Published in final edited form as:
Pharmacogenet Genomics. 2010 February ; 20(2): 112–120. doi:10.1097/FPC.0b013e328335b02d.

HIV protease inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3 and lopinavir plasma concentrations are influenced by *SLCO1B1* polymorphisms

Ruben C Hartkoorn[*,1], **Wai San Kwan**[*,1], **Victoria Shallcross**[1], **Ammara Chaikan**[1], **Neill Liptrott**[2], **Deirdre Egan**[2], **J Enrique Salcedo Sora**[3], **Chloe E James**[1], **Sara Gibbons**[1], **Pat G Bray**[3], **David J Back**[1], **Saye H Khoo**[1], and **Andrew Owen**[1]

[1]Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool, UK

[2]NIHR Biomedical Research Centre, Royal Liverpool & Broadgreen University Hospitals Trust, UK

[3]Molecular and Biochemical Parasitology, Liverpool School of Tropical Medicine, Liverpool, UK

Abstract

OATP1B1 and OATP1B3 are major hepatic drug transporters whilst OATP1A2 is mainly located in the brain but is also located in liver and several other organs. These transporters affect the distribution and clearance of many endo- and xenobiotics and have been reported to have functional SNPs. We have assessed the substrate specificites of these transporters for a panel of antiretrovirals and investigated the effects of SNPs within these transporters on the pharmacokinetics of lopinavir. *SLCO1A2, SLCO1B1* and *SLCO1B3* were cloned, verified and used to generate cRNA for use in the Xenopus *laevis* oocyte transport system. Using the oocyte system, antiretrovirals were tested for their substrate specificities. Plasma samples (n=349) from the Liverpool therapeutic drug monitoring registry were genotyped for SNPs in *SLCO1A2*, *SLCO1B1* and *SLCO1B3* and associations between SNPs and lopinavir plasma concentrations were analysed. Antiretroviral protease inhibitors, but not non-nucleoside reverse transcriptase inhibitors are substrates for OATP1A2, OATP1B1 and OATP1B3. Furthermore, ritonavir was not an inhibitor of OATP1B1. The 521T>C polymorphism in *SLCO1B1* was significantly associated with higher lopinavir plasma concentrations. No associations were observed with functional variants of *SLCO1A2* and *SLCO1B3*. These data add to our understanding of the factors that contribute to variability in plasma concentrations of protease inhibitors. Further studies are now required to confirm the association of *SLCO1B1* 521T>C with lopinavir plasma concentrations and to assess the influence of other polymorphisms in the *SLCO* family.

Keywords

Pharmacogenetics; pharmacokinetics; drug disposition; OATP

Author for correspondence and reprints: Dr A Owen, Department of Pharmacology and Therapeutics, University of Liverpool, 70 Pembroke Place, Liverpool, L69 3GF, U.K, **Tel No** +44 (0) 151 794 5919, **Fax No** + 44 (0) 151 794 5656 aowen@liv.ac.uk.
*should be considered as joint authors

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Introduction

There is marked inter-individual variability in plasma concentrations of anti-HIV drugs [1, 2]. This is particularly true for HIV protease inhibitors and non-nucleoside reverse transcriptase inhibitors, for which therapeutic drug monitoring should be considered for optimizing therapy in some clinical situations. Achieving target plasma protease inhibitor and non-nucleoside reverse transcriptase inhibitor concentrations is vital for HIV therapy as sub-optimal concentrations leads to the emergence of drug resistance [3–5], and high concentrations may lead to toxicity [4, 6]. To date, sex [7, 8], age, liver function [9] and drug interactions have all been identified to affect plasma drug concentrations. However, even after taking these covariates into consideration variability is still observed.

Protease inhibitors are principally metabolised by CYP3A enzymes but are also substrates for the efflux transporters ABCB1, ABCC1 and ABCC2. Since these proteins affect the absorption, distribution and clearance of protease inhibitors as well as permeation of cellular sanctuary sites for HIV, they have attracted attention as candidate genes in pharmacogenetic studies [10]. Many of the genes encoding these proteins contain functional polymorphisms. However, genotype-phenotype studies in HIV+ patients receiving protease inhibitors have to date failed to show conclusive associations. One factor that may partially explain this is that ritonavir is a potent inhibitor of ABCB1, CYP3A4 and CYP3A5 [11, 12]. While this is the basis of its ‘boosting’ effects, this may also explain why these polymorphisms do not influence protease inhibitor pharmacokinetics in patients [13]. We have recently observed an association between a SNP in the pregnane-X-receptor (PXR) and unboosted atazanavir plasma concentrations [14]. Since ritonavir is also a ligand for PXR this association may be more relevant for unboosted than for boosted atazanavir.

Organic anion transporting polypeptides (OATP), coded for by the *SLCO* genes, are a family of solute carrier membrane transport proteins which influx numerous endogenous and xenobiotic compounds. Many of the OATPs (of which there are currently 13 members) have wide substrate specificity and are expressed ubiquitously [15–19]. The main OATPs associated with hepatic drug uptake are OATP1B1 (OATP-C) [19, 20] and OATP1B3 (OATP-8) whilst OATP1A2 (OATP-A) has also been located in the liver [19, 21, 22]. Interestingly, it has been demonstrated using human embryonic kidney (HEK 293) cells stably transfected with *SLCO1B1*, that the protease inhibitors indinavir and saquinavir inhibit bilirubin uptake through OATP1B1 [23]. Also, saquinavir is a substrate for OATP1A2 in the X. *laevis* oocyte expression system [24].

