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Influence of *MDR1* C1236T polymorphism on lopinavir plasma concentration and virological response in HIV-1-infected children

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ABSTRACT

Background: Variability in *MDR1* and PXR has been associated with differences in drug plasma levels and response to antiretroviral therapy. We investigated whether polymorphisms in *MDR1* (T-129C, C1236T and C3435T) and PXR (C63396T) affect lopinavir plasma concentration and the virological or immunological response to HAART in HIV-1-infected children.
Methods: Genotypes were identified in 100 blood donors and 38 HIV-1-infected children. All children received HAART with lopinavir boosted with ritonavir (LPV/r) at the time of LPV plasma level quantification, before (C_{trough}) and between 1 and 2 h after ($C_{post\text{-}dose}$) the administration of the next dose of drug. $CD4^+$ T-cell counts and plasma viral load were analyzed before and after the initiation of LPV/r.
Results: MDR1 1236T, *MDR1* 3435T and PXR 63396T alleles showed a frequency of ~50% while the *MDR1* -129C allele only reached 5%. Children heterozygotes 1236CT showed a significantly lower LPV $C_{post\text{-}dose}$ than homozygotes 1236TT (median $C_{post\text{-}dose}$ = 3.04 μg/ml and 6.50 μg/ml, respectively; $p = 0.016$). Children heterozygotes 1236CT also had a lower decrease of viral load after 36 weeks of LPV/r exposure compared with homozygotes 1236CC (median viral load changes = −0.50 $\log_{10}$ copies/ml and −2.08 $\log_{10}$ copies/ml, respectively; $p = 0.047$). No effect on the immunological response was observed for polymorphisms of *MDR1* or PXR.
Conclusions: Our results suggest that the *MDR1* C1236T SNP significantly reduces LPV plasma concentration affecting the virological response to HAART. Heterozygotes 1236CT might have an altered level of P-gp expression/activity in enterocytes and $CD4^+$ T lymphocytes that limits the absorption of LPV leading to an impaired virological suppression.

1. Introduction

Highly active antiretroviral therapy (HAART) significantly improved HIV-1 infection reducing AIDS incidence and mortality. Nevertheless, at present, virological failure and treatment-associated adverse effects are still major medical concerns. Almost all antiretroviral drugs exhibit high inter-individual pharmacokinetic variability and narrow therapeutic range, which can lead to suboptimal or toxic plasma concentrations. In the last years, pharmacogenetic studies have been developed to understand the factors that contribute to the variability of antiretroviral drug plasma concentration and its impact on drug response. Most of the studies have been focused on two groups of proteins: the metabolizing enzymes, mainly CYP450 (Cytochrome P450), and drug transporters, such as P-glycoprotein (ABCB1) and MRP (Multidrug Resistance Protein).

Lopinavir/ritonavir (LPV/r) is a co-formulation widely used as first-line protease inhibitors (PI) for the treatment of pediatric patients. As most PIs, they are substrates for the P-glycoprotein (P-gp), a member of the ATP-binding cassette (ABC) transporters, that actively pumps the drugs out of the cells influencing their absorption and elimination (Cianfriglia et al., 2007; Lee et al., 1998). P-gp is expressed on a variety of cells and tissues, including enterocytes (Albermann et al., 2005) and $CD4^+$ T lymphocytes, the primary target of HIV (Ford et al., 2003).

Single nucleotide polymorphisms (SNPs) in the human multidrug resistance (*MDR1*) gene – that encodes for P-gp – could affect protein expression and have been associated with variability of drug absorption

Abbreviations: ABC, ATP-binding cassette; CDC, Centers for Disease Control and Prevention; CYP450, Cytochrome P450; HAART, highly active antiretroviral therapy; HPLC, high-performance liquid chromatography; LPV, lopinavir; LPV/r, HAART with lopinavir boosted with ritonavir; MDR, human multidrug resistance; MRP, Multidrug Resistance Protein; NNRTIs, non-nucleoside reverse transcriptase inhibitors; NRTIs, nucleoside reverse transcriptase inhibitors; PBMCs, peripheral blood mononuclear cells; PCR, polymerase chain reaction; P-gp, P-glycoprotein; PI, protease inhibitors; PXR, Pregnane X Receptor; RFLP, restriction fragment length polymorphism; RTV, ritonavir; SNPs, single nucleotide polymorphisms.

