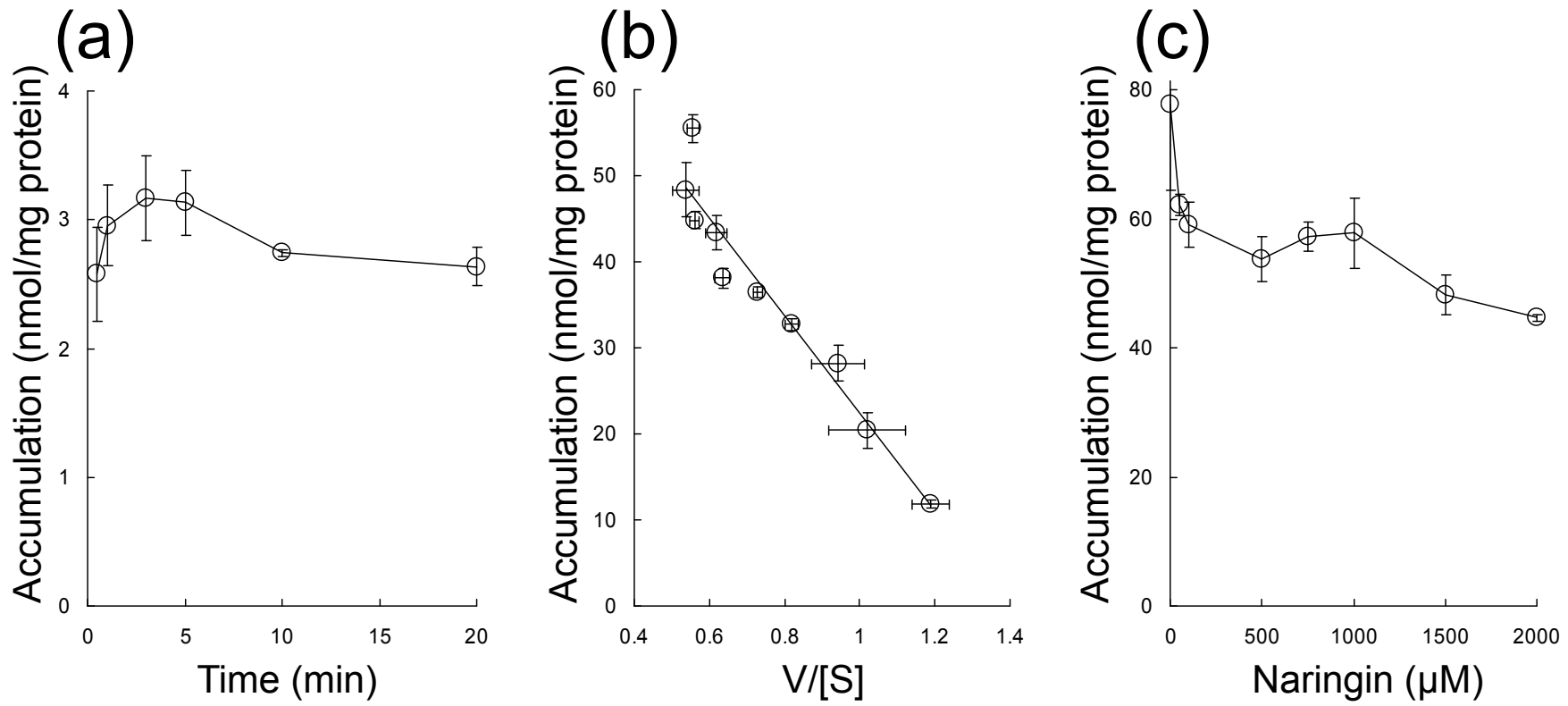

(a)
Accumulation (nmol/mg protein)
0
1
2
3
4
0
5
10
15
20
Time (min)
(b)
Accumulation (nmol/mg protein)
0
10
20
30
40
50
60
0.4
0.6
0.8
1
1.2
1.4
V/[S]
(c)
Accumulation (nmol/mg protein)
0
20
40
60
80
0
500
1000
1500
2000
Naringin (μM)

