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CYP3A5, ABCB1 and SLCO1B1 Polymorphisms and Pharmacokinetics and Virologic Outcome of Lopinavir/Ritonavir in HIV-infected Children

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Abstract

Objective—*CYP3A5*, *MDR1 (ABCB1)* and *OATP1 (SLCO1B1)* polymorphisms have been associated with variability in the pharmacokinetics (PK) of protease inhibitors. The aim of this study was to investigate the influence of *CYP3A5* A6986G, *ABCB1* (C3435T and G2677T), and *SLCO1B1* (T521C and A388AG) polymorphisms on the PK and virologic outcome of lopinavir/ritonavir (LPV/RTV) in HIV-infected children.

Design and Methods—Prospective cohort study in children (4–18 yrs old) on stable antiretroviral therapy with LPV/RTV. *CYP3A5*, *ABCB1* and *SLCO1B1* genotypes were determined using PCR amplification with allelic discrimination assays. The 12 hr plasma area under the concentration-time curves (AUC) and clearances (CL) of LPV and RTV were estimated using non-compartmental models. HIV RNA viral load was evaluated every 12 weeks for a total study period of 52 weeks. Analysis of covariance models with adjustment for age and adherence, and allometric adjustment of CL were used to assess associations between studied polymorphisms and AUC, CL and HIV RNA.

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Results—50 children (median age 11.2 yrs) were enrolled. Allele frequencies of studied genotypes were in Hardy-Weinberg equilibrium. There was no statistically significant association between LPV or RTV AUC or CL, and *CYP3A5*, *ABCB1* or *SLCO1B1* A388G polymorphisms. There was a significant association between *SLCO1B1* T521C genotype and increased LPV AUC ($p=0.042$) and a nearly significant association with decreased LPV CL ($p=0.063$). None of the studied polymorphisms including *SLCO1B1* T521C were associated with virologic outcome during 52 weeks of study follow up.

Conclusions—There was no statistically significant influence of the *CYP3A5*, *ABCB1* or *SLCO1B1* A388AG polymorphisms on the PK and virologic outcome of LPV/RTV in HIV-infected children. *SLCO1B1* T521C polymorphism was significantly associated with increase in LPV AUC, but was not associated with undetectable HIV RNA during the study period.

Keywords

Lopinavir; Ritonavir; CYP3A5; ABCB1; SLCO1B1; HIV; children

INTRODUCTION

Lopinavir/ritonavir (LPV/RTV, Kaletra®, Abbott, Abbott Laboratories, North Chicago, Illinois) is a widely used first line protease inhibitor (PI) component of the antiretroviral (ARV) therapy of pediatric patients due to co-formulation of LPV with RTV in liquid and tablet forms suitable for young patients. Limited data are available regarding the effect of genetic polymorphisms of drug metabolizing enzymes and drug transporters on the pharmacokinetics (PK) and pharmacodynamics (PD) of this boosted PI in children.

LPV and RTV are both essential substrates and inhibitors of the CYP3A enzymes, and variations in the CYP3A5 expression have been suggested to be potentially responsible for the inter-individual variability in the absorption and disposition of several PIs.^[1-4] In addition to CYP3A enzymes, drug transporters may affect the disposition of PIs into tissues and cells.^[5] P-glycoprotein (P-gp) expression and polymorphisms in the multidrug resistant (*MDR1*) (*ABCB1*) gene encoding for P-gp have been associated with variability in drug absorption and disposition, drug response, and toxicity of several PIs in HIV-infected adult patients.^[2, 6-8] Recent reports suggest an important role in the disposition of PIs of another major hepatic drug transporter: organic anion transporting polypeptide (OATP), coded by the *SLCO* genes.^[9, 10] The trough concentrations of LPV have been reported to be significantly increased in patients with the *SLCO1B1* 521T>C polymorphism.^[10] Reduced uptake of LPV by hepatocytes in carriers of *SLCO1B1* T521T>C genotype has been suggested to be responsible for this effect, but the data on the clinical importance of *SLCO1B1* polymorphisms on LPV PK are lacking.^[9]