Numerous genetic polymorphisms have been described in the *SLCO1B1* gene [25–27] and to a lesser extent in the *SLCO1A2* and *SLCO1B3* genes [28, 29]. Furthermore, single nucleotide polymorphisms (SNPs) located in the transmembrane domains of OATP1B1 are associated with a decrease in transport function, both *in vitro* and *in vivo*. One of these SNPs, 521T>C (rs4149056) has a high allelic frequency in American and European Caucasians, but is rare in sub-Saharan Africa and Oceania [26]. In clinical studies, individuals homozygous for the C allele at position 521 have higher peak concentrations and an increased area under the concentration-time curve (AUC) for some statins [25, 30–36]. A haplotype constructed from the 521T>C and another polymorphism, 388A>G (rs2306283)

has also been shown to be of functional relevance for OATP1B1 substrates [37, 38]. Recently, another polymorphism in *SLCO1B1* (rs4149032), which is located in intron 2 of the *SLCO1B1* gene was shown to be functionally important [39]. A trend for an association of this SNP with statin induced myopathy was also previously reported in a genome wide association study (OR = 2.3; $P = 3 \times 10^{-5}$) [40]**.** For *SLCO1A2*, the 334T>G SNP (rs4149117) is in complete linkage disequilibrium with 699G>A and appears to be functional in at least some studies [41, 42]. Six non-synonomous SNPs have been reported in *SLCO1A2* but only two of these (rs10841795, 38T>C; rs11568563, 516T>G) have been detected in Europeans [28].

In this study, the tissue distribution of SLCO1A2, SLCO1B1 and SLCO1B3 mRNA was first confirmed. X. *laevis* oocyte expression systems were then utilised to determine whether protease inhibitors (saquinavir, lopinavir and darunavir) or non-nucleoside reverse transcriptase inhibitors (nevirapine and efavirenz) are substrates for these transporters. Since ritonavir inhibition may obviate any influence of *SLCO1B1* polymorphisms on plasma protease inhibitor concentrations, this system was also utilised to assess inhibition of OATP1B1 by ritonavir. Finally, the influence of previously reported functional polymorphisms in these transporters was evaluated using lopinavir as a paradigm.

Methods

Materials

Total human mRNA was purchased from Stratagene. TaqMan Reverse Transcription Reagents and TaqMan Gene Expression Assays were purchased from Applied Biosystems Inc. (Warrington, UK). Absolute QPCR Mix was purchased from ThermoFisherScientific (Loughborough, UK), TOPO TA cloning kit was purchased from Invitrogen Ltd (Paisley, UK), and pBluescriptII-KSM vector was kindly provided by Dr. William Joiner (Yale University). DH5-α competent *E. coli* cells, T4 DNA ligase, Antarctic phosphatase and all restriction enzymes were purchased from New England Biolabs (Hitchen, UK). T3 mMessage mMachine Transcription kits were purchased from Ambion Ltd. (Warrington, UK). Adult female *X. laevis* frogs were purchased from Xenopus Express (France). [^{3}H] – estrone-3-sulphate (specific activity, 50 Ci/mmol) was purchased from American Radiolabeled Company Inc. (Missouri, USA), [^{3}H] – saquinavir (specific activity, 1Ci/mmol), [^{3}H] – lopinavir (specific activity, 1Ci/mmol), and [^{3}H] – nevirapine (specific activity, 1.6Ci/mmol) was purchased from Moravek Chemicals (California, USA). [C^{14}] – darunavir (specific activity, 7.6 Bq/mmol) and [^{14}C] – efavirenz (specific activity, 12.7μCi/μmol) was kindly provided by Tibotec and Bristol-Myers-Squibb respectively. All other chemicals were purchased from Sigma-Aldrich (Poole, UK).

SLCO1A2, SLCO1B1 and SLCO1B3 mRNA tissue distribution

Total human RNA samples for various tissues were purchased from Ambion (UK). Total human mRNA (2ug) was reverse transcribed using the TaqMan Reverse Transcription kit according to manufacturer's protocols. Real-time PCR using Absolute QPCR Mix and TaqMan assays (SLCO1A2, Hs00366488_m1; SLCO1B1, Hs00272374_m1; SLCO1B3, Hs00251986_m1) were combined with cDNA (40ng) and used to quantify mRNA

expression by standard methodology. Comparative C(t) calculation was used to assess relative gene expression utilising GAPDH as the housekeeping gene.

