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High Interpatient Variability of Raltegravir Cerebrospinal Fluid Concentrations in HIV-positive Patients: a Pharmacogenetic Analysis.

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Objectives: To analyse the determinants of raltegravir CSF penetration including pharmacogenetics of drug transporters located at the brain-blood-barrier or blood-CSF barrier.

Methods: Plasma and CSF raltegravir concentrations were determined by a validated High Performance Liquid Chromatography coupled with Mass Spectrometry method in adults on raltegravir-based combination antiretroviral therapy undergoing a lumbar puncture. Single nucleotide polymorphisms in the genes encoding drugs transporters (*ABCB1* 3435, *SLCO1A2*, *ABCC2* and *SLC22A6*) and for the nuclear factor HNF4α were determined by real-time PCR.

Results: In 41 patients (73.2% male, 96.3% Caucasians) medianraltegravir plasma and CSF concentrations were 165 ng/mL (83-552) and 31 ng/mL (21-56), respectively. CSF-to-plasma ratios (CPR) ranged from 0.005 to 1.33 [median 0.20, IQR (0.04-0.36)].raltegravir trough CSF concentrations (n=35) correlated withraltegravir plasma levels (rho=0.39, p=0.019); CPRs were higher in patients with blood brain barrier damage (0.47 versus 0.18, p=0.02). Hepatocyte nuclear factor 4 alpha (HNF4α) 613 CG genotype carriers had lower trough CSF concentrations (20 versus 37 ng/mL, p=0.03) and CPRs (0.12 versus 0.27, p=0.02). At multivariate linear regression analysis CSF to serum albumin ratio was the only independent predictor ofraltegravir penetration in the CSF.

Conclusions: Raltegravir penetration into the CSF shows a large inter-patient variability although cerebrospinal fluid concentrations result above wild type IC_{50} in all patients (and above IC_{95} in 28.6%). In this cohort blood brain barrier permeability is the only independent predictor ofraltegravir CSF to plasma ratio. The impact of single nucleotide polymorphisms in selected genes on raltegravir penetration warrants further studies.

Introduction

Antiretrovirals (ARVs) penetration into the central nervous system (measured as drug concentrations in the cerebrospinal fluid) has been associated with control of HIV replication and to neurocognitive function. Raltegravir (RAL) in combination with other ARVs has been proven to be effective and well-tolerated and to elicit a very fast viral load decay after treatment initiation.[1] Data on raltegravir CSF penetration derives from two papers and a small case-series:[2-4] drug concentrations in the CSF have been described to be 3-7.8% of plasma ones even if a wide inter-patient variability has been reported (with CSF-to-plasma ratios ranging from 0.01 to 0.61). In the first report[2] altered blood-brain barrier (BBB) was associated with higherraltegravir cerebrospinal fluid concentrations. Furthermore raltegravir has been proven to be p-glycoprotein and OAT1 substrate[5] and both transporters are expressed at the blood brain barrier or at the CSF-blood barrier (BCB).[6,7] Furthermore Hepatocyte nuclear factor 4 alpha (HNF4α), a zinc-finger protein, plays a role in the transcriptional control of drug transporters: among the genes regulated by HNF4α are a broad range of xenobiotic-metabolizing cytochrome P450 iso-enzymes, UDP-glucuronosyltransferases, sulfotransferases and transporters including organic anion transporter 2, organic cation transporter 1, the ABC transporter ABCC2, ABCC6, ABCG5 and ABCG8.[8,9] Recent data have shown that both OAT1 (and OAT3) and HNF4 are expressed at the choroid plexus and thus at the blood-CSF barrier.[10]

The primary objective of this study was to analyse the determinants of raltegravir cerebrospinal fluid penetration including plasma concentrations, blood brain barrier damage, concomitant antiretroviral drugs and single nucleotide polymorphisms (SNPs) in the genes encoding enzymes present at the blood-brain barrier (*ABCB1, SLCO1A2, ABCC2, SLC22A6* and *HNF4*).