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and disposition, drug response and toxicity of several PIs in HIV-1-infected patients. The most extensively studied is the *MDR1* C3435T SNP on exon 26 which has been associated with low P-gp expression in enterocytes (Hoffmeyer et al., 2000) and peripheral blood mononuclear cells (PBMCs) (Owen et al., 2004). Several studies have evaluated the influence of the *MDR1* C3435T SNP on different antiretroviral drug responses in HIV-1-infected patients with controversial findings (Curras et al., 2009; Estrela et al., 2009; Fellay et al., 2002; Ma et al., 2007; Rakhmanina et al., 2011; Saitoh et al., 2005; Winzer et al., 2003). With regard to lopinavir (LPV) or ritonavir (RTV), *MDR1* polymorphisms do not seem to modify trough plasma concentration in adult patients (Estrela et al., 2009; Ma et al., 2007; Winzer et al., 2003). However, in pediatric patients, the effect of *MDR1* C3435T SNP remains controversial. Saitoh et al. (2005) and Curras et al. (2009) reported an effect on drug response to nelfinavir and indinavir, respectively, while a recent report showed no influence of the *MDR1* variants, C3435T and G2677T, on the pharmacokinetic and virologic outcome of LPV/r in HIV-infected children (Rakhmanina et al., 2011).

The expression of P-gp is regulated by the nuclear transcription factor Pregnane X Receptor (PXR, *NR1I2*) in response to endobiotics and xenobiotics (Geick et al., 2001). Several polymorphisms have been identified in the *NR1I2* gene but only the C63396T SNP on intron 1b has been reported to affect plasma concentration of another PI, atazanavir (Siccardi et al., 2008).

The aim of this study was to investigate whether genetic polymorphisms in *MDR1* (T-129C, C1236T and C3435T) and in PXR (C63396T) affect the lopinavir plasma concentration and the virological or immunological response to HAART in HIV-infected children.

2. Materials and methods

2.1. Study population

The study included 100 randomly selected blood donors and 38 HIV-1 perinatally infected children with LPV plasma concentration determined in 2006 and followed-up at the single reference pediatric hospital "Juan P. Garrahan" (Buenos Aires, Argentina). The ethnic background of the Argentinean children and blood donors is considered "Hispanic-Caucasian" and mainly composed by European descendants. HIV-1 infection status and AIDS definition were established according to the 1994 criteria of the U. S. Centers for Disease Control and Prevention (CDC) classification for children (Centers for Disease Control and Prevention (CDC), 1994). Clinical and immunological data were collected by retrospective chart review. At the moment of lopinavir determination, all of the children received HAART regimen with lopinavir boosted with ritonavir (LPV/r) as the only PIs, except for 2 patients who also received amprenavir. The oral doses of LPV were 230 and 300 mg/m^2/12 h for children older and younger than 6 months, respectively. The Ethics Committee and the Institutional Review Board of the hospital approved the study. Written informed consent was obtained from the blood donors and the parents or legal guardians of the children.

2.2. Genotyping analysis

MDR1 and PXR genotypes were identified by polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP) assays. The *MDR1* C1236T (rs1128503) and *MDR1* C3435T (rs1045642) SNPs were genotyped based on the previous report of Cascorbi et al. (2001), and the *MDR1* T-129C (rs3213619) SNP was genotyped based on the report of Furuno et al. (2002). A PCR-RFLP assay for the PXR C63396T SNP (rs2472677) was designed based on the primer sequences kindly provided by Siccardi et al. (2008).

Briefly, PBMC lysates or DNA samples were used for PCR assays and the amplicons of 366 bp (C1236T), 197 bp (C3435T), 215 bp (T-129C) and 134 bp (C63396T) were subjected to digestion with the restriction enzymes *HaeIII*, *MboI*, *MspA1I* and *Hpy188I*, respectively. Finally, digested products were separated on agarose gel (3.5%–4.5%) and visualized by ethidium bromide staining under UV light with the *El Logic 200 imaging system* (*Kodak*, *Rochester*, New York, USA).