SLCO1A2, *SLCO1B1* and *SLCO1B3* cloning

SLCO1A2, *SLCO1B1* and *SLCO1B3* were cloned from cDNA obtained from A549 and Huh-7D12cells. *SLCO1A2* was amplified from A549 cDNA in two fragments using Expand High Fidelity PCR reagents. Fragment 1 (the 5' fragment) underwent two rounds of PCR (nested PCR), initially to amplify the fragment (forward primer; 5'- atgggagaaactgagaaaa-3', reverse primer; 5'- taacgaatgcattgaactgt-3') and secondly to add a Kozak consensus sequence (CCACC), and *BglII* restriction site prior to the start codon (forward primer; 5'- agatctccaccatgggagaaactgagaaaa-3', reverse primer; 5'- taacgaatgcattgaactgt-3'). Fragment 2 (the 3' fragment) was also amplified by nested PCR, initially to amplify the fragment (forward primer; 5'- acagttcaatgcattcgtta-3', reverse primer; 5'-ttacaatttagttttcaattc-3'), and secondly, to add a *BglII* restriction site after the stop codon (forward primer; 5'- acagttcaatgcattcgtta-3', reverse primer; 5'-agatctttacaatttagttttcaattc-3'). Both of the purified fragments were then digested (*NsiI*) and ligated together to form full length *SLCO1A2*. *SLCO1B1* and *SLCO1B3* were amplified from Huh-7D12 cDNA by nested PCR. Initially *SLCO1B1* and *SLCO1B3* were amplified using primers in the untranslated regions (UTRs) of the genes (*SLCO1B1* forward primer; 5'-ggattctaaatccaggtgattg-3', reverse primer; 5'-aaacacagaagcagaagtgg-3' and *SLCO1B3* forward primer; 5'-gcattcaaagtcaaggtgatca-3', reverse primer 5'-gttagttggcagcagcattgtc-3'). These products were then used as a template to amplify the genes without UTRs (*SLCO1B1* forward primer; 5'-atggaccaaaatcaacatttg-3, same reverse primer' and *SLCO1B3* forward primer; 5'-atggaccaacatcaacatttg-3', same reverse primer), and then to include a Kozak sequence (*SLCO1B1* forward primer; 5'-ccaccatggaccaaaatcaacatttg-3', same reverse primer and *SLCO1B3* forward primer; 5'-ccaccatggaccaacatcaacatttg-3', same reverse primer). The final products were cloned into a pCRII-TOPO vector (according to manufacturer's instructions; Invitrogen) forming pCRII-*SLCO1A2*, pCRII-*SLCO1B1* and pCRII-*SLCO1B3*.

pCRII-*SLCO1A2*, pCRII-*SLCO1B1* and pCRII-*SLCO1B3* were sequenced by primer walking (GATC biotech, Germany). Sequences were aligned to NM_134431 (*SLCO1A2* transcript variant 1), and NM_006446 (*SLCO1B1*) and NM_019844 (*SLCO1B3*). Non-synonymous mutations were then corrected using the QuikChange Multi Site-Directed Mutagenesis kit according to manufacturer's instructions (Stratagene).

Sub-cloning *SLCO1A2* and *SLCO1B1* into pBluescriptII-KSM

To form pBluescriptII-KSM-*SLCO1A2*, pBluescriptII-KSM-*SLCO1B1* and pBluescriptII-KSM-*SLCO1B3*, *SLCO1A2*, *SLCO1B1* and *SLCO1B3* were digested from their corresponding pCRII vectors using *BglII* for *SLCO1A2* and both *SpeI* and *XbaI* for *SLCO1B1* and *SLCO1B3*. pBluescriptII-KSM was linearised using either *BglII* (for *SLCO1A2*) or *SpeI* and *XbaI* (for *SLCO1B1* and *SLCO1B3*) and dephosphorylated using Antarctic phosphatase. *SLCO1A2*, *SLCO1B1* and *SLCO1B3* were then ligated into pBluescriptII-KSM (T4 ligase, overnight, 4°C), transformed into DH5-α competent *E. coli* and clones confirmed by restriction digestion.

In vitro transcription

pBluescriptII-KSM-*SLCO1A2*, pBluescriptII-KSM-*SLCO1B1* and pBluescriptII-KSM-*SLCO1B3* were linearised with *SacI* and used as a template for cRNA synthesis by T3 mMessage mMachine *in vitro* transcription kit (as per manufacturer's protocol).

X. laevis maintenance, oocyte isolation and micro-injection

Adult female *X. laevis* frogs were sacrificed by an anaesthetic solution (MS222, 45mins) and oocytes were isolated and transferred into modified Barth's solution without calcium (NaCl 88mM, KCl 1mM, HEPES 15mM) containing collagenase (1mg/ml, shaking, 2h). The oocytes were washed twice with Barth's solution without calcium, followed by a wash with Barth's solution containing calcium (NaCl 88mM, KCl 1mM, HEPES 15mM, $CaCNO_3 \cdot 6H_2O$ 0.3mM, $CaCl_2 \cdot 6H_2O$ 41μM, $MgSO_4 \cdot 7H_2O$, 0.82mM). The cells were incubated (18°C, 1h) and healthy oocytes were selected and maintained in Barth's solution (18°C, overnight). *SLCO1A2* cRNA (1μg/μl), *SLCO1B1* cRNA (1μg/μl) *SLCO1B3* cRNA (1μg/μl) or DEPC treated water was then injected (± 50nL) into the oocytes. Oocytes were maintained in Barth's solution with penicillin and streptomycin (10mg/l, 3 days for *SLCO1A2* and *SLCO1B1* injected oocytes and 5 days for *SLCO1B3* injected oocytes, 18°C), making sure that dead oocytes were removed and media changed daily.