Material and Methods

Adults on stable raltegravir-based combination antiretroviral therapy (more than two weeks on treatment) undergoing a lumbar puncture for clinical reasons were included. Patients signed a written informed consent and this protocol was approved by the our Institution Ethics Committee.

Plasma and CSF raltegravir concentrations (2 to15 hours after drug intake) were determined by validated High Performance Liquid Chromatography coupled with photo diode array detection (HPLC-PDA) and (modified for the CSF) Mass Spectrometry (HPLC-MS) methods, respectively.[11,12]

Trough concentrations were considered the ones collected after 10 to 14 hours after drug intake; less then 30 minutes passed from CSF withdrawal to plasma sampling.

SNPs in selected genes were obtained trough Real-time PCR [TaqMan Drug Metabolism Genotyping Assays (Applied Biosystem)]. The eight SNPs selected were (1) ABCB1 (encodes P-glycoprotein) 3435C→T (Ile1145Ile; rs1045642); 1236C→T (Gly412Gly; rs1128503); 2677G → A/T (A:Ala893Thr, T:Ala893Ser; rs2032582), (2) SLCO1A2 (encodes OATP1A2) 38A→G (Ile13Thr; rs10841795); 516A→C (Glu172Asp; rs11568563); (3) ABCC2 (encodes MRP-2) -24G→A (in the promoter; rs717620); (4) SLC22A6 (encodes OAT1) 453G→A (in the 5' UTR, rs4149170); (5) HNF4α (encodes HNF4α) 613C→G (in the promoter, rs1884613).

BBB damage was measured through Reibergram and measurement of albumin CSF to plasma ratios (CSARs): normal valued were considered below 6.5 below the age of 60 years and below 9.5 above this age threshold. [13]

Baseline characteristics were tested for correlation to raltegravir CSF concentration and ratio by the Spearman's test for continuous variables and by Mann-Whitney test for categorical variables. Associations between genotypes andraltegravir CSF penetration were tested by univariate and multivariate stepwise linear regression analyses: SNPs were categorized as dichotomous variables according to the results of univariate analysis. The impact of other variables was estimated with univariate analysis, and those with $P < 0.20$ were incorporated into multivariate analysis, in addition to the basic demographics such as age and sex. Statistical significance was defined at 2-sided P

value <0.05 while for the effect of single SNPs a correction for multiple comparison defined a P value <0.005. The online Hardy-Weinberg equilibrium calculator was used to test the selected SNPs. (available at http://www.oege.org/software/hwe-mr-calc.shtmL). All other statistical analyses were performed with the Statistical Package for Social Sciences ver. 20.0 (IBM Corp. Released 2011. Armonk, NY: IBM Corp). Data are presented as medians (interquartile ranges).

Results

Forty-one patients (30, 73.2% male) were enrolled; median age and BMI were respectively 44 years (39-50) and 20.9 kg/m^2 (18.7-22.7). Spinal tabs were performed in patients with HIV-associated neurological disorders [19, 46.3%; mostly neurological symptoms in the course of non CNS opportunistic infections (10, 24.4%), HIV-associated neurocognitive disorders (6, 14.6%) and non JCV-related leucoencephalopathy (3, 7.3%)], follow-up of opportunistic diseases [15, 36.6%; non-Hodgkin's lymphomas (4, 9.7%), Burkitt's lymphoma (4, 9.7%), previous neurotoxoplasmosis (3, 7.3%), previous tubercular meningitis (2, 4.9%), previous cryptococcal meningitis (2, 4.9%)] or for differential diagnosis of other clinical conditions (4, 9.7% such as seizures and hepatic encephalopathy). Median CD4 cell count was 256 cells/uL (140-471), median plasma HIV RNA level 1.76 $\log_{10}$ copies/mL (1.28-2.61), and median CSF HIV RNA level 1.96 $\log_{10}$ copies/mL (1.28-2.95). The majority of patients presented concordant plasma and CSF viral loads: either both below 20 copies/mL (13, 31.7%) or above 20 copies/mL (19, 46.3%); patients with neurological complaints in the course of non CNS opportunistic infections had the highest plasma and CSF viral loads (10 patients, 1947 copies/mL and 1117 copies/mL) while the remaining 31 subjects had HIV RNA in both compartments below 1000 copies/mL.