2.3. Quantification of lopinavir in plasma

LPV plasma level was determined from blood drawn immediately before the administration of the next dose of drug (C_{trough}; n = 38) and between 1 and 2 h after the corresponding morning dose administration ($C_{post\text{-}dose}$; n = 34). Only one determination (C_{trough} and $C_{post\text{-}dose}$) per patient was performed. A data record of dosing and sampling time was obtained. Adherence to antiretroviral treatment in the week previous to drug monitoring was assessed by means of a questionnaire specifically designed for this study. Additional information included demographic and anthropometric data, clinical, virological and immunological parameters, previous exposure to PIs and antiretroviral treatment duration. Plasma concentration was measured by high-performance liquid chromatography (HPLC) with ultraviolet detection as previously described (Droste et al., 2003). The lower limit of quantification for LPV was 50 ng.

2.4. HIV-1 plasma viral load and CD4$^+$ T-cell counts

Plasma HIV-1 RNA was quantified using *Amplicor HIV-1 Monitor test v 1.5* (*Roche Diagnostics Systems*, Branchburg, New Jersey, USA) until June 2005 and since then *HIV-1 RNA Cobas® TaqMan 48* (*Roche Diagnostic Systems*, Branchburg, New Jersey, USA). The lower limits of quantification for viral load were 1.7 and 1.67 $\log_{10}$ copies/ml, respectively. CD4$^+$ T-cell counts were measured using flow cytometry (*FACS sorter*, *Becton Dickinson*, San Jose, USA) on whole blood samples. Virological and immunological response was retrospectively evaluated. Available CD4$^+$ T-cell counts and plasma viral load were analyzed before and after the initiation of HAART containing LPV/r. Baseline data corresponded to the determination performed up to 20 weeks prior to LPV/r exposure. Viral load was analyzed at weeks 18 (2–46) [mean (range)] and 36 (10–94), and CD4$^+$ T-cell counts at weeks 21 (2–57) and 41 (8–93) after the initiation of treatment.

2.5. Statistical analysis

Genotypic frequencies were estimated by direct allele counting. Fit to Hardy–Weinberg equilibrium was tested with Pearson's χ^2. Differences between *MDR1* and PXR genotypes in relation to trough and post-dose concentrations, baseline and changes in plasma viral load and CD4$^+$ T-cell counts were analyzed with Kruskal–Wallis test applying the Bonferroni correction. Dunn's Multiple Comparison test was used to assess pairwise differences. Statistical tests were performed using GraphPad Prism version 2.01. All tests were two-tailed with a significance level of 0.05.

3. Results

3.1. Characteristics of the patients

We studied 38 HIV-1-infected children that received HAART regimen containing LPV/r. Characteristics of the studied group at the time of the determination of LPV plasma concentration are summarized in Table 1. The children were equally distributed among girls and boys. The median age was 109.7 months, although the range was very wide including children from 7 months up to 19 years. The median time LPV/r exposure was around 24 months. At the moment of the determination of drug plasma concentration, the median plasma viral load was 3.62 $\log_{10}$ copies/ml, the median CD4$^+$ T-cell count was 23%, and 76% of the patients had developed AIDS (stage C). Three patients received HAART containing LPV/r by first time, while the majority had HAART experience with other PIs than LPV (mainly ritonavir full dose and

Table 1
Clinical characteristics of patients at time of lopinavir plasma level determination.

Characteristics		
Females—n (%)		17 (45%)
Age—median, months [range]		109.70 [7.83–233.47]
Body weight—median, kg [range]		23 [9–60]
Treatment duration—median, months [range]		24.68 [2.03–62.33]
Clinical stage—n (%)	A	2 (5%)
	B	6 (16%)
	C	30 (79%)
Plasma VL—median, $\log_{10}$ copies/ml [range]		3.62 [≤1.70–5.90]
$CD4^+$ T-cell count—median, % [range]		23 [1–43]

Treatment duration: time since treatment initiation to drug plasma level determination. VL: viral load.

nelfinavir). At the time of LPV measurement, background regimens consisted of: 2 or 3 NRTIs (nucleoside reverse transcriptase inhibitors) in 35 patients (92%), 2 or 3 NRTIs plus 1 PI (amprenavir) in 2 patients (5%) and 4 NRTIs plus 1 NNRTI (non-nucleoside reverse transcriptase inhibitor, efavirenz) in one patient (3%).

