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1 **Pharmacokinetic Changes during Pregnancy According to**
2 **Genetic Variants: A Prospective Study in HIV-infected**
3 **Patients Receiving Atazanavir/Ritonavir**

4
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20 **Abstract**

21 Atazanavir/ritonavir concentrations change over pregnancy in HIV-positive patients; the impact of
22 genetic variants is unknown. 20 patients were enrolled: antiretrovirals' plasma and intracellular
23 concentrations were measured in addition to single nucleotide polymorphisms in transport affecting
24 genes. Linear logistic regression showed that genetic variants in organic-anion-transporter-1B1 and
25 pregnane-X-receptor encoding genes affected third trimester atazanavir exposure. In this
26 prospective study genetic variants partially explained the observed inter-patient variability in third-
27 trimester antiretrovirals' exposure.

28

29 **Main text**

30 Antiretroviral (ARV) treatment of HIV-infected pregnant women is a key element to prevent mother-
31 to-child HIV transmission (MTCT) [1]. Despite ARVs' target being intracellular the knowledge on
32 these compounds pharmacokinetics mostly derives from plasma concentration. Some reports
33 suggested a potential correlation between PIs' ICs, their activity and clinical outcomes [2, 3, 4, 5, 6,
34 7]. One of the most preferred third agents of ARV regimen during pregnancy are protease Inhibitors
35 (PIs); however several changes in PIs exposure have been reported in third trimester. Following oral
36 administration this class of drugs is primarily metabolized by cytochrome P450 (CYP) 3A4 and is
37 substrate of the transmembrane efflux pump P-glycoprotein (P-gp) and of the Organic Anion
38 Transporters Protein 1B1 (OATP1B1) [8] [9].

39 All of these steps may be mediated by proteins regulated by different genes. Single-nucleotide
40 polymorphisms (SNPs) in Pregnane-X-Receptor (PXR), P-gp and OATP1B1 have been involved in
41 intracellular drug penetration [11, 12]. PXR is a nuclear transcription factor that influences the
42 expression of P-glycoprotein; PXR regulates the expression of CYP3A4 and ABCB1 and is associated
43 with atazanavir plasma concentration (T allele at position 63396) [13,14]. Several studies have
44 reported a relationship between the ABCB1 3435 C/T polymorphism and PIs activity. [15, 16] SNPs
45 in OATP1B1 encoding gene, in particular the 521C allele, have been associated with a decrease in
46 PIs transport, both *in vitro* and *in vivo*. [17,18,19,20]. Pregnancy induces changes in systemic gene
47 expression but there is lack of data about transporting genes changes during pregnancy [21].

48 Aim of this study was to evaluate changes in atazanavir (ATV) and ritonavir (RTV)
49 plasma/intracellular (IC) concentrations during pregnancy according to SNPs in PXR, P-gp and
50 OATP1B1 encoding genes.

51 HIV- pregnant patients treated with ATV/RTV (300/100 mg once daily, with food) based regimens
52 were prospectively enrolled after signing a written informed consent.

53 Plasma and IC (intra-peripheral blood mononuclear cell, PBMC) antiviral concentration of ATV and RTV
54 were measured at every visit using validated high-performance liquid chromatography (HPLC)/mass
55 (MS) spectrometry methods (with direct evaluation of cells volume) [22, 23].

56 PBMC-associated and plasma ATV and RTV concentrations were measured by validated HPLC–MS
57 and HPLC–photodiode array (PDA) methods, respectively. Median values of individual
58 measurements were considered and expressed as ng/mL.

59 Whole blood was stored at -20°C for pharmacogenetic analysis. Genomic DNA was extracted using
60 QIAamp whole blood mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's

61 instructions. Genotyping was conducted by real time-based allelic discrimination with the use of
62 standard methods (Applied Biosystems, Foster City, CA, USA). All primers, probes and PCR
63 conditions are available on request. The following single nucleotide polymorphisms were analysed:
64 *PXR* 63396 C>T (rs2472677, encoding for PXR), *ABCB1* 3435 C>T (rs1045642, encoding for P-
65 glycoprotein), *SLCO1B1* 521 T>C (rs4149056, encoding for OATP1B1) and grouped as dichotomous
66 variables (according to available literature data).