Raltegravir was used in combination with different drugs in dual-regimens [with a boosted protease inhibitor (PI), n=8], in three-drugs combination [n=15, mainly with two nucleos(t)ide reverse transcriptase inhibitors (NRTI), n=7] or in intensified four-drugs treatments [n=18, associated with 2 NRTIs and a boosted PI (n=12) or a non-nucleoside reverse transcriptase inhibitor (n=6)].

CSF cells were absent in the majority of patients (38, 92,7%): one presented 7 cell/mL while two patients in the follow up of cryptococcal meningitis showed 40 and 60 cells/mL). Median CSF-serum albumin ratio (CSAR) was 5.6 (3.7-7.2) defining altered BBBs in 12 patients (29.2%). Patients with previous opportunistic infections had the highest prevalence of impaired BBB [10/15, 66.7% with median CSAR of 7 (6.2-8].

CSF and plasma raltegravir concentrations were 31 ng/mL (21-56) (Fig. 1) and 165 ng/mL (83-552) accounting for 20.6% (3.8-36.3) of plasma drug concentrations.

In patients with trough determinations (n=35), CSF and plasma concentrations and CSF-to-plasma ratios (CPRs) were 32 ng/mL (21-57), 147 ng/mL (65-307) and 0.22 (0.12-0.47) respectively. Coefficients of variation for the three variables were 108%, 188% and 100%. Using recently published reference values[14] no patient's concentration was below IC_{50} (3.6 ng/mL), 25 (71.4%) were between IC_{50} and IC_{95} (44 ng/mL) and 10 (28.6%) were above IC_{95}.

CSF raltegravir concentrations correlated with plasma concentrations (rho=0.395, p=0.019). Gender, age, BMI, time after drug intake and concomitant protease inhibitors in the regimen did not significantly influence raltegravir CSF levels and ratios (Spearmen's correlations test). Although a direct correlation betweenraltegravir CPR and CSAR was not statistically significant (rho=0.306, p=0.10) patients with BBB damage showed higherraltegravir CSF-to-plasma ratios [0.47 (0.23-1.13) versus 0.18 (0.06-0.29), p=0.02, Mann-Whitney] (Fig.2b) but not CSF concentrations [42 ng/mL (21-73) versus 30 ng/mL (20-43), p=0.23] (Fig.2a).

Data of single nucleotide polymorphisms prevalence and effect on trough CSF concentrations and CSF to plasma ratios are resumed in Table 1.

All polymorphisms were in Hardy-Weinberg equilibrium but the ABCB1 3435C→T and the ABCB1 2677G→A/T.

At multivariate linear regression analysis (including alsoraltegravir plasma concentrations and HNF4α CG genotype with backward elimination) CSAR was the only independent predictor ofraltegravir CSF concentrations (adjusted R^2=0.61, Beta=0.79, P<0.001, 95% CI 5.50-10.19). At

multivariate linear regression analysis CSAR was the only independent predictor ofraltegravir CSF-to-plasma ratios (adjusted R^2=0.30, Beta=0.57, P=0.001, 95% CI 0.02-0.06) with a non-significant effect of HNF4α CG genotype (Beta=-0.26, p=0.09, 95% CI -0.04+0.03).