3.2. Influence of MDR1 and PXR polymorphisms on lopinavir plasma concentration and response to HAART

To estimate the allelic and genotypic frequencies of *MDR1* (T-129C, C1236T and C3435T) and PXR (C63396T) SNPs in Argentinean population, we studied a group of 231 blood donors as shown in Table 2. We further compared the genotypic distribution between blood donors and HIV-1-infected children included in the pharmacogenetic evaluation and no significant differences were found. The *MDR1* 1236T, *MDR1* 3435T and PXR 63396T alleles were highly frequent (around 47%) in both groups, whereas the frequency of the *MDR1* -129C allele was only 5% and no homozygotes *MDR1* -129CC were found. All the genotypes studied fitted to Hardy–Weinberg equilibrium indicating that there was no bias in the study groups.

Next, we investigated whether *MDR1* and PXR polymorphisms affected LPV plasma level in HIV-1-infected children. Based on the adherence questionnaire, all the children had taken the corresponding LPV/r dose in the week previous to drug monitoring. We found that the *MDR1* C1236T SNP has a significant effect on LPV post-dose concentration ($p = 0.016$; Table 3). Children heterozygotes *MDR1* 1236CT showed lower LPV post-dose levels than homozygotes *MDR1* 1236TT, with a median $C_{post\text{-}dose}$ of 3.04 μg/ml and 6.50 μg/ml, respectively (Fig. 1A). In addition, LPV trough concentration was also lower in patients with the *MDR1* 1236CT genotype (median $C_{trough} = 2.29$ μg/ml) than in patients with the *MDR1* 1236TT genotype (median $C_{trough} = 6.30$ μg/ml), although the difference was not statistically significant (Table 3). There was no significant association between LPV trough and post-dose concentrations and the *MDR1* C3435T, *MDR1* T-129C and PXR C63396T SNPs. No significant differences were found between *MDR1* or PXR genotypes and plasma viral load or $CD4^+$ T-cell counts at the time of the determination of LPV plasma concentration (Table 1).

We further explored if the *MDR1* and PXR polymorphisms affected the virological and immunological response to HAART containing LPV/r. $CD4^+$ T-cell counts and plasma viral load were analyzed before (baseline) and after LPV/r exposure. Due to the retrospective design of the study, the virological and immunological response could only be analyzed in the available time points (Supplementary Table 1). Since there was a wide variation in the baseline of plasma viral load and $CD4^+$ T-cell counts, we established 20 weeks as the maximum period evaluated before the initiation of HAART containing LPV/r. The first and second time points for plasma viral load and $CD4^+$ T-cell counts were chosen close to the mean of the available data; 18 and 36 weeks for plasma viral load, and 21 and 41 weeks for $CD4^+$ T-cell counts. The median baseline viral load was similar among the four analyzed polymorphisms. However, we found that the *MDR1* C1236T polymorphism significantly affects the virological response to HAART containing LPV/r ($p = 0.047$; Table 4). Children heterozygotes *MDR1* 1236CT had a lower decrease in plasma viral load at week 36 than wild type homozygotes *MDR1* 1236CC, with median viral load changes of -0.50 $\log_{10}$ copies/ml and -2.08 $\log_{10}$ copies/ml, respectively (Fig. 1B). This difference was also observed at week 18, but it did not reach statistical significance (Table 4). Plasma viral load could be evaluated for the 38 children, 13 of them (34%) achieved virological suppression at week 18, 5 homozygotes 1236CC, 4 heterozygotes 1236CT and 4 homozygotes 1236TT. Virological response to HAART containing LPV/r was not associated with *MDR1* C3435T, *MDR1* T-129C and PXR C63396T SNPs. *MDR1* and PXR polymorphisms had no significant association with immunological change either at week 21 or at week 41 after initiation of LPV/r regimens.

These findings suggest that only the *MDR1* C1236T SNP significantly affects the LPV plasma levels with a consequent impact on drug efficiency to reduce viral replication.

