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**High interpatient variability of raltegravir CSF concentrations in HIV-positive patients: a pharmacogenetic analysis.**

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*Original Citation:*

*Availability:*

This version is available <http://hdl.handle.net/2318/140932> since 2016-06-09T15:56:01Z

*Published version:*

DOI:10.1093/jac/dkt339

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## UNIVERSITÀ DEGLI STUDI DI TORINO

This is an author version of the contribution published on:

Calcagno A, Cusato J, Simiele M, Motta I, Audagnotto S, Bracchi M, D'Avolio  
A, Di Perri G, Bonora S

High interpatient variability of raltegravir CSF concentrations in HIV-positive  
patients: a pharmacogenetic analysis.

JOURNAL OF ANTIMICROBIAL CHEMOTHERAPY (2014) 69

DOI: 10.1093/jac/dkt339

The definitive version is available at:

<http://www.jac.oxfordjournals.org/cgi/doi/10.1093/jac/dkt339>

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

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7  
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12 **Running Head:** Raltegravir PK/PG in the CSF

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14 **Type of article:** Concise Communication

15  
16 **Key words:** antiretroviral; pharmacokinetics; blood brain barrier; p-glycoprotein; hepatic nuclear  
17 factor 4 alpha.

18  
19 **Word count:** 1760

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

42 **Methods:** Plasma and CSF raltegravir concentrations were determined by a validated High  
43 Performance Liquid Chromatography coupled with Mass Spectrometry method in adults on  
44 raltegravir-based combination antiretroviral therapy undergoing a lumbar puncture. Single  
45 nucleotide polymorphisms in the genes encoding drugs transporters (*ABCB1* 3435, *SLCO1A2*,  
46 *ABCC2* and *SLC22A6*) and for the nuclear factor HNF4 $\alpha$  were determined by real-time PCR.

47 **Results:** In 41 patients (73.2% male, 96.3% Caucasians) medianraltegravir plasma and CSF  
48 concentrations were 165 ng/mL (83-552) and 31 ng/mL (21-56), respectively. CSF-to-plasma ratios  
49 (CPR) ranged from 0.005 to 1.33 [median 0.20, IQR (0.04-0.36)].raltegravir trough CSF  
50 concentrations (n=35) correlated withraltegravir plasma levels ( $\rho=0.39$ ,  $p=0.019$ ); CPRs were  
51 higher in patients with blood brain barrier damage (0.47 versus 0.18,  $p=0.02$ ). Hepatocyte nuclear  
52 factor 4 alpha (HNF4 $\alpha$ ) 613 CG genotype carriers had lower trough CSF concentrations (20 versus  
53 37 ng/mL,  $p=0.03$ ) and CPRs (0.12 versus 0.27,  $p=0.02$ ). At multivariate linear regression analysis  
54 CSF to serum albumin ratio was the only independent predictor ofraltegravir penetration in the CSF.

55 **Conclusions:** Raltegravir penetration into the CSF shows a large inter-patient variability although  
56 cerebrospinal fluid concentrations result above wild type IC<sub>50</sub> in all patients (and above IC<sub>95</sub> in  
57 28.6%). In this cohort blood brain barrier permeability is the only independent predictor  
58 ofraltegravir CSF to plasma ratio. The impact of single nucleotide polymorphisms in selected genes  
59 on raltegravir penetration warrants further studies.

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## 66 **Introduction**

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

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

88

## 89 **Material and Methods**

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

93 Plasma and CSF raltegravir concentrations (2 to 15 hours after drug intake) were determined by  
94 validated High Performance Liquid Chromatography coupled with photo diode array detection  
95 (HPLC-PDA) and (modified for the CSF) Mass Spectrometry (HPLC-MS) methods,  
96 respectively.<sup>11,12</sup>

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

99 SNPs in selected genes were obtained through Real-time PCR [TaqMan Drug Metabolism  
100 Genotyping Assays (Applied Biosystem)]. The eight SNPs selected were (1) ABCB1 (encodes P-  
101 glycoprotein) 3435C→T (Ile1145Ile; rs1045642); 1236C→T (Gly412Gly; rs1128503); 2677G →  
102 A/T (A:Ala893Thr, T:Ala893Ser; rs2032582), (2) SLCO1A2 (encodes OATP1A2) 38A→G  
103 (Ile13Thr; rs10841795); 516A→C (Glu172Asp; rs11568563); (3) ABCC2 (encodes MRP-2) -  
104 24G→A (in the promoter; rs717620); (4) SLC22A6 (encodes OAT1) 453G→A (in the 5' UTR,  
105 rs4149170); (5) HNF4α (encodes HNF4α) 613C→G (in the promoter, rs1884613).

106 BBB damage was measured through Reibergram and measurement of albumin CSF to plasma ratios  
107 (CSARs): normal values were considered below 6.5 below the age of 60 years and below 9.5 above  
108 this age threshold.<sup>13</sup>

109 Baseline characteristics were tested for correlation to raltegravir CSF concentration and ratio by the  
110 Spearman's test for continuous variables and by Mann-Whitney test for categorical variables.