Discussion

These data confirm the penetration of raltegravir in the cerebrospinal fluid although reporting increased CSF to plasma ratios (22% versus the previously reported 3-8%). In the other studies the percentage of patients with significant blood brain barrier impairment was not reported but a small effect was noted in one of those: furthermore one patient with three samples showed a reduction inraltegravir CPRs with the concomitant decrease in CSARs. This aspect suggests that CSF pharmacokinetic studies should be performed in patients with different BBB and BCB permeability since other drugs have shown a similar pattern[15] but reporting the extent of BBB damage. The clinical impact of such increased penetration is unclear since it may reflect higher total drug levels bound to albumin or to other proteins present in the CSF.[16,17] Furthermore a efficacy cut offs in the CSF have not been validated: CSF and brain parenchyma levels can differ substantially although drugs with higher neuropenetration/neuroefficacy have been associated with the decreased likelihood of CSF viral replication.[18,19] The report of all patients with CSF levels above the published IC_{50} suggests that the measured concentrations are potentially effective although we have no data on the drug free fraction.

A linear correlation was noted between CSF and plasma concentrations as in the other papers. Nevertheless at multivariate analysis the CSF to serum albumin ratio is the only independent factor that partially explains the variability in CSF levels (60%) and in CSF penetration (30%). Being blood brain barrier impairment quite common in the course of HIV infection[20] this could have potential long-term effects: recently age and CSAR have been described as risk factors for the development of HIV-associated Neurocognitive Disorders.[21]

Although SNPs in genes encoding enzymes involved in raltegravir transport (P-glycoprotein and OAT1 and potentially OATP1A2 and MRP-2) at the BBB or BCB could potentially modulate drug passage into the CSF, this study showed no such significant relationship. Furthermore it should be noted that the precise effect of the different transporters present at the CNS barriers on CSF or parenchyma drugs exposure is currently unclear. Anyhow, the effect of SNPs in the HNF4α gene is an interesting finding although multiple comparison may probably explain this results since after Bonferroni correction it did not retain statistical significancy. This intra nuclear factor has been described to regulate (along with PXR and CAR) several pathways and specifically the ones leading to the expression of OAT1, OAT2 and OCT1.[9] OAT1 is present at choroid plexus and has the potential to regulate the passage of drugs at the blood CSF barrier[10] and being raltegravir substrate of this transporter a possible mechanism could be foreseen. Nevertheless with a limited samples size and with ABCB1 polymorphisms not in Hardy-Weinberg equilibrium (possibly representing population selection bias) we are not able to show clear effect of the studied SNPs. Furthermore the co-administered drugs may potentially modulate drug transport at the BBB: while we found no effect of protease inhibitors on raltegravir CSF penetration we had insufficient patients groups to analyse other drugs influence (NNRTIs, NRTIs).

In conclusion, this study shows that raltegravir concentrations in the cerebrospinal fluid are above the IC_{50} in all studied patients with a very high inter-patient variability. Blood brain barrier permeability is associated with raltegravir CSF concentrations and CSF-to-plasma ratios; larger sample sizes are needed to fully investigate the effect on raltegravir neuropenetration of single nucleotide polymorphisms in transporters-encoding genes.

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References

1. Rockstroh JK, Dejesus E, Lennox JL, *et al.* Durable Efficacy and Safety of Raltegravir versusEfavirenz When Combined With Tenofovir/Emtricitabine In Treatment-Naive HIV-1Infected Patients: Final Five-Year Results From STARTMRK. *J Acquir Immune DeficSyndr*. 2013; **63**:77-85.
2. Yilmaz A, Gisslén M, Spudich S, *et al.* Raltegravir cerebrospinal fluid concentrations in HIV-1 infection. *PLoS One*. 2009; **4**:e6877.
3. Calcagno A, Bonora S, Bertucci R, *et al.* Raltegravir penetration in the cerebrospinal fluid of HIV-positive patients. *AIDS*. 2010; **24**:931-2.
4. Croteau D, Letendre S, Best BM, *et al.* Total raltegravir concentrations in cerebrospinal fluid exceed the 50-percent inhibitory concentration for wild-type HIV-1. *Antimicrob Agents Chemother*. 2010; **54**:5156-60.
5. Moss DM, Kwan WS, Liptrott NJ, *et al.* Raltegravir is a substrate for SLC22A6: a putative mechanism for the interaction between raltegravir and tenofovir. *Antimicrob Agents Chemother*. 2011; **55**:879-87.
6. Sykes D, Sweet DH, Lowes S, *et al*. Organic anion transport in choroid plexus from wild-type and organic anion transporter 3 (Slc22a8)-null mice. *Am J Physiol Renal Phys*iol. 2004; **286**:972-8.
7. Thuerauf N, Fromm MF. The role of the transporter P-glycoprotein for disposition and effects of centrally acting drugs and for the pathogenesis of CNS diseases. *Eur Arch Psychiatry Clin Neurosci*. 2006; **256**:281-6.
8. Niehof M, Borlak J. Expression of HNF4alpha in the human and rat choroid plexus: implications for drug transport across the blood-cerebrospinal-fluid (CSF) barrier. *BMC Mol Biol*. 2009; **10**:68.
9. Ogasawara K, Terada T, Asaka J, *et al.* Hepatocyte nuclear factor-4{alpha} regulates the human organic anion transporter 1 gene in the kidney. *Am J Physiol Renal Physiol*. 2007; **292**:1819-26