While no association between the *ABCB1* polymorphisms and LPV or RTV plasma trough concentrations has been reported in adult patients with HIV infection, in pediatric patients the *ABCB1* G3435T polymorphism has been reported to affect plasma concentrations and virologic response to another PI, nelfinavir.^[11] In addition, recent studies of immunosuppressive therapy in children have shown that the effect of *ABCB1* polymorphisms on the PK of cyclosporine is related to age, and thus developmental stage.^[12, 13] These findings suggest that developmental changes in *ABCB1* activity may need to be considered when evaluating the impact of genetic variants in children. To date no studies have evaluated the role of the CYP3A5, *ABCB1* and OATP1B1 expression on the PK and PD parameters of LPV or RTV in HIV-infected children.

This study was originally designed to investigate whether genetic polymorphisms in *CYP3A5* A6986G, *ABCB1* (C3435T and G2677T) affect the steady-state PK and PD of LPV

and/or RTV in HIV-infected children. Following recent reports on the significance of *SLCO1B1* expression on the LPV PK in adults, we have also studied the effect of the genetic polymorphisms of *SLCO1B1* (T521C and A388G) on the steady-state PK of LPV/RTV and evaluated the effect of these polymorphisms on the virologic outcome of LPV/RTV based therapy in pediatric patients in our initial study cohort.

PATIENTS AND METHODS

The study was performed between 2004 and 2009. Pediatric patients with HIV-1 infection (ages 4-18 years), receiving care at a large metropolitan pediatric HIV program at Children's National Medical Center and treated with ARV regimen based on a single PI (including boosted PI), were eligible for study participation. The study represents a subset analysis from the larger pediatric study evaluating the relationship between polymorphisms of drug metabolizing enzymes and drug transporters and the PK of PIs in children.

All approached patients were recruited from a single perinatal pediatric HIV treatment center with a total of 165 patients with similar characteristics in care. The study protocol was reviewed and approved by the IRB at CNMC. Written informed consent was obtained from parents/legal guardians of children younger than 18 years of age. The subject's assent was obtained in children older than 7 years of age, unless a waiver of assent was required. Consent included permission to store the genetic samples at the genetic bank. Prior to study entry, all subjects had received uninterrupted, unchanged single PI based combination antiretroviral therapy, which included LPV/RTV, for a minimum of 4 weeks. Co-administration of medications with a potential interaction with the PI metabolism (such as non-nucleoside reverse transcriptase inhibitors and others) was an exclusion criterion. Kaletra® was administered in liquid, capsule and tablet form depending on the child's age, tolerability and formulation availability (Kaletra® [200 mg LPV/50 mg RTV] tablets became approved after the first year of the study).

Blood samples were collected for genotyping during the enrollment. *CYP3A5*, *ABCB1* and *SLCO1B1* genotypes were determined using PCR amplification followed by allelic discrimination assays based on the use of fluorogenic oligonucleotide probes (TaqMan) (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands) and direct sequencing analysis performed on ABI Prism 3130 Genetic Analyzer (Applied Biosystems, Nieuwerkerk a/d IJssel, The Netherlands). Allele frequencies were identified and all three SNPs were determined to be in Hardy-Weinberg Equilibrium (HWE) using a 1 degree of freedom chi-square test.

Blood samples for LPV PK measurement were obtained during a 12-hour admission to the Clinical Research Center within 8-12 weeks after enrollment. The subjects were administered their standard prescribed LPV/RTV dose under direct observation with a standard light snack. The plasma PK samples were obtained before and 0.5, 1, 2, 4, 8, and 12 h after observed intake of LPV/RTV. LPV and RTV concentrations were determined by a published validated tandem-mass spectrometric method using the Applied Biosystems/Sciex API-2000 (Foster City, California, USA).^[14] Within-run precision (%CV) was below 7% and between-day precision was below 10% for all analytes at the tested concentrations. Accuracy ranged between 95% and 105%. Loss of assay precision (more than 15%) occurred at concentrations less than 10 ng/mL; hence, results below this limit were censored. The ARV therapeutic drug monitoring (TDM) laboratory at CNMC was an accredited member of the International Quality Control Program for Therapeutic Drug Monitoring in HIV infection (University Medical Center Nijmegen, The Netherlands).