Uptake experiments in *X. laevis* oocytes

Oocytes were washed and incubated in [^{3}H]estrone-3-sulphate (1μM, 0.33μCi/ml), [^{3}H]saquinavir (1μM, 0.33μCi/ml), [^{3}H]lopinavir (1μM, 0.33μCi/ml), [^{14}C]darunavir (3μM, 0.60μCi/ml), [^{14}C]efavirenz (1μM, 0.33μCi/ml) or [^{3}H]nevirapine (1μM, 0.33μCi/ml) in the presence and absence of human serum albumin (HSA; 2%) in a 24 well plate (>=7 oocytes per condition, 18°C, 1hr, shaking at 150rpm). In order to assess inhibition by ritonavir, the uptake of both [^{3}H]estrone-3-sulphate (1μM, 0.33μCi/ml) and [^{3}H]lopinavir (1μM, 0.33μCi/ml) was also determined in *X. laevis* oocytes injected with either water or *SLCO1B1* cRNA in the presence of ritonavir (10-1000 ng/mL). To preclude displacement of protein binding, this experiment was performed in the absence of human serum albumin. After all incubations, oocytes were transferred into cell strainers, washed 3 times with Hanks balanced solution (4°C) and individual oocytes transferred into scintillation vials. Scintillation fluid was added to the vials and radioactivity was counted by liquid scintillation spectroscopy.

Selection of plasma samples from the TDM registry for genotyping

This study received ethics committee approval for anonomysed genotyping of stored plasma samples (North West Multi-centre Research Ethics Committee, reference number 05/MRE08/67). Plasma samples were selected from 400 individuals that received lopinavir/ritonavir 400mg/100mg b.d., were aged above 18 years and had recorded lopinavir concentrations determined as part of routine TDM as previously described [43]. 326/400 patients had plasma samples 10-14h post dose (designated Cmin) and 293/400 patients had plasma samples 2-6h post dose (designated $C_{2\text{-}6}$). Exclusion criteria included pregnancy, undetectable plasma lopinavir concentrations (suggesting non-adherence to the regimes) and concomitant medications with known enzyme inducers. Of the samples included in the

analysis, 78% were male (4% unspecified), median age was 40 (range 19 to 64), median weight was 72 Kg (range 44 to 113) and median lopinavir concentration was 5072 ng/ml (range 114 to 24432).

Plasma DNA extraction and *SLCO1A2*, *SLCO1B1* and *SLCO1B3* genotyping

DNA was extracted from plasma (600 μL) using a GenElute Blood Genomic DNA kit (Sigma-Aldrich, Poole, UK), according to manufacturer's instructions. Genotyping for the *SLCO1A2* (rs10841795, 38T>C; rs11568563, 516T>G), *SLCO1B1* (rs4149032, intron 2; rs2306283, 388A>G; rs4149056, 521T>C) and *SLCO1B3* (rs4149117, 334T>G) was carried out on 2 μL of the final eluent (100 μL) using a real-time qPCR based allelic discrimination assay as previously described [25]. All genotyping experiments were performed in duplicate and contained negative controls (no template) and three positive controls for each of the possible genotypes. Genotype was only assigned when both duplicates were in agreement.

Statistical analysis

Drug accumulation into oocytes was expressed as the amount of drug associated with each oocyte (pmol/oocyte). The median drug accumulation of each experiment was determined and each experimental condition was performed at least 4 times except for *SLCO1B3* injected oocytes incubated with darunavir, which was performed once (due to limited availability of radiolabelled compound). Normality of the oocyte data was assessed using a Shapiro-Wilk test and based on this statistical analysis was performed using the Kruskal-Wallis non-parametric test. Plasma lopinavir concentrations are given as median (range). Unless otherwise stated, genetic and demographic associations were tested by linear regression on log transformed plasma concentrations. Multivariate analyses were also conducted by linear regression with backward subtraction using SPSS 16. Throughout the manuscript, 0.05 was taken as the P value threshold for statistical significance.

Results

SLCO1A2, *SLCO1B1* and *SLCO1B3* detection in total human mRNA

SLCO1A2 was found to be highly expressed in the brain with modest expression in the kidney, liver and lung tissues. *SLCO1B1* was found to be predominantly expressed in the liver and *SLCO1B3* was expressed in liver and pancreas (Figure 1).

SLCO1A2, *SLCO1B1* and *SLCO1B3* cloning

SLCO1A2 and *SLCO1B1* were successfully cloned into the X. *laevis* expression vector pBluescriptII-KSM. Sequencing of the inserts revealed no mutations in *SLCO1A2* compared to the reference sequence (NM_134431). Two synonymous mutations in *SLCO1B1* (571 C>T, 597C>T) and five non-synonymous mutations (296T>C, 388A>G, 833T>C, 1541A>G and 1910T>C) in *SLCO1B1* compared to the reference sequence (NM_006446) were observed. *SLCO1B3* contained two synonymous mutations (33A>G, 1557A>G) and two non-synonymous mutations (334T>G and 699G>A) when compared to reference sequence (NM_019844). Non-synonymous mutations in *SLCO1B1* and *SLCO1B3* were successfully corrected by site directed mutagenesis.

Drug transport by OATP1A2, OATP1B1 and OATP1B3

Both in the presence and in the absence of 2% HSA, estrone-3-sulphate and all the tested protease inhibitors showed a significant increase in accumulation in oocytes injected with *SLCO1A2* and *SLCO1B1* cRNA compared to water injected control oocytes (Tables 1 and 2). A significant increase of saquinavir was observed, whereas lopinavir and estrone-3-sulphate showed a trend towards increased accumulation in *SLCO1B3* injected compared to water injected control. No significant increase was observed in the accumulation of efavirenz or nevirapine in *SLCO1A2*, *SLCO1B1* or *SLCO1B3* cRNA injected compared to water injected control oocytes (Tables 1, 2 and 3). For OATP1A2 and OATP1B1, the accumulation of drug into the oocyte decreased in the presence of 2% human serum albumin.