10. Nagle MA, Wu W, Eraly SA, Nigam SK. Organic anion transport pathways in antiviral handling in choroid plexus in Oat1 (Slc22a6) and Oat3 (Slc22a8) deficient tissue. *Neurosci Lett.* 2013; **534**:133-8.
11. D'Avolio A, Baietto L, Siccardi M, *et al.* An HPLC-PDA method for the simultaneous quantification of the HIV integrase inhibitor raltegravir, the new nonnucleoside reverse transcriptase inhibitor etravirine, and 11 other antiretroviral agents in the plasma of HIV-infected patients. *Ther Drug Monit*. 2008; **30**:662-9.
12. D'Avolio A, Siccardi M, Sciandra M, *et al.* HPLC-MS method for the simultaneous quantification of the new HIV protease inhibitor darunavir, and 11 other antiretroviral agents in plasma of HIV-infected patients. *J Chromatogr B Analyt Technol Biomed Life* Sci. 2007; **859**:234-40. Erratum in: *J Chromatogr B Analyt Technol Biomed Life Sci*. 2008; **862**:265.
13. Tibbling G, Link H, Ohman S. Principles of albumin and IgG analyses in neurological disorders. I. Establishment of reference values. *Scand J Clin Lab Invest* 1977; **37**: 385–390.
14. Acosta EP, Limoli KL, Trinh L, *et al.* Novel method to assess antiretroviral target trough concentrations using in vitro susceptibility data. *Antimicrob Agents Chemother*. 2012; **56**:5938-45.
15. Calcagno A, Bonora S, Simiele M, *et al.* Tenofovir and emtricitabine cerebrospinal fluid-to-plasma ratios correlate to the extent of blood-brainbarrier damage. *AIDS*. 2011; **25**:1437-9.
16. Nguyen A, Rossi S, Croteau D, *et al*. Etravirine in CSF is highly protein bound. *J Antimicrob Chemother* 2013; **68**:1161-8.
17. Marchi N, Betto G, Fazio V, *et al.* Blood-brain barrier damage and brain penetration of antiepileptic drugs: role of serum proteins and brain edema. *Epilepsia*. 2009;**50**:664-77.
18. Letendre S, Ellis R, Deutsch R *et al*. Correlates of time-to-loss-of-viral-response in CSF and plasma in the CHARTER Cohort. *In Abstracts from the Seventeenth Conference on Retroviruses and Opportunistic Infection*s, 2010, San Francisco, USA. Abstract 430.
19. Giancola ML, Lorenzini P, Cingolani A, *et al*. Virological Response in Cerebrospinal Fluid to Antiretroviral Therapy in a Large Italian Cohort of HIV-Infected Patients with Neurological Disorders. *AIDS Res Treat.* 2012; **2012**:708456.
20. Abdulle S, Hagberg L, Gisslén M. Effects of antiretroviral treatment on blood-brain barrier integrity and intrathecal immunoglobulin production in neuroasymptomatic HIV-1-infected patients. *HIV Med*. 2005; **6**:164-9.
21. Letendre S, Croteau D, Ellis R, *et al*. Lower CSAR Are Associated with Global Neurocognitive Impairment in Antiretroviral-treated People with HIV. *In Abstracts from the Eighteenth Conference on Retroviruses and Opportunistic Infections*, 2011, Boston, USA. Abstract 408.