Table 2
Genotypic and allelic frequencies of *MDR1* and PXR SNPs in HIV-seronegative blood donors and perinatally HIV-1-infected children.

Genotypes	HIV-1-infected children n (%)	Adult blood donors[a] n (%)	p[b]
MDR1 -129TT	34 (89%)	90 (90%)	0.927
MDR1 -129TC	4 (11%)	10 (10%)	
MDR1 -129CC	0 (0%)	0 (0%)	
Allele -129C frequency	0.05	0.05	
MDR1 1236CC	11 (29%)	72 (31%)	0.780
MDR1 1236CT	18 (47%)	115 (50%)	
MDR1 1236TT	9 (24%)	44 (19%)	
Allele 1236T frequency	0.47	0.44	
MDR1 3435CC	7 (18%)	80 (35%)	0.059
MDR1 3435CT	24 (63%)	100 (43%)	
MDR1 3435TT	7 (18%)	51 (22%)	
Allele 3435T frequency	0.50	0.44	
PXR 63396CC	8 (21%)	29 (29%)	0.518
PXR 63396CT	23 (61%)	50 (50%)	
PXR 63396TT	7 (18%)	21 (21%)	
Allele 63396T frequency	0.49	0.46	

Frequencies of the minor allele for each polymorphism are shown. All genotypic frequencies fitted to Hardy–Weinberg equilibrium.

[a] Data of *MDR1* C1236T and *MDR1* C3435T SNPs was previously published in Bellusci et al. (2010).

[b] Differences between HIV-1-infected children and adult blood donors in relation to genotypic frequencies were evaluated with Pearson's χ^2 test.

Table 3
Lopinavir plasma level according to *MDR1* and PXR genotypes.

Genotypes	Lopinavir plasma concentration—median, μg/ml [range]			
	n	C_{trough}	n	$C_{post\text{-}dose}$
MDR1 -129TT	34	5.12 [0.02–15.00]	30	5.26 [0.63–15.00]
MDR1 -129TC	4	2.96 [0.60–10.20]	4	4.15 [0.50–11.80]
MDR1 -129CC	0	–	0	–
p		1		1
MDR1 1236CC	11	7.90 [0.33–15.00]	11	9.70 [2.35–15.00]
MDR1 1236CT	18	2.29 [0.02–10.70]	15	3.04 [0.50–14.22]
MDR1 1236TT	9	6.30 [1.45–11.02]	8	6.50 [4.75–12.77]
p		0.152		0.016
MDR1 3435CC	7	3.57 [0.33–10.20]	7	3.04 [1.54–11.80]
MDR1 3435CT	24	3.22 [0.02–15.00]	21	5.00 [0.50–15.00]
MDR1 3435TT	7	6.30 [1.45–11.02]	6	6.32 [4.75–12.77]
p		0.988		0.656
PXR 63396CC	8	4.58 [0.33–15.00]	7	5.00 [2.35–11.90]
PXR 63396CT	23	3.57 [0.29–11.90]	20	4.93 [0.50–15.00]
PXR 63396TT	7	8.20 [0.02–10.70]	7	6.50 [1.12–14.22]
p		1		1

Differences between *MDR1* and PXR genotypes in relation to lopinavir trough (C_{trough}) and post-dose ($C_{post\text{-}dose}$) concentrations were evaluated with Kruskal–Wallis test applying the Bonferroni correction. In four patients the LPV post-dose concentration was not available.