HIV RNA viral load (VL) (Roche Amplicor, Branchburg, NJ, USA) was measured every 12 weeks for 52 weeks. The lower limit of quantitation for HIV RNA VL was 400 copies/mL. The virological outcome was determined as Study Undetectable VL (SUVL) if ever achieving VL <400 copies/mL during the study duration (52 weeks) or Ever Undetectable VL (EUVL) if ever achieving VL <400 copies/mL while on ARV therapy with LPV/RTV. The historical data on the length of LPV/RTV therapy and associated VL were collected from medical charts and clinical database. Patient adherence to ART was assessed by interactive 3-day-recall interview of the caregivers and children older than 10 years of age and was calculated as the percentage of the number of doses taken over the number of doses prescribed. Adherence was assessed at enrollment, during PK study and at follow up visits every 12 weeks for 52 weeks.

LPV and RTV area under the time-concentration curves from 0 to 12 hours (AUC) were estimated by the linear trapezoidal algorithm using the *R* statistical package (version 2.9.2, R Foundation, Vienna, Austria, available at <http://cran.r-project.org/>). Apparent oral clearance (CL) was estimated using the formula $CL = F \cdot \text{Dose} / \text{AUC}$, where *F* is bioavailability. Since *F* could not be estimated without intravenous dosing, it was fixed at a value of 1 (note that apparent oral clearance will be larger than clearance after intravenous dosing if *F* is less than 1).

Several statistical methods were used to measure the relationships between *CYP3A5*, *ABCB1* and *SLCO1B1* polymorphisms and LPV/RTV AUC, CL and virologic outcome. Analysis of covariance models with adjustment for age was used to assess associations between polymorphisms and single parameters, multivariate analysis of covariance was used for associations between polymorphisms and multiple parameters, and logistic regression models were used to assess relationships between polymorphisms and virologic outcome. LPV and RTV AUC and CL parameters were log-transformed to conform to normality. For the evaluation of the relationship of the CL with selected SNPs CL of LPV and RTV was allometrically scaled. By scaling CL to weight raised to the 0.75 power, size was accounted for, and any remaining relationship with age was independent of body size. All analyses were performed using Stata V10 (College Station, TX, USA).

RESULTS

The total number of recruited subjects in the study was 78 with 50 subjects on LPV/RTV based ART. Six patients on LPV/RTV based ART were not included in the study due to medical non-adherence. The LPV/RTV PK and virologic outcome data were collected from 50 children (24 boys, 26 girls) with a median (range) age of 11.2 years (4.3-17.2 years). The majority of the children were African American (43), with 3 patients of African origin, 1 Asian patient, and 3 Caucasian patients, 2 of whom were Hispanic. Median weight was 38.5 kg (15.4-90.5 kg), median height was 139.4 cm (108.7-168.3 cm) and a median BMI was 19.0 (12.8-33.0). Background regimens consisted of 2 NRTIs in 44 (88%) patients, 3 NRTIs in 2 patients, and 1 or 2 NRTIs plus NNRTI in 4 patients (LPV dose adjusted for NNRTI exposure). The median LPV/RTV dose was 275/69 mg/m² (246/62-287/72 mg/m²), which is 19.6% higher than currently recommended pediatric dose of 230 mg LPV/57.5 mg RTV/m². The median LPV exposure was 2.2 yrs (0.5-5.2 yrs). Mean self-reported adherence throughout the study period was 88% (41-100%). Of the 50 participants, 27 (54%) patients achieved VL < 400 copies/mL at least once during the study period (52 weeks) and 36 (72%) achieved VL <400 copies/mL at least once ever on LPV/RTV therapy.