67 For descriptive statistics, continuous variables were described as medians (25th–75th percentiles,
68 IQR) and categorical variables as frequencies (percentages). All polymorphisms were tested for
69 Hardy–Weinberg equilibrium (<http://www.oege.org/software/hwe-mr-calc.shtml>). Mann-Whitney
70 analysis was used to compare previously identified relevant genotypes (*PXR* 63396TT, *ABCB1*
71 3435CT/TT and *SLCO1B1* 521TC/CC) with plasma and IC concentrations as well as IC-to-plasma-
72 ratios (IPr) and changes over time. Linear regression analysis was used to investigate the potential
73 influences of the three SNPs on plasma and IC concentrations changes over time. Statistical analyses
74 were performed using the PASW software package ver. 23.0 (SPSS, Chicago, IL, USA), accepting
75 statistical significance at two-sided p-values <0.05.

76 Twenty HIV-positive pregnant women were enrolled from November 2013 to January 2016. Baseline
77 characteristics are reported in Table 1. Viro-immunological, clinical and pharmacokinetic results have
78 been reported elsewhere [24].

79 *PXR* 63396 CC, CT and TT genotypes were observed in 6 (30%), 8 (40%) and 6 (30%) individuals,
80 respectively, *ABCB1* 3435 CC, CT and TT genotypes were observed in 12 (60%), 5 (25%) and 3 (15%)
81 and *SLCO1B1* 521 TT, TC and CC genotypes were observed in 16 (80%), 3 (15%) and 1 (5%). All SNPs
82 were in Hardy-Weinberg equilibrium ($p>0.05$). In *PXR* 63396 TT carriers third trimester ATV
83 concentrations were lower in plasma [468 ng/mL (range 262-746) vs. 810 ng/mL (341-1338), $p=0.39$]
84 and higher intracellularly [1224 ng/mL (823-1967) vs. 640 ng/mL (394-1906), $p=0.11$]; IPr were
85 significantly higher [2.6 (1.6-3.5) vs. 0.96 (0.5-2), $p=0.03$].

86 As compared to post-partum, ATV plasma concentrations were not significantly lower in the third
87 trimester [-315 ng/mL (-1121; +129)] with stable IPr [+0.12 (-0.44; +1.35)]. *PXR* 63396 TT carriers,
88 despite a higher decrease in plasma concentrations (-397 vs. -247 ng/mL) showed an increase in IC
89 levels (+95 vs. -241 ng/mL) and in IPr (+0.42 vs. +0.12). The association between ATV/RTV plasma,
90 IC and IPr according to the studied SNPs are shown in Figure 1 and Supplementary Table 1.

91 Logistic regression analysis (including all three genotypes) identifying predictors of third trimester
92 changes (versus post-partum concentrations) showed *SLCO1B1* 521 TC/CC genotypes as predictors

93 of ATV changes ($p=0.059$ for plasma and $p=0.051$ for IC) and *PXR* 63396 TT genotypes of RTV changes
94 (IPr, $p=0.025$) in the third trimester.

95 We observed minimal changes in ATV and RTV concentrations in HIV-positive pregnant patients;
96 this variability could be explained by genetic variants [24].

97 For this purpose, we studied SNPs in genes encoding OATP1B1, P-gp and *PXR*. *PXR* may influence
98 the expression of P-gp as well as other membrane transporters and it is activated by a huge variety
99 of endobiotics and xenobiotics, including several drugs [25, 26]. It has been demonstrated that
100 *PXR* regulates the expression of CYP3A4/5 and p-gp encoding genes and it has been associated
101 with ATV plasma exposure (T allele at position 63396) [14, 13]. We observed that a TT genotype in
102 *PXR* 63396 was associated with higher ATV IPr and less variability in IC concentrations during the
103 third trimester. Furthermore multivariate logistic regression analysis identified single nucleotide
104 polymorphisms in OATP1B1 encoding genes as being relevant for ATV plasma concentrations
105 hanges during the third trimester. Available data are limited but suggest that hepatic uptake (and
106 therefore the amount of drug available for metabolism) may be mediated by OATP1B1 transport
107 [8].

108 Possible limitations of this study are the limited sample size, the limited amount of drug
109 measurement and the lack of serum markers that could help understanding the complex interplay
110 between genetically determined and pregnancy-induced changes.

111 Further studies may clarify the role of different SNP on antiretroviral transport during pregnancy
112 as well as the clinical impact of IC ATV and RTV accumulation according to different genotypes.

113 These results may be relevant for allowing tailored treatment strategies during pregnancy.

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201

202 **Figure 1 Legend**

203

204 **Figure 1. Intracellular to plasma ratios of atazanavir (above) and ritonavir (below) according to**
205 **single nucleotide polymorphisms in the studied genes.** Boxes (ordered according to trimester of
206 pregnancy) represent interquartile ranges, horizontal lines median values and whiskers standard
207 deviations; circles and stars represent outliers.