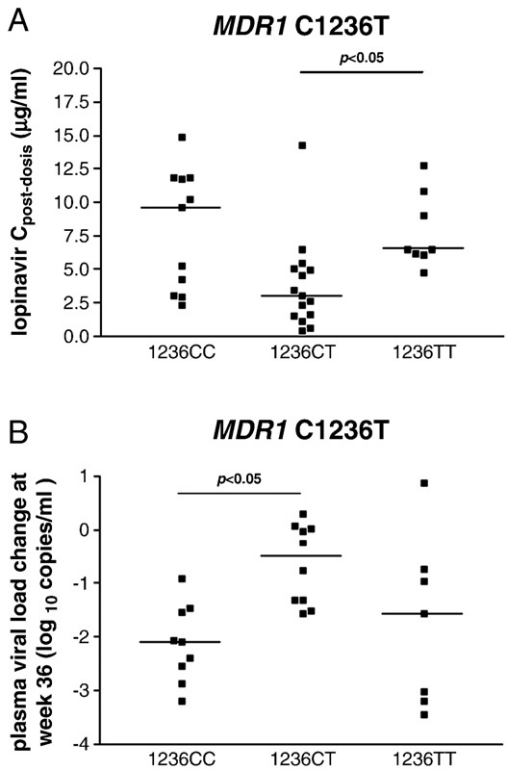

Fig. 1. Effect of the C1236T SNP on lopinavir post-dose concentration and change in plasma viral load. A. Differences between *MDR1* C1236T pairwise genotypes in relation to lopinavir post-dose concentration ($C_{post\text{-}dose}$, μg/ml) were evaluated using Dunn's Multiple Comparison test. Median $C_{post\text{-}dose}$ of each genotype and the significant p value are shown. B. Differences between *MDR1* C1236T pairwise genotypes in relation to change in plasma viral load at 36 weeks post-treatment initiation were evaluated using Dunn's Multiple Comparison test. Median viral load change of each genotype and the significant p value are depicted.

4. Discussion

In this study, we evaluated the effect of *MDR1* and PXR polymorphisms on the LPV plasma concentration and virological or immunological response in HIV-1-infected children of Caucasian descent. We found that heterozygosity for the *MDR1* C1236T polymorphism was significantly associated with a lower LPV level and a higher viral load at week 36 of LPV exposure.

MDR1 C1236T, *MDR1* C3435T and PXR C63396T polymorphisms were highly frequent in the adult Argentinean healthy group (~50%). Conversely, the *MDR1* -129C allele showed a frequency of only 5% and no homozygotes *MDR1* -129CC were found. The PXR 63396T allele is less frequent in the Argentinean group (46%) compared to Spanish (57%) (Schipani et al., 2010) and Italian populations (63%) (Schipani et al., 2010). Reports from Africa showed PXR 63396T allelic frequencies of 39% for Ghana and 45% for Kenya similar to our group (Schipani et al., 2010). The frequency of the *MDR1* T-129C SNP widely varies among populations, being 3% in Italian (Furuno et al., 2002), 8% in Japanese (Tanabe et al., 2001) and around 21% in African (Parathyras et al., 2009) populations. The frequency found in the Argentinean population (5%) was similar to the Caucasian and Asian groups but significantly different from the African population ($p < 0.05$).

Differences in allelic frequencies of the *MDR1* C1236T and C3435T SNPs in relation to other ethnic populations have been previously discussed by Bellusci et al. (2010). Briefly, the allelic frequencies of the *MDR1* 3435T allele in the Argentinean population are similar to those previously reported in European white populations of Germany, Italy and Spain (Bernal et al., 2003; Furuno et al., 2002; Hoffmeyer et al., 2000), but they significantly differ from those reported in African populations ($p < 0.05$). No difference in the distribution of *MDR1* variants between our population and populations from Asia was observed (China and Japan) (Ameyaw et al., 2001; Komoto et al., 2006). The frequency of the *MDR1* C1236T SNP was only reported in German (48%) (Hoffmeyer et al., 2000) and Japanese (66%) (Komoto et al., 2006) populations. The frequencies found in the Argentinean population were similar to the white group but significantly different from those found in the Asian population ($p < 0.05$).

There is a wide variation in the response to antiretroviral treatment, especially in children. In our study, 8/38 (21%) of the children did not reach the recommended LPV trough concentration of 1 μg/ml (van Luin et al., 2008). However, other studies suggested a LPV trough concentration of 4 μg/ml (Breilh et al., 2004) and 19/38 (50%) of the children of our group did not achieve this recommended level. Several studies tried to assess the influence of the *MDR1* C3435T SNP on antiretroviral response; however, it remains elusive (Estrela et al., 2009; Ma et al., 2007; Rakhmanina et al., 2011; Winzer et al., 2003). We found no association between the *MDR1* C3435T SNP and LPV plasma concentration. Our findings are in agreement with the recent report of Rakhmanina et al. (2011) that showed no significant differences in AUC and clearance

Table 4
Virological and immunological response to HAART regimen containing lopinavir according to *MDR1* and PXR genotypes.