Inhibition of OATP1B1 mediated transport of estrone-3-sulphate and lopinavir by ritonavir

Ritonavir (0 – 1000 ng/mL) did not significantly decrease the accumulation of either estrone-3-sulphate (Figure 2A) or lopinavir (Figure 2B) in water or *SLCO1B1* cRNA injected oocytes.

Lopinavir plasma concentrations and *SLCO* genotyping in patients from the Liverpool TDM registry

The frequencies of the minor alleles for *SLCO1A2* 38 (C), *SLCO1A2* 516 (C), *SLCO1B1* rs4149032(T), *SLCO1B1* 388 (A), *SLCO1B1* 521 (C) and *SLCO1B3* 334 (T) were 9%, 4%, 44%, 43%, 11% and 32%, respectively. All SNPs were in Hardy-Weinburg equilibrium as assessed by Chi Square test of observed versus predicted genotype frequencies. The influence of individual *SLCO1B1* SNPs and the previously reported [37, 38] haplotype (*1A, *1B, *5 and *15) constructed from 388A>G and 521T>C on log transformed lopinavir Cmin and $C_{2\text{-}6}$ are shown in Figure 3.

Univariate and Multivariate regression analyses are summarised in table 4. For Cmin, the multivariate model had an adjusted $R^2 = 0.072$; $F = 11.3$, $p < 0.001$. Body weight, coadministered protease inhibitor and *SLCO1B1* rs4149032 genotype were all independently associated with lopinavir Cmin. Cmin was 17.2% lower for every 10 Kg increase in body weight, 15.4% higher in patients receiving an additional protease inhibitor and 11% lower for each variant allele of *SLCO1B1* rs4149032. For $C_{2\text{-}6}$, the multivariate model had an adjusted $R^2 = 0.052$; $F = 8.2$, $p < 0.001$. Body weight, coadministered protease inhibitor and *SLCO1B1* 521T>C genotype were all independently associated with lopinavir $C_{2\text{-}6}$. $C_{2\text{-}6}$ was 13.5% lower for every 10 Kg increase in body weight, 14.4% higher in patients receiving an additional protease inhibitor and 11.1% higher for each variant allele of *SLCO1B1* 521T>C. No differences in Cmin or $C_{2\text{-}6}$ between *1B/*1B versus *1A/*1A homozygotes were observed in univariate or multivariate regression analyses (data not shown).

Discussion

efavirenz and nevirapine are extensively metabolised by CYP2B6 (although CYP3A4 is also implicated in the metabolism of nevirapine) and polymorphisms in this gene have been well

documented to influence plasma concentrations of these drugs [44–46]. However, relatively little is known about pharmacogenetic determinants of protease inhibitor disposition [10, 13]. Therefore, there is a need to identify novel mechanisms that regulate the disposition of this important class of antiretroviral drug.

Recent data have shown that OATP influx transporters influence the bioavailability of statins [25, 30–36] and have also indicated a potential role in saquinavir uptake into hepatocytes [23, 24]. Taken collectively with the observation that there is an interaction between statins and protease inhibitors [47], this suggests that drug uptake into the liver might be an important factor in protease inhibitor disposition. Our data from the X. *laevis* oocyte model clearly show that the protease inhibitors saquinavir and lopinavir are substrates for OATP1A2, OATP1B1 and OATP1B3 and darunavir is a substrate for OATP1A2 and OATP1B1, while the non-nucleoside reverse transcriptase inhibitors are not substrates. These observations hold true in the presence and absence of human serum albumin and suggest that OATP1A2, OATP1B1 and/or OATP1B3 may be additional determinants of variability in plasma concentrations of protease inhibitors. Therefore we examined the potential role of pharmacogenetics.

Although some SNPs in *SLCO1A2* and *SLCO1B3* have been indentified [28, 29], there is an absence of clearly validated genotype – phenotype relationships. Therefore, more extensive studies are now needed to determine other potential functional SNPs in these transporters. Indeed, we observed no associations between currently identified functional SNPs in these genes and lopinavir plasma concentrations; despite showing that lopinavir is a substrate for both transporters. In contrast, the *SLCO1B1* 521T>C SNP has been reproducibly associated with an increase in Cmax and AUC for many of its substrates including pravastatin [30, 31], rosuvastatin [32, 33], pitavastatin [34, 35] and simvastatin [25]. Since lopinavir and other protease inhibitors are given in combination with low dose ritonavir, it was first necessary to establish whether ritonavir inhibited OATP1B1. In the concentration range of 10-1000 ng/ml, ritonavir did not inhibit either estrone-3-sulphate or lopinavir uptake by OATP1B1, thus confirming the validity of examining the impact of *SLCO1B1* SNPs on lopinavir plasma concentrations. We note that the lack of ritonavir inhibition contrasts with a report of inhibition of pitavastatin uptake in OATP1B1 - expressing HEK293 cells [48]. However, these cells express endogenous uptake transporters and the ki for OATP1B1 (0.8 μM) is 25 fold higher than that reported for inhibition of CYP3A4 (0.03 μM).

Here we demonstrate significantly higher median plasma lopinavir Cmin and $C_{2\text{-}6}$ in patients that are homozygous for the C allele at position 521 of *SLCO1B1* compared to either T homozygotes or heterozygotes. A significantly lower lopinavir Cmin was also observed with a SNP located in intron 2 of the *SLCO1B1* gene. The 521T>C polymorphism has been extensively studied for other known substrates of OATP1B1 and further studies to assess the impact of the intron 2 SNP on statins are now warranted. For lopinavir, this latter association may be more clinically important because it is more frequent within the population and the phenotype would be predicted to predispose patients to an increased risk of acquisition of resistance.