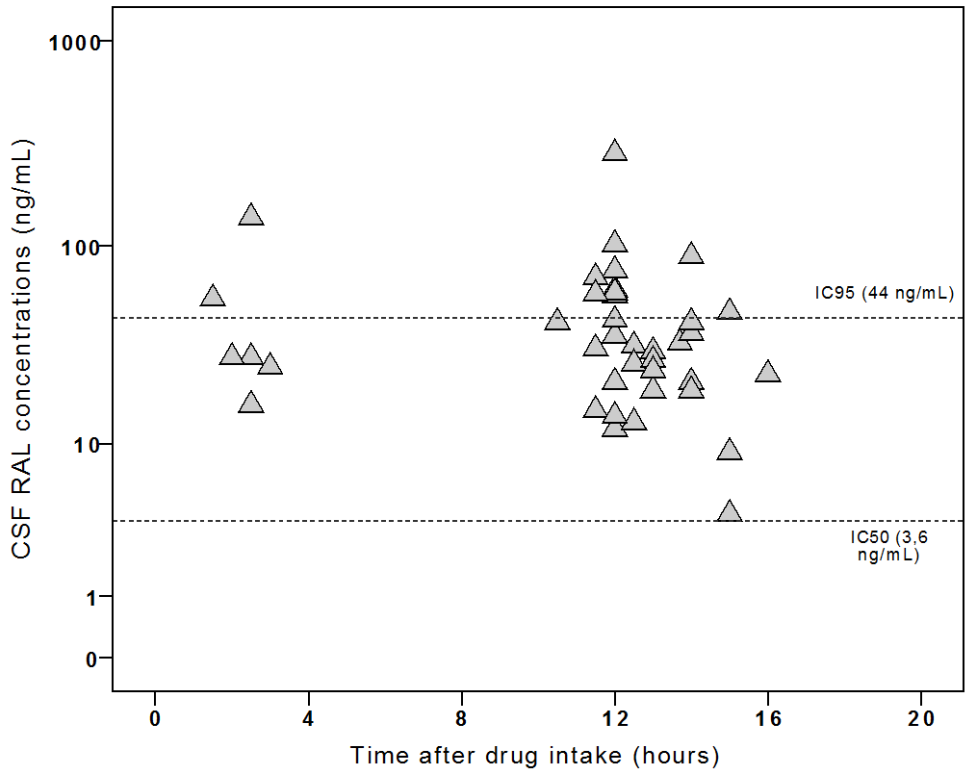
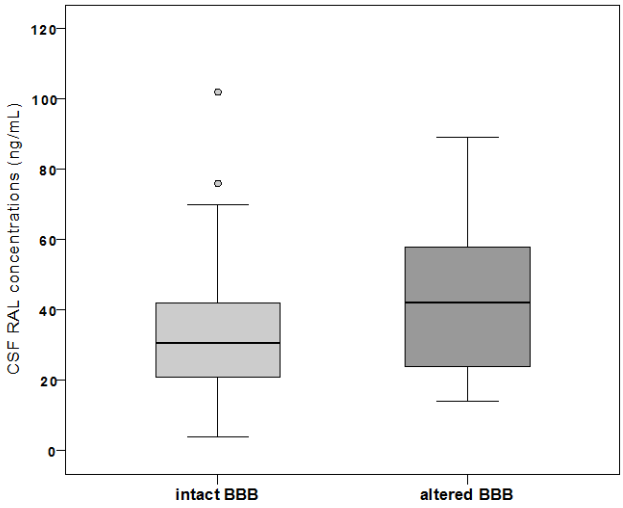
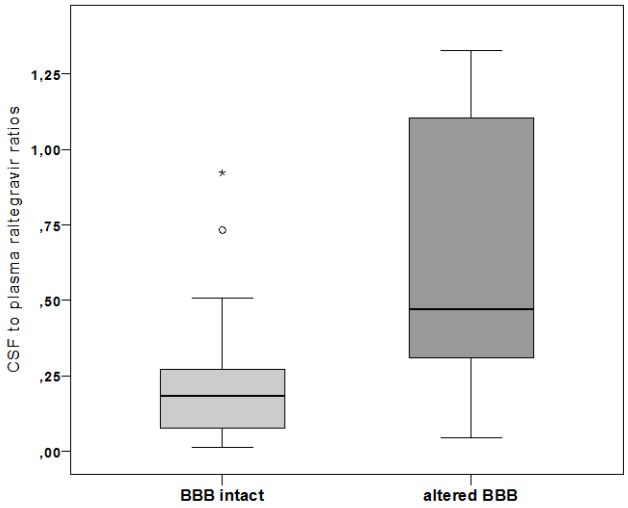