Of the 300 possible PK samples, 17 (5.6%) were missing or censored, and were equally distributed within the dosing interval. The median LPV AUC and CL were 96.87 µg·h/L (15.29 - 228.77 µg·h/L) and 0.09 L/h/kg (0.04-0.44 L/h/kg), respectively. For RTV, AUC

and CL were 44.73 $\mu\text{g}\cdot\text{h}/\text{L}$ (0.45-14.90 $\mu\text{g}\cdot\text{h}/\text{L}$) and 0.53 L/kg/h (0.15-3.74 L/kg/h), respectively. There was no significant relationship between the age and AUC for either LPV or RTV. As anticipated the AUC and CL of LPV had direct linear relationship with AUC and CL of RTV ($r^2=0.755$; $p<0.0001$ and $r^2=0.755$; $p<0.0001$, respectively). The decrease in CL of LPV and RTV was statistically significantly associated with advanced age ($r^2=0.136$, $p=0.008$ and $r^2=0.178$, $p=0.002$ respectively).

The allelic frequencies of the polymorphisms for *CYP3A5* A6986G and *ABCB1* (C3435T and G2677T) and for *SLCO1B1* (T521C and A388G) were as follows: *CYP3A5* (A6986G) - $p(\text{A})=0.583$, $p(\text{G})=0.417$ (HWE $p\text{-value}=0.113$); *ABCB1* (C3435T) - $p(\text{C})=0.688$, $p(\text{T})=0.312$ (HWE $p\text{-value}=0.834$); *ABCB1* (G2677T) - $p(\text{G})=0.800$, $p(\text{T})=0.200$ (HWE $p\text{-value}=0.693$); *SLCO1B1* (T521C) - $p(\text{T})=0.958$, $p(\text{C})=0.042$ (HWE $p\text{-value}=0.763$); *SLCO1B1* (A388G) - $p(\text{G})=0.702$, $p(\text{A})=0.298$ (HWE $p\text{-value}=0.595$).

There was no statistically significant association between LPV or RTV AUC₀₋₁₂ and *CYP3A5*, *ABCB1* C3435T and G2677T, and *SLCO1B1* A388G genotypes. There was a significant association between *SLCO1B1* T521C genotype and LPV AUC ($p=0.042$), but no association between the same genotype and RTV AUC ($p=0.3453$). (Figure 1) The *CYP3A5*, *ABCB1* and *SLCO1B1* polymorphisms did not significantly influence CL of the LPV and RTV in children, although *SLCO1B1* 521T>C genotype was nearly significantly associated with an increase in LPV CL ($p=0.063$). (Figure 2) When linear models were adjusted by age no statistical difference was observed. The combined PK of LPV and RTV (RTV AUC + LPV CL) were equally not affected by the *CYP3A5*, *ABCB1* and *SLCO1B1* genotypes. (Table 1)

CYP3A5, *ABCB1* and *SLCO1B1* polymorphisms had no statistically significant association with virologic suppression ($\text{VL}<400$ copies/mL) during the study period or ever achieved with LPV/RTV therapy). (Table 2) The multiple SNP analysis did not reveal significant association between *CYP3A5*, *ABCB1* and *SLCO1B1* combined genotypes and PK and virologic outcome of LPV/RTV therapy. (Table 3)

In order to minimize any confounding of size on age effects we also used allometric (size-adjusted) CL by scaling CL to weight raised to the 0.75 power in our model. For every year of life, LPV allometric CL (Allo CL) decreased by 0.02 L/kg^{0.75}/h ($r^2=0.06$, $p=0.14$) and RTV Allo CL decreased by 0.03 L/kg^{0.75}/h ($r^2=0.112$, $p=0.03$). *SLCO1B1* T521C appeared to have an effect on Allo CL of LPV, although it just did not reach statistical significance ($p=0.053$). When adjusted for age, that effect decreased ($p=0.067$). *SLCO1B1* T521C appeared to have an effect on LPV Allo CL regardless of age. (Table 4) In addition, *SLCO1B1* A388G had clearly some effect on LPV Allo CL. (Table 4) Genotype $\times$ age interaction was tested for all SNPs except *SLCO1B1* T521C (not have enough subjects with mutant allele) and was not found to be statistically significant.