Genotypes	Plasma VL—median, log_{10} copies/ml [range]				$CD4^+$ T-cell count—median, % [range]			
	n	Baseline[a]	Change at week 18	Change at week 36	n	Baseline[b]	Change at week 21	Change at week 41
MDR1 -129TT	25	5.17 [2.93–6.92]	−1.16 [−3.44–0.78]	−1.38 [−3.44–0.88]	19	14.00 [1.00–31.00]	2.00 [−4.00–30.00]	4.00 [−5.00–30.00]
MDR1 -129TC	2	5.17 [4.89–5.45]	−2.63 [−3.56–(−1.70)]	−3.02 [−3.19–(−2.85)]	3	38.00 [15.00–39.00]	0.00 [−5.00–8.00]	7.00 [2.00–8.00]
MDR1 -129CC	0	–	–	–		–	–	–
p		1	0.316	0.182		0.208	1	1
MDR1 1236CC	10	5.36 [3.04–6.92]	−1.47 [−3.56–(−0.04)]	−2.08 [−3.19–(−0.90)]	6	18.50 [7.00–38.00]	5.50 [0.00–30.00]	7.50 [0.00–30.00]
MDR1 1236CT	10	5.14 [4.60–5.84]	−0.64 [−1.72–0.78]	−0.50 [−1.57–0.31]	9	22.00 [10.00–39.00]	0.00 [−5.00–13.00]	3.00 [−5.00–10.00]
MDR1 1236TT	7	5.14 [2.93–5.99]	−1.91 [−3.44–(−0.65)]	−1.56 [−3.44–0.88]	7	2.00 [1.00–28.00]	2.00 [−2.00–15.00]	4.00 [−1.00–7.00]
p		1	0.120	0.047		0.365	0.627	0.725
MDR1 3435CC	5	5.45 [4.84–6.92]	−0.74 [−3.56–0.20]	−1.45 [−2.85–(−0.24)]	5	23.00 [10.00–39.00]	0.00 [−5.00–5.00]	2.00 [−5.00–8.00]
MDR1 3435CT	17	5.14 [3.04–6.38]	−1.26 [−3.44–0.78]	−1.51 [−3.44–0.31]	11	19.00 [7.00–31.00]	5.00 [−4.00–30.00]	7.00 [−4.00–30.00]
MDR1 3435TT	5	5.62 [2.93–5.99]	−1.16 [−3.01–(−0.65)]	−0.95 [−3.02–0.88]	6	2.00 [1.00–28.00]	3.50 [−2.00–15.00]	4.00 [0.00–7.00]
p		1	1	1		0.077	1	0.767
PXR 63396CC	7	5.59 [3.04–6.92]	−0.74 [−1.70–(−0.19)]	−1.10 [−3.19–0.31]	3	18.00 [15.00–26.00]	5.00 [0.00–8.00]	7.00 [0.00–8.00]
PXR 63396CT	15	5.14 [2.93–6.38]	−1.19 [−3.44–0.78]	−1.53 [−3.44–0.88]	14	12.50 [1.00–39.00]	3.50 [−5.00–30.00]	4.00 [−5.00–30.00]
PXR 63396TT	5	5.17 [5.00–5.45]	−1.72 [−3.56–(−0.09)]	−1.50 [−3.18–0.10]	5	24.00 [10.00–38.00]	0.00 [−4.00–5.00]	7.00 [−4.00–8.00]
p		1	1	1		0.948	0.685	1

Differences between *MDR1* and PXR genotypes in relation to baseline and changes in plasma VL and $CD4^+$ T-cell counts were evaluated with Kruskal–Wallis test applying the Bonferroni correction. VL: viral load.

[a] Plasma VL data was available in 27 patients.

[b] $CD4^+$ T-cell counts data was available in 22 patients.

of LPV among the *MDR1* C3435T and C2677T genotypes in a cohort of predominantly African-American HIV-1-infected children. Other studies performed in adult patients showed similar results when LPV trough levels were evaluated (Estrela et al., 2009; Ma et al., 2007; Winzer et al., 2003).