Bioinformatic analysis suggests that the allele switch from C to T in intron 2 may slightly alter an octamer factor 1 (oct-1) transcription factor binding motif. Oct-1 is ubiquitously expressed [49] and one could speculate that transcription of the *SLCO1B1* gene may therefore be affected by this SNP. Clearly, this would require experimental confirmation and another possibility is that this SNP is in linkage disequilibrium with another, as yet unidentified functional variant.

These findings indicate that OATP1B1-mediated uptake of lopinavir into the liver is an important determinant of lopinavir disposition. However, the degree of variability in each genotype group is still large and thus other factors clearly play a role. Given that lopinavir is a substrate of at least two other OATP transporters (i.e. OATP1A2 and OATP1B3) a more comprehensive pharmacogenetic analysis is now warranted, which includes other members of this family.

These results must be interpreted in the context that TDM cohorts have inherent limitations resulting from selection bias and lack of information on factors such as ethnicity. However, they represent a potentially valuable resource for pharmacogenetic studies, providing these limitations are understood and we have underpinned this analysis with a mechanistic assessment for biologically plausibility. Nonetheless, as with any pharmacogenetic association, these results should now be validated in other cohorts.

Funding

This work was funded by the UK Medical Research Council and the authors also thank the National Institute of Health Research (NIHR – Department of Health) and the Northwest Development Agency (NWDA) for infrastructural and project support. WSK is funded by Tibotec pharmaceuticals.

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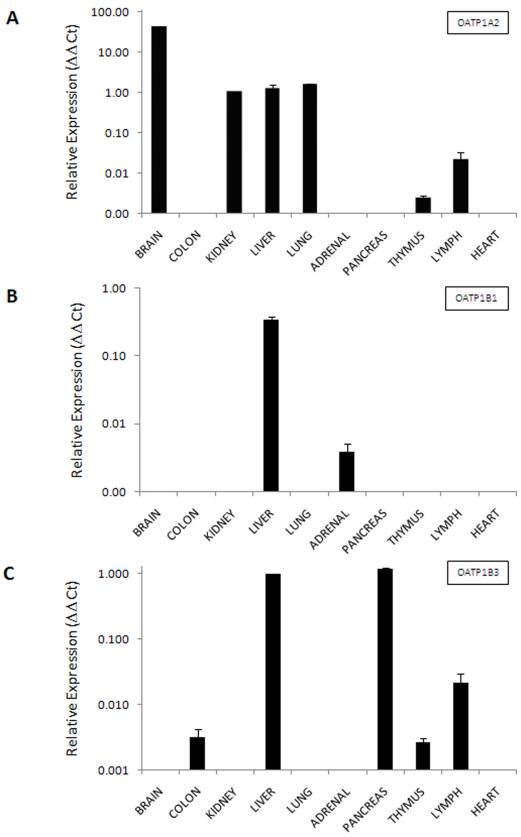

Figure 1.

OATP1A2 (A), OATP1B1 (B) and OATP1B3 (C) mRNA expression across a panel of ten different human tissues.

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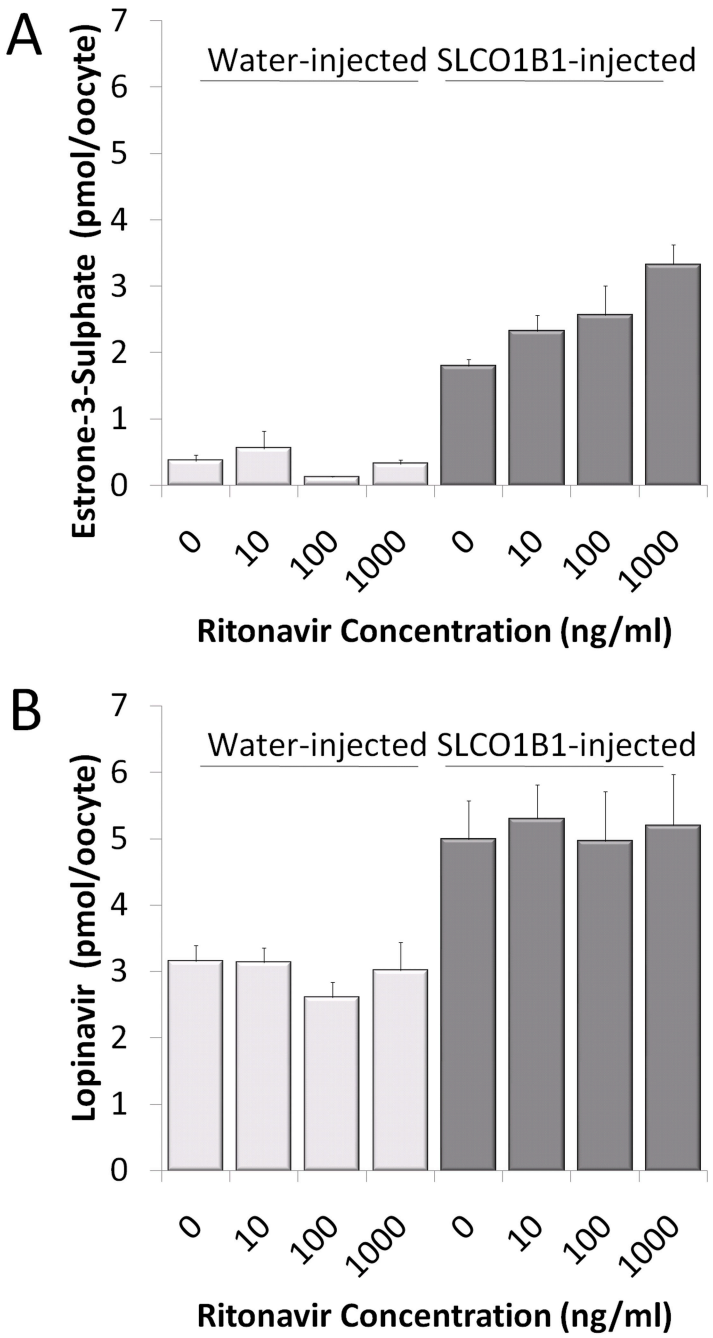