DISCUSSION

This study evaluated the *CYP3A5*, *ABCB1* and *SLCO1B1* polymorphisms in a cohort of predominantly African American (86%) HIV-infected children. The *CYP3A5* 6986A allelic frequency is present among approximately 50% in African American populations, and *CYP3A5* has been detected in measurable amounts in up to 60% of African American adults.^[15-17] The allelic frequencies in our study of predominantly African American children equally demonstrate the higher prevalence of wild-type, active enzyme encoding allele of the *CYP3A5* gene. The allelic frequency of *ABCB1* 3435C was seen in majority of our patients in accordance with previously reported high frequency of this allele in the African American population.^[15, 18] The incidence of *ABCB1* 2677G allele in our study

cohort was higher than reported in the literature for African American adults.^[15, 19] The allelic frequencies of *SLCO1B1* 521C polymorphisms are known to be rare in patients of African origin, and were very low in our study population of predominantly African American children (comparable with the data from Brazilian HIV-infected population).^[10, 20, 21] The incidence of *SLCO1B1* 388G allele was predominant in our population in accordance with published distribution in African subjects.^[20, 21]

In our study neither LPV nor RTV AUC and CL were affected by the *CYP3A5* A6986G polymorphism. These data correlate with previously published data on the lack of effect of *CYP3A5* A6986G on LPV and RTV trough plasma concentrations in adults.^[22] The study by Estrela et al. also did not find an association between another *CYP3A5* G14690A polymorphism and trough plasma concentrations of LPV/RTV in HIV-infected adults and ascribed the overall lack of the *CYP3A5* influence on the PK of LPV/RTV to the inhibition of the *CYP3A5* activity by RTV.^[22] Both *ABCB1* C3435T and G2677T polymorphisms and their haplotypes also had no effect on plasma AUC and CL of LPV/RTV as previously reported in adult studies.^[23-26] The most recent adult study evaluating LPV plasma trough concentrations in plasma, semen and saliva of adult HIV-infected men found no association with *ABCB1* C1236T, T2677A and C3435T genotypes and haplotypes.^[27]

The reported effects of the *ABCB1* SNPs on the outcome of LPV/RTV based ART have been controversial. *ABCB1* C3435T polymorphism has been associated with CD4 cell recovery in HIV-infected adult patients by Fellay et al.,^[24] but not by other studies.^[23, 28] *ABCB1* C3435T polymorphism has been associated with HIV viral load outcome with earlier virologic failure reported in adult HIV-infected patients with 3435CC genotype.^[29] These data have not been confirmed by Haas et al.^[23], who reported that the allelic *ABCB1* G2677T and C3435T variants were not associated with difference in the rate of phase 1 viral decay. Our study found no association between the *ABCB1* polymorphisms and the virologic outcome in treatment-experienced HIV-infected children.

This is the first pediatric study to evaluate the effect of the *SLCO1B1* polymorphisms on the PK and PD of LPV/RTV in HIV-infected children. Our data on the significance of *SLCO1B1* T521C polymorphism on the trough of LPV are consistent with recently published reports from the adult data from Brazil and England.^[9, 10] The study by Hartkoorn et al. has evaluated the effect of *SLCO1A1*, *SLCO1B1* and *SLCO1B3* on the LPV plasma concentrations in adult subjects (>18 years) at 10-14 hours post dose (326 patients) and 2-6 hours post dose (293 patients). No data on ethnic/racial background or virologic outcome were available. The study combined the data from clinical database with *in vitro* research evaluating the PI as substrates to the OATP1A2, OATP1B1 and OATP1B3 in *Xenopus laevis* oocyte model. The data from the *X. laevis* oocyte model showed that the PIs saquinavir and LPV are the two PIs substrates to OATP1A2, OATP1B1 and OATP1B3, and RTV had no inhibitory effect on OATP1B1. In human samples, however, no association was found between *SLCO1A2* and *SLCO1B3* and LPV plasma concentrations. In contrast, the data from human samples showed a significant association between *SLCO1B1* 521T to C polymorphism with higher LPV plasma concentrations at both time points studied.^[9] Significantly higher median plasma LPV C_{min} and C_{2-6} were observed in patients that were homozygous for the C allele at position 521 of *SLCO1B1* compared with either T homozygotes or heterozygotes. The study by Kohlrausch et al. in Brazilian HIV-infected men showed similar association between *SLCO1B1* 521 T to C polymorphism with higher LPV C_{min} when compared with wild-type genotype.^[10]

In our analysis of the effect of *SLCO1B1* 521 T to C polymorphism on the PK of LPV and RTV in the pediatric cohort with wide range of ages (4.3-17.2 yrs) the relationship between outcome and genotype could be confounded by a relationship between the outcome and age.