Figure 3

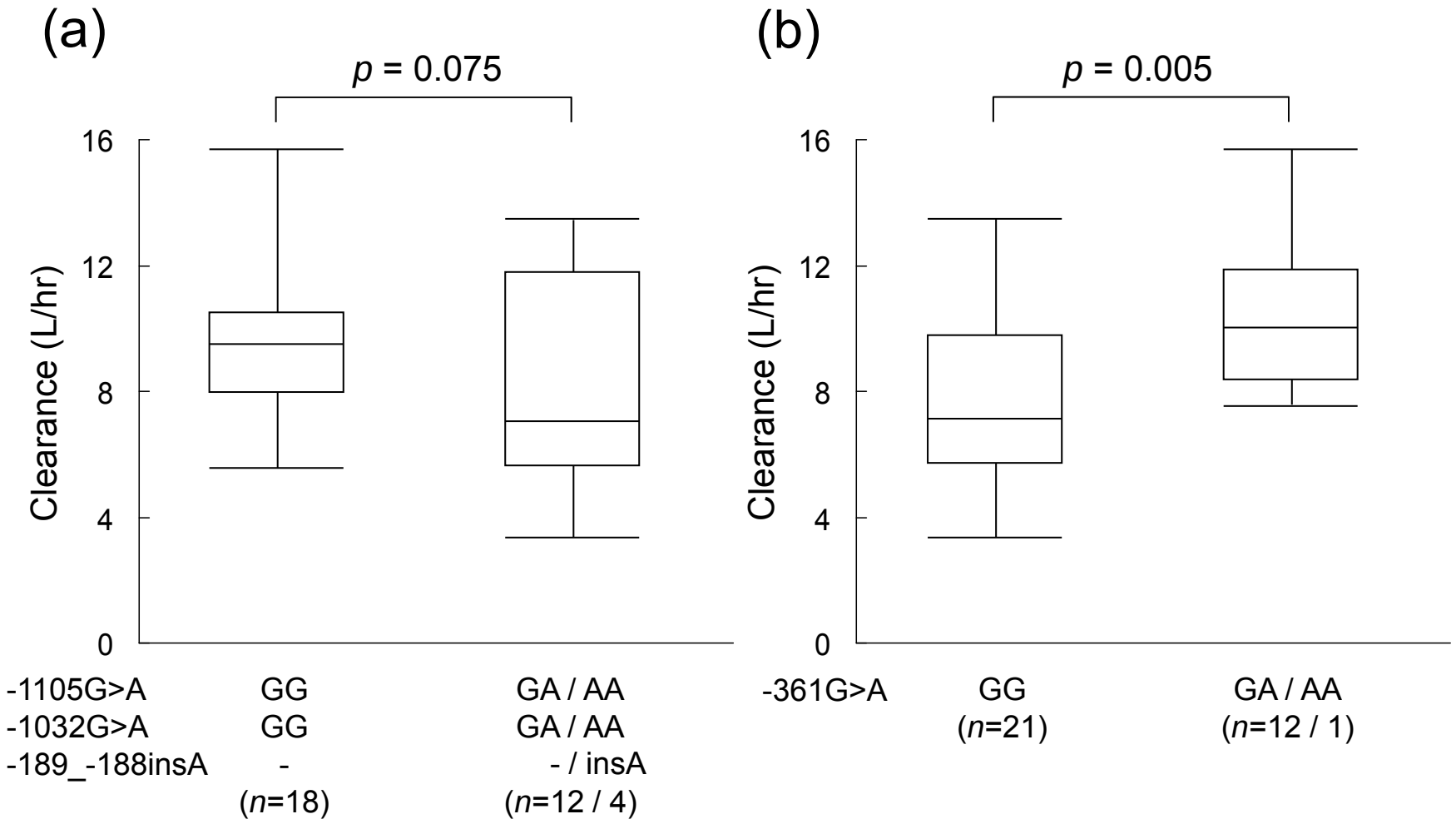
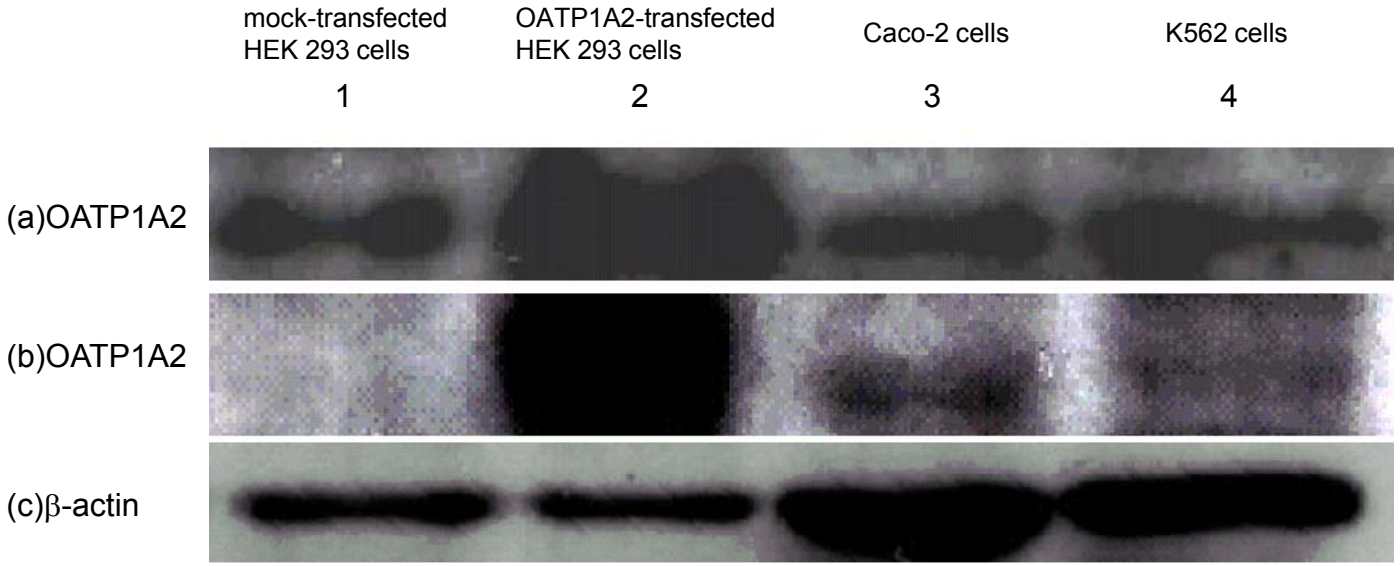
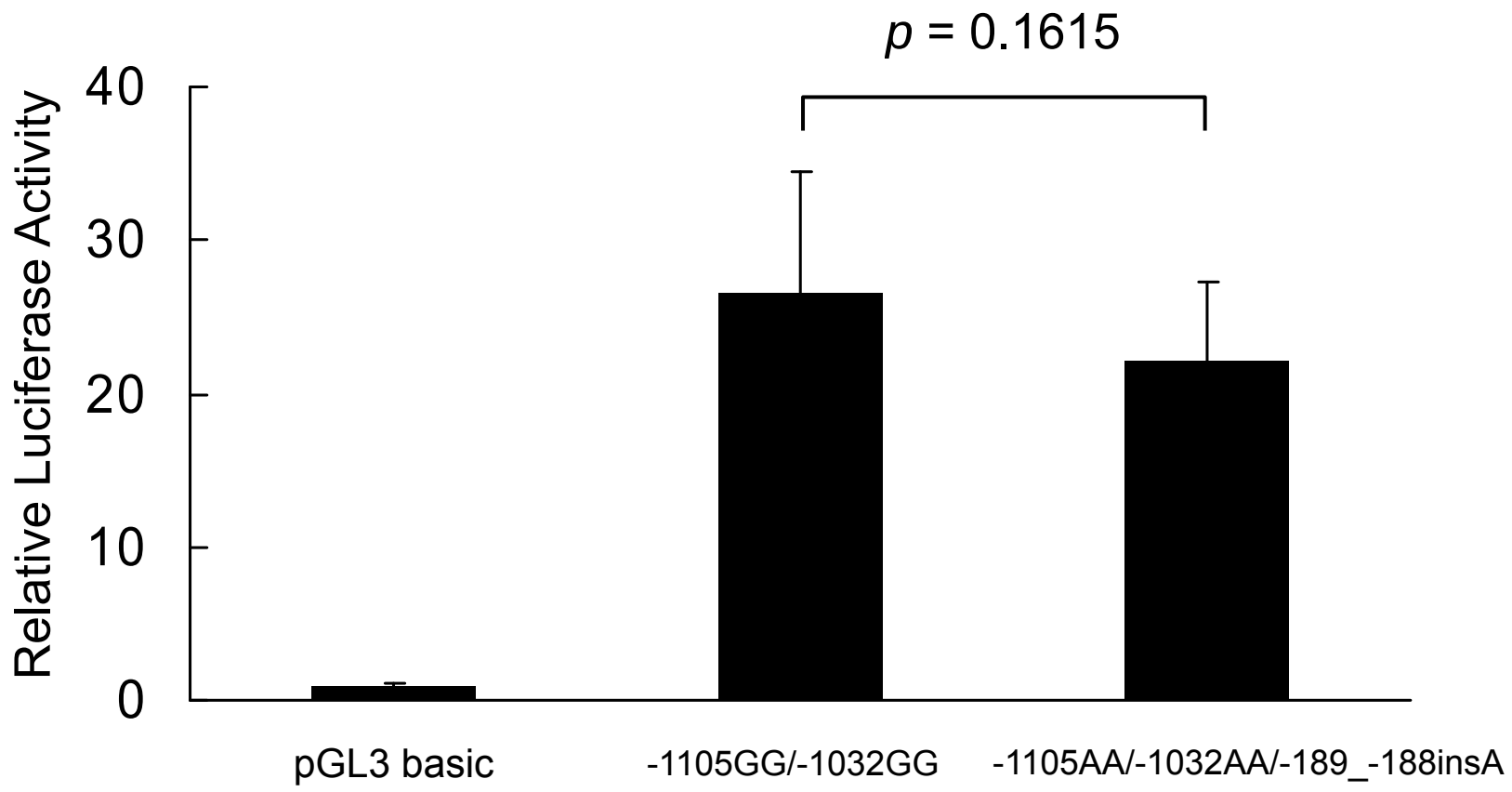

(a)
p = 0.075
Clearance (L/hr)
16
12
8
4
0
-1105G>A
-1032G>A
-189_-188insA
GG
GG
-
(n=18)
GA / AA
GA / AA
- / insA
(n=12 / 4)
(b)
p = 0.005
Clearance (L/hr)
16
12
8
4
0
-361G>A
GG
(n=21)
GA / AA
(n=12 / 1)

Figure 4


mock-transfected
HEK 293 cells
OATP1A2-transfected
HEK 293 cells
Caco-2 cells
K562 cells
1
2
3
4
(a)OATP1A2
(b)OATP1A2
(c)β-actin

Appendix 1


p = 0.1615
Relative Luciferase Activity
40
30
20
10
0
pGL3 basic
-1105GG/-1032GG
-1105AA/-1032AA/-189_-188insA

Appendix 2