111 Associations between genotypes and raltegravir CSF penetration were tested by univariate and  
112 multivariate stepwise linear regression analyses: SNPs were categorized as dichotomous variables  
113 according to the results of univariate analysis. The impact of other variables was estimated with  
114 univariate analysis, and those with  $P < 0.20$  were incorporated into multivariate analysis, in addition  
115 to the basic demographics such as age and sex. Statistical significance was defined at 2-sided  $P$

116 value <0.05 while for the effect of single SNPs a correction for multiple comparison defined a P  
117 value <0.005. The online Hardy-Weinberg equilibrium calculator was used to test the selected  
118 SNPs. (available at <http://www.oege.org/software/hwe-mr-calc.shtml>). All other statistical analyses  
119 were performed with the Statistical Package for Social Sciences ver. 20.0 (IBM Corp. Released  
120 2011. Armonk, NY: IBM Corp). Data are presented as medians (interquartile ranges).

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## 122 **Results**

123 Forty-one patients (30, 73.2% male) were enrolled; median age and BMI were respectively 44 years  
124 (39-50) and 20.9 kg/m<sup>2</sup> (18.7-22.7). Spinal taps were performed in patients with HIV-associated  
125 neurological disorders [19, 46.3%; mostly neurological symptoms in the course of non CNS  
126 opportunistic infections (10, 24.4%), HIV-associated neurocognitive disorders (6, 14.6%) and non  
127 JCV-related leucoencephalopathy (3, 7.3%)], follow-up of opportunistic diseases [15, 36.6%; non-  
128 Hodgkin's lymphomas (4, 9.7%), Burkitt's lymphoma (4, 9.7%), previous neurotoxoplasmosis (3,  
129 7.3%), previous tubercular meningitis (2, 4.9%), previous cryptococcal meningitis (2, 4.9%)] or for  
130 differential diagnosis of other clinical conditions (4, 9.7% such as seizures and hepatic  
131 encephalopathy). Median CD4 cell count was 256 cells/uL (140-471), median plasma HIV RNA  
132 level 1.76 log<sub>10</sub> copies/mL (1.28-2.61), and median CSF HIV RNA level 1.96 log<sub>10</sub> copies/mL  
133 (1.28-2.95). The majority of patients presented concordant plasma and CSF viral loads: either both  
134 below 20 copies/mL (13, 31.7%) or above 20 copies/mL (19, 46.3%); patients with neurological  
135 complaints in the course of non CNS opportunistic infections had the highest plasma and CSF viral  
136 loads (10 patients, 1947 copies/mL and 1117 copies/mL) while the remaining 31 subjects had HIV  
137 RNA in both compartments below 1000 copies/mL.

138 Raltegravir was used in combination with different drugs in dual-regimens [with a boosted protease  
139 inhibitor (PI), n=8], in three-drugs combination [n=15, mainly with two nucleos(t)ide reverse  
140 transcriptase inhibitors (NRTI), n=7] or in intensified four-drugs treatments [n=18, associated with  
141 2 NRTIs and a boosted PI (n=12) or a non-nucleoside reverse transcriptase inhibitor (n=6)].

142 CSF cells were absent in the majority of patients (38, 92,7%): one presented 7 cell/mL while two  
143 patients in the follow up of cryptococcal meningitis showed 40 and 60 cells/mL). Median CSF-  
144 serum albumin ratio (CSAR) was 5.6 (3.7-7.2) defining altered BBBs in 12 patients (29.2%).  
145 Patients with previous opportunistic infections had the highest prevalence of impaired BBB [10/15,  
146 66.7% with median CSAR of 7 (6.2-8)].

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

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

154 CSF raltegravir concentrations correlated with plasma concentrations ( $\rho=0.395$ ,  $p=0.019$ ).  
155 Gender, age, BMI, time after drug intake and concomitant protease inhibitors in the regimen did not  
156 significantly influence raltegravir CSF levels and ratios (Spearman's correlations test). Although a  
157 direct correlation between raltegravir CPR and CSAR was not statistically significant ( $\rho=0.306$ ,  
158  $p=0.10$ ) patients with BBB damage showed high raltegravir CSF-to-plasma ratios [0.47 (0.23-  
159 1.13) versus 0.18 (0.06-0.29),  $p=0.02$ , Mann-Whitney] (Fig.2b) but not CSF concentrations [42  
160 ng/mL (21-73) versus 30 ng/mL (20-43),  $p=0.23$ ] (Fig.2a).

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

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

165 At multivariate linear regression analysis (including also raltegravir plasma concentrations and  
166 HNF4 $\alpha$  CG genotype with backward elimination) CSAR was the only independent predictor  
167 of raltegravir CSF concentrations (adjusted R<sup>2</sup>=0.61, Beta=0.79, P<0.001, 95% CI 5.50-10.19). At



168 multivariate linear regression analysis CSAR was the only independent predictor of raltegravir CSF-  
169 to-plasma ratios (adjusted  $R^2=0.30$ ,  $\text{Beta}=0.57$ ,  $P=0.001$ , 95% CI 0.02-0.06) with a non-significant  
170 effect of HNF4 $\alpha$  CG genotype ( $\text{Beta}=-0.26$ ,  $p=0.09$ , 95% CI -0.04+0.03).

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## 173 Discussion

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

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

194 Although SNPs in genes encoding enzymes involved in raltegravir transport (P-glycoprotein and  
195 OAT1 and potentially OATP1A2 and MRP-2) at the BBB or BCB could potentially modulate drug  
196 passage into the CSF, this study showed no such significant relationship. Furthermore it should be  
197 noted that the precise effect of the different