When looking at pediatric PK parameters and trying to minimize any confounding of size on age effects, it definitely is best to use size-adjusted parameters (i.e. allometrical adjustment). From our analysis using allometrically scaled CL it appears that LPV CL is related to both age and *SLCO1B1* genotype, and that the effect of genotype is weakly moderated by age, but we acknowledge that the present study has insufficient numbers and low minor allele frequency to make definitive statement about such relationship. It is also difficult to make conclusion about the effect of the *SLCO1B1* A388G on LPV Allo CL, since this SNP had only two genotypes in the database (compared to three means for all other SNPs) which can disproportionally increase the power of analysis for this SNP in the study. (Table 4)

Findings of the significant effect of *SLCO1B1* polymorphisms (T521C and A388G) on the PK of other OATP substrates such as statins have been reported and the *SLCO1B1* polymorphism has been linked to be associated with statin-induced myopathy.^[30-33] As a result of these findings, the speculation about the association of *SLCO1B1* polymorphism and increased risk of resistance to LPV and virologic failure has been made. Our study is the first to evaluate the clinical significance of the *SLCO1B1* polymorphisms in HIV-infected patients. In our study cohort of experienced HIV-infected pediatric patients, the *SLCO1B1* polymorphisms (A388G and T521C) had no statistically significant association with virologic outcome (undetectable HIV RNA viral load <400 copies/mL) during the study period or ever achieved with LPV/RTV based antiretroviral treatment regimen. We recognize that CYP3A, ABCB1 and *SLCO1B1* expression may be affected by HIV infection and concomitant ARV therapy, and hence may be a confounding variable in determining drug response.^[34-37] The full spectrum of the substrates/suppressor/inducer interaction between CYP3A enzymes, P-gp, OATP and ARV therapy and HIV infection is complex and remains to be determined.

Finally, as we reported in our population PK analysis for LPV^[38], in this study we also found a significant association between RTV CL and age, due to age related changes in apparent volume of distribution that were independent of weight. For both drugs, the weight-adjusted volume of distribution in L/kg on average diminishes with age, which is likely a reflection of the age-related reduction in extracellular body water as a percentage of total body weight.^[39]

The major limitation of our study is the relatively small sample size of this pediatric cohort from our single center. Second, we did not evaluate the contribution of the resistance to ARV drugs to the virologic outcome. Finally, the adherence in the study was evaluated through guardians and an older child self-report and is subject to recall bias.

CONCLUSIONS

These data add to our understanding of the factors that contribute to variability in plasma concentrations of protease inhibitors. Our data showed the impact of the *SLCO1B1* T521C polymorphism on LPV AUC and CL. There was no statistically significant influence of the *CYP3A5* A6986G, *ABCB1* (C3435T and G2677T) and *SLCO1B1* A388AG polymorphisms on the PK of LPV and RTV in children. This is the first study to evaluate the clinical significance of *ABCB1* and *SLCO1B1* polymorphisms on the outcome of HIV infection. The virologic outcome of LPV/RTV based antiretroviral therapy was not affected by the *ABCB1* or *SLCO1B1* polymorphisms in the treatment experienced HIV-infected children. Age had a statistically significant effect on the clearance of LPV and RTV. The full spectrum of the substrates/suppressor/inducer interaction between CYP450 enzymes, P-gp, OATP and antiretroviral therapy and HIV infection is complex and remains to be determined.

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Figure 1. Association between LPV (a) and RTV (b) AUC and *SLCO1B1* T512C genotype.

Figure 2. Association between LPV (a) and RTV (b) CL and *SLCO1B1* T512C genotype.

Table 1

Analysis of combined PK parameters (LPV AUC and RTV CL) in association with each SNP.