Figure 2.

Effect of ritonavir (0 – 1000 ng/ml) on accumulation of estrone-3-sulphate (A) and lopinavir (B) in OATP1B1 and water injected X. *laevis* oocytes.

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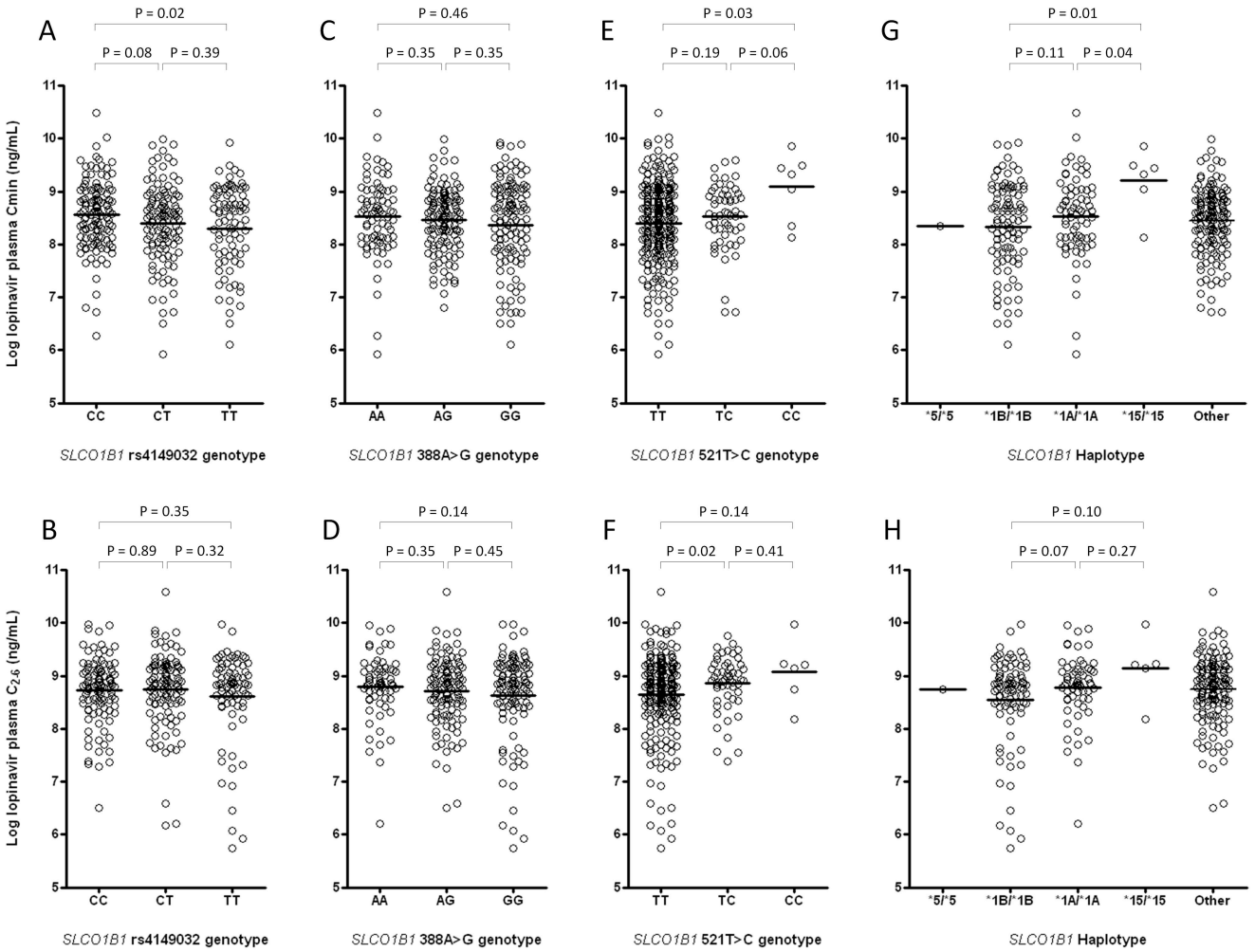

Figure 3.

Plasma lopinavir concentrations in patients from the Liverpool therapeutic drug monitoring registry according to *SLCO1B1* rs4149032 (A & B) and 521T>C (C &D) genotypes. Association with Cmin and $C_{2\text{-}6}$ Are given. Bars indicate the median lopinavir concentrations for each genotype.

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Table 1.