We found no effect of the *MDR1* C3435T SNP neither on immunological nor virological response to HAART containing LPV/r. Conversely, Fellay et al. (2002) reported that HIV-1-infected adults with the *MDR1* 3435TT genotype had the highest $CD4^+$ T-cell count recovery while Saitoh et al. (2005) found that pediatric patient heterozygotes *MDR1* 3435CT showed the highest decrease in viral load. However, several studies performed in adult cohorts were not able to corroborate these findings (Brumme et al., 2003; Haas et al., 2003; Hendrickson et al., 2008; Nasi et al., 2003; Parathyras et al., 2009; Verstuyft et al., 2005; Winzer et al., 2005; Zhu et al., 2004). Up to now, most of the evidence supports no effect of the *MDR1* C3435T SNP on the virological and immunological response to antiretroviral therapy.

The *MDR1* C3435T SNP presents strong but not complete linkage disequilibrium with the *MDR1* C1236T SNP on exon 12 (Kim et al., 2001). Therefore, we analyzed the influence of this SNP on LPV/r pharmacokinetic and virological or immunological response. Surprisingly, we found that HIV-1-infected children heterozygotes *MDR1* 1236CT had a significantly lower LPV plasma concentration ($C_{post\text{-}dose}$) compared to homozygotes *MDR1* 1236TT. However, la Porte et al. (2007) did not found significant correlation between this polymorphism and saquinavir C_{max} in adult healthy volunteers. In addition, we did not observed a significant association between *MDR1* C1236T genotypes and LPV trough concentration in accordance with a previous study of Estrela et al. (2009) performed in HIV-infected Brazilian adult males.

We also found that children heterozygotes *MDR1* 1236CT had a lower decrease of plasma viral load after 36 weeks of treatment initiation with LPV/r when compared with homozygotes *MDR1* 1236CC. However, we did not observe an impact of the *MDR1* C1236T SNP on the immunological response to HAART as has been reported by Parathyras et al. (2009). In contrast, Zhu et al. (2004) reported that patients with the *MDR1* 1236CC genotype showed a lower increase in $CD4^+$ T-cell counts at 1 and 9 months after initiation of HAART than those with the *MDR1* 1236TT genotype, but they did not find differences in rates of viral suppression. The main difference with our study is that they carried out the analysis in a group of adult patients that received different treatment combinations of PIs as part of HAART, whereas in our cohort LPV/r was used as the only PIs except for two patients who also had amprenavir. When the analysis was performed excluding these patients, the same trends were observed (data not shown).

The C63396T polymorphism of PXR was recently identified (Lamba et al., 2008) and associated with atazanavir trough level below the minimum effective concentration (150 ng/ml) in a cohort of HIV-1-infected adults (Caucasians) (Siccardi et al., 2008). However, we did not observe an effect of this SNP on LPV trough and post-dose plasma concentration or response to therapy in HIV-1-infected children. Extensive studies are needed to further determine the potential impact of PXR as a modifier of the response to antiretroviral therapy.

It should be noted that several factors, such as differences in ethnicity, age, HAART regimens and criteria applied for the analysis of the immunological and virological response to therapy (e.g. time points evaluated before and after treatment), disable the adequate comparison among pharmacogenetic studies.

We have previously reported that the *MDR1* 1236T allele significantly delays the onset of pediatric AIDS independently of the initiation of HAART (Bellusci et al., 2010), supporting the notion that the P-glycoprotein plays a role in the HIV-1 infection separately from its function in drug transport. In addition, the present study suggests that the *MDR1* C1236T polymorphism also has an important effect on the pharmacokinetic of LPV and virological response to HAART. Although the effect of the *MDR1* C1236T SNP on P-gp expression and/or its activity has not yet been clearly established, we can hypothesize that those carriers of the *MDR1* 1236CT genotype might have an altered level of P-gp expression/activity in enterocytes that limit the absorption of LPV and consequently reduce its concentration in $CD4^+$ T-cells, leading to a lower decrease of viral load. Further research is needed to support this hypothesis.

Conflict of interest

The authors declare that all actual or potential conflict of interests including any financial, personal or other relationships with other people or organizations within three years of beginning the submitted work that could inappropriately influence, or be perceived to influence, our work, have been disclosed.

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