Covariates	Phenotypes	SNP	Wald's p-value for SNP effect
None *	RTV AUC ^{\$} + LPV CL ^{\$\$}	<i>CYP3A5</i> A6986G	0.6638
	RTV AUC + LPV CL	<i>ABCB1</i> C3435T	0.9317
	RTV AUC + LPV CL	<i>ABCB1</i> G2677T	0.9626
	RTV AUC + LPV CL	<i>SLCO1B1</i> A388G	0.8730
	RTV AUC + LPV CL	<i>SLCO1B1</i> T521C	0.1017
Age	RTV AUC + LPV CL	<i>CYP3A5</i> A6986G	0.6580
	RTV AUC + LPV CL	<i>ABCB1</i> C3435T	0.7367
	RTV AUC + LPV CL	<i>ABCB1</i> G2677T	0.9316
	RTV AUC + LPV CL	<i>SLCO1B1</i> A388G	0.8959
	RTV AUC + LPV CL	<i>SLCO1B1</i> T521C	0.1209

^{\$} AUC₀₋₁₂

^{\$\$} CL₀₋₁₂

* Unadjusted analysis

Table 2
Associations between virologic outcome and SNPs: analysis adjusted for study medical adherence and age.

Phenotype	SNP	Genotype	Number of No	Number of Yes	OR*	P-value	95% CI	P-value for comparison of homozygous mutants and heterozygotes
SUVL400 [§]	CYP3A5 A6986G	AA	9	10	1.00			
		AG	7	11	1.072	0.924	0.255 – 4.522	
		GG	6	5	2.119	0.366	0.416 – 10.790	0.3889
	ABCB1 C3435T	CC	11	12	1.00			
		CT	9	11	0.955	0.942	0.275 – 3.312	
		TT	2	3	0.656	0.684	0.086 – 5.001	0.7216
	ABCB1 G2677T	GG	15	11	1.00			
		GT	7	5	0.971	0.968	0.236 – 3.988	
		TT	0	2	---	---	---	---
	SLCO1B1 A388G	GG	8	12	1.00			
		AG	9	10	1.281	0.714	0.341 – 4.816	
		AA	2	1	2.667	0.468	0.189 – 37.712	0.5921
EUVL400 ^{§§}	CYP3A5 A6986G	TT	24	20	1.00			
		TC	2	2	1.828	0.576	0.220 – 15.166	
		CC	0	0	---	---	---	---
	CYP3A5 A6986G	AA	6	13	1.00			
		AG	4	14	0.762	0.736	0.157 – 3.702	
		GG	3	8	1.012	0.990	0.176 – 5.836	0.7488
	ABCB1 C3435T	CC	6	17	1.00			
		CT	7	13	1.676	0.451	0.437 – 6.421	
		TT	0	5	---	---	---	---
	ABCB1 G2677T	GG	10	16	1.00			
		GT	2	10	0.280	0.156	0.048 – 1.623	
		TT	0	2	---	---	---	---
SLCO1B1 A388G	SLCO1B1 A388G	GG	4	16	1.00			
		AG	5	14	1.321	0.723	0.283 – 6.157	
		AA	1	2	1.862	0.653	0.123 – 28.042	0.8054

Phenotype	SNP	Genotype	Number of No	Number of Yes	OR*	P-value	95% CI	P-value for comparison of homozygous mutants and heterozygotes
	<i>SLCO1B1</i> <i>T521C</i>	TT	13	31	1.00			
		TC	0	4	---	---	---	
		CC	0	0	---	---	---	---

[§] Study Undetectable Viral Load (<400 copies/mL) during the study follow up (52 weeks)

^{§§} Ever Undetectable Viral Load (<400 copies/mL) on ARV therapy that included LPV/RTV

Table 3

Multiple SNP analysis: for each phenotype as the outcome (dependent) variable, all 5 polymorphisms are included in the model, with and without age as an additional covariate. P-values are for the effect of each covariate.