Accumulation in X. *laevis* oocytes injected with water or OATP1A2 cRNA, in the presence or in the absence of 2% HSA. The ratio indicates fold increase in OATP1A2 cRNA injected oocytes compared to water injected oocytes. (* = P < 0.05 by Kruskal-Wallis test).

	Drug uptake (pmol/oocyte)					
	Without human serum albumin			With 2% human serum albumin		
Drug	**Water**	**OATP1A2**	**Ratio**	**Water**	**OATP1A2**	**Ratio**
estrone-3-sulphate	0.19 ± 0.05	2.02 ± 0.52	**10.9 ***	0.10 ± 0.03	0.35 ± 0.15	**3.55 ***
Saquinavir	1.01 ± 0.21	2.16 ± 0.71	**2.14 ***	0.27 ± 0.03	0.45 ± 0.05	**1.65 ***
Lopinavir	2.53 ± 0.36	4.07 ± 0.67	**1.61 ***	0.82 ± 0.24	1.65 ± 0.59	**2.02 ***
Darunavir	2.18 ± 0.40	4.32 ± 0.75	**1.98 ***	0.96 ± 0.37	2.56 ± 0.78	**2.67 ***
Nevirapine	2.65 ± 0.54	2.92 ± 0.59	**1.10**	2.23 ± 0.93	2.17 ± 0.26	**0.97**
Efavirenz	12.6 ± 2.49	10.3 ± 2.90	**0.81**	1.47 ± 0.28	1.63 ± 0.24	**1.11**

Data are given as mean ± standard deviation.

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Table 2.

Accumulation in X. *laevis* oocytes injected with water or OATP1B1 cRNA, in the presence or in the absence of 2% HSA. The ratio indicates fold increase in OATP1B1 cRNA injected oocytes compared to water injected oocytes. (* = P < 0.05 by Kruskal-Wallis test).

	Drug uptake (pmol/oocyte)					
	Without human serum albumin			With 2% human serum albumin		
Drug	**Water**	**OATP1B1**	**Ratio**	**Water**	**OATP1B1**	**Ratio**
estrone-3-sulphate	0.16 ± 0.04	0.51 ± 0.17	**3.21 ***	0.08 ± 0.03	0.15 ± 0.03	**1.90 ***
Saquinavir	0.94 ± 0.26	1.78 ± 0.44	**1.90 ***	0.21 ± 0.06	0.64 ± 0.23	**3.07 ***
Lopinavir	2.14 ± 0.43	4.31 ± 0.72	**2.01 ***	0.66 ± 0.22	1.14 ± 0.41	**1.73 ***
Darunavir	2.12 ± 0.55	4.13 ± 0.40	**1.95 ***	0.72 ± 0.18	2.14 ± 0.82	**2.96 ***
Nevirapine	2.14 ± 0.22	2.26 ± 0.20	**1.06**	1.80 ± 0.46	2.01 ± 0.40	**1.12**
Efavirenz	15.9 ± 1.70	16.0 ± 4.17	**1.00**	1.46 ± 0.21	1.51 ± 0.12	**1.03**

Data are given as mean ± standard deviation.

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Table 3.

Accumulation in X. *laevis* oocytes injected with water or OATP1B3 cRNA. The ratio indicates fold increase in OATP1B3 cRNA injected oocytes compared to water injected oocytes. (* = P < 0.05 by Kruskal-Wallis test).

	Drug uptake (pmol/oocyte)		
	Without human serum albumin		
Drug	**Water**	**OATP1B3**	**Ratio**
estrone-3-sulphate	0.21 ± 0.01	0.50 ± 0.20	**2.38 ***
Saquinavir	1.62 ± 0.45	2.17 ± 0.61	**1.34 ***
Lopinavir	1.69 ± 0.78	2.30 ± 0.66	**1.36**
Darunavir	3.29	5.41	**1.64**
Nevirapine	2.98 ± 0.59	3.12 ± 0.47	**1.05**
Efavirenz	16.56 ± 6.84	18.21 ± 6.21	**1.10**

Data are given as mean ± standard deviation.

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Table 4.

Multivariate analysis by best subset selection of factors influencing lopinavir plasma concentrations in the Liverpool TDM registry. The minor allele frequencies (MAF) are also given.

Independent Variable	Dependent variable	Univariate P (Effect size %)	Multivariate P (Effect size %)
***SLCO1B1* Intron 2**	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	**0.024 (−11.9)** 0.43 (−4.6)	**0.016 (−11)** -
***SLCO1B1* 388A>G**	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	0.17 (−7.3) 0.21 (−7.1)	- -
***SLCO1B1* 521T>C**	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	**0.039 (12.2)** **0.040 (12.8)**	- **0.033 (11.1)**
***SLCO1B3* 334T>G**	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	0.75 (−1.8) 0.98 (−0.2)	- -
***SLCO1A2* 38T>C**	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	0.42 (4.7) 0.34 (5.8)	- -
***SLCO1A2* 516A>C**	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	0.86 (−1.0) 0.36 (5.4)	- -
Age (Years)	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	0.85 (1.1) 0.26 (6.8)	- -
Male Gender	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	0.31 (−5.5) 0.49 (4.2)	- -
Weight (per 10kg)	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	**0.001 (−17.4)** **0.018 (−13.7)**	**<0.001 (−17.2)** **0.003 (13.5)**
Coadministered protease inhibitor	Cmin (ng/mL) $C_{2\text{-}6}$ (ng/mL)	**0.002 (−15.5)** **0.006 (−14.9)**	**0.001 (−15.4)** **0.002 (−14.4)**