Figure 1. Raltegravir cerebrospinal fluid concentrations (Log_{10} ng/mL) according to time after drug intake (hours). Dotted lines represent IC_{50} and IC_{95} (in ng/mL).





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344 **Figure 2.** Raltegravir CSF concentrations (ng/mL, Figure 2a) and CSF-to-plasma ratios (Figure 2b)
345 in patients with altered and intact blood brain barrier. Central lines and boxes represents medians
346 and interquartile ranges; open circles and asterisks respectively represent outliers and extreme
347 outliers.
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genotype		n	CSF RAL conc	p value	CSF-P RAL ratio	p value
ABCB1						
3435C→T	rs1045642					
C/C		15	47 (19-70)		0.22 (0.12-0.50)	
C/T		8	26 (21-41)	0.40	0.23 (0.20-0.31)	0.97
T/T		12	31 (19-43)		0.24 (0.06-0.80)	
1236C→T	rs1128503					
C/C		14	32 (18-59)		0.21 (0.11-0.55)	
C/T		13	37 (22-56)	0.51	0.23 (0.14-0.32)	0.69
T/T		8	25 (16-40)		0.28 (0.07-1.05)	
2677G→A/T	rs2032582					
G/G		15	31 (19-59)		0.20 (0.07-0.50)	
G/A		1	102		0.17	
G/T		11	28 (21-42)	0.50	0.22 (0.22-0.31)	0.98
T/T		14	31(20-46)		0.17 (0.02-0.58)	
A/A		0	-		-	
SLCO1A2						
38A→G	rs10841795					
A/A		25	32 (21-57)		0.27 (0.14-0.92)	
A/G		9	36 (20-56)	0.43	0.17 (0.07-0.35)	0.29
G/G		1	14		0.05	
516A→C	rs11568563					
A/A		34	31 (20-57)		0.22 (0.12-0.39)	
A/C		1	42	0.71	1.10	0.17
C/C		0	-		-	
ABCC2						
-24G→A	rs717620					
G/G		19	33 (23-47)		0.20 (0.13-0.47)	
G/A		15	30 (19-59)	0.40	0.27 (0.12-0.48)	0.42
A/A		1	14		0.05	
SLC22A6						
555G→A	rs4149170					
G/G		28	32.5 (21-59)		0.22 (0.11-0.44)	
G/A		6	30 (13-43)	0.50	0.20 (0.10-0.63)	0.92
A/A		1	21		0.19	
HNF4α						
4613X→Y	rs1884613					
C/C		25	37 (26-58)		0.27 (0.17-0.49)	
C/G		10	20 (15-29)	0.03	0.12 (0.04-0.24)	0.02
G/G		0	-		-	

Table 1. Genotype frequencies of different single nucleotide polymorphisms and their effect on Raltegravir cerebrospinal fluid concentrations (ng/mL) and CSF-to-plasma ratios. Abbreviations: n=number, conc=concentration