Phenotype	Age effect P-value	CYP3A5 A6986G effect P-value	ABCB1 C3435T effect P-value	ABCB1 G2677 effect P-value	SCLCO1B1 A388G effect P-value	SLCO1B1 T521C effect P-value
LPV AUC [§]	None [*]	0.725	0.950	0.982	0.945	0.190
LPV CL ^{§§}		0.861	0.973	0.938	0.9075	0.328
RTV AUC [§]		0.705	0.935	0.908	0.750	0.716
RTV CL ^{§§}		0.952	0.952	0.902	0.797	0.658
SUVL400		0.594	0.803	0.401	0.894	---
EUVL400		0.149	0.907	0.098	0.744	---
LPV AUC	0.126	0.710	0.897	0.990	0.986	0.258
LPV CL 12	0.033	0.959	0.708	0.928	0.893	0.455
RTV AUC	0.027	0.499	0.947	0.952	0.967	0.474
RTV CL 12	0.008	0.764	0.746	0.931	0.999	0.372
SUVL400 ^{**}	0.803	0.577	0.816	0.410	0.876	---
EUVL400 ^{***}	0.991	0.153	0.907	0.099	0.745	---

[§] AUC₀₋₁₂

^{§§} CL₀₋₁₂

^{*} Unadjusted analysis

^{**} Study Undetectable Viral Load (<400 copies/mL) during the study follow up (52 weeks)

^{***} Ever Undetectable Viral Load (<400 copies/mL) on ARV therapy that included LPV/RTV

Table 4

Associations between Allometric Clearance (AlloCL) and SNPs: age analysis

Phenotype *	SNP	Genotype effect		Age effect		Genotype (N: adjusted mean $\pm$ SEM)
		F-test	P-value	F-test	P-value	
Allo LPV CL 12	CYP3A5 A6986G	0.30	0.7440	4.36	0.043	AA (N=19; -1.31 $\pm$ 0.13) AG (N=18; -1.32 $\pm$ 0.14) GG (N=11; -1.47 $\pm$ 0.17)
Allo RTV CL 12		0.64	0.5313	7.13	0.011	AA (N=19; 0.38 $\pm$ 0.17) AG (N=18; 0.20 $\pm$ 0.18) GG (N=11; 0.06 $\pm$ 0.22)
Allo LPV CL 12	ABCB1 C3435T	0.92	0.4055	5.11	0.029	CC (N=23; -1.39 $\pm$ 0.11) CT (N=20; -1.37 $\pm$ 0.11) TT (N=5; -1.71 $\pm$ 0.23)
Allo RTV CL 12		0.54	0.5838	9.47	0.004	CC (N=23; 0.14 $\pm$ 0.13) CT (N=20; 0.24 $\pm$ 0.14) TT (N=5; -0.08 $\pm$ 0.29)
Allo LPV CL 12	ABCB1 G2677T	0.04	0.9627	1.75	0.194	GG (N=26; -1.32 $\pm$ 0.11) GT (N=12; 1.36 $\pm$ 0.16) TT (N=2; 1.41 $\pm$ 0.40)
Allo RTV CL 12		0.15	0.8612	6.40	0.016	GG (N=26; 0.34 $\pm$ 0.13) GT (N=12; 0.30 $\pm$ 0.20) TT (N=2; 0.07 $\pm$ 0.48)
Allo LPV CL 12	SLCO1B1 A388G	0.10	0.9017	6.48	0.015	GG (N=20; -1.45 $\pm$ 0.12) AG (N=19; -1.37 $\pm$ 0.12) AA (N=3; -1.38 $\pm$ 0.32)
Allo RTV CL 12		0.07	0.9295	8.61	0.006	GG (N=20; 0.14 $\pm$ 0.16) AG (N=19; 0.19 $\pm$ 0.16) AA (N=3; 0.31 $\pm$ 0.41)
Allo LPV CL 12	SLCO1B1 T521C	3.52	0.0671	3.06	0.087	TT (N=44; -1.33 $\pm$ 0.08) TC (N=4; -1.87 $\pm$ 0.28)
Allo RTV CL 12		0.12	0.7316	5.65	0.022	TT (N=44; 0.25 $\pm$ 0.11) TC (N=4; 0.11 $\pm$ 0.37)

* All phenotypes log-transformed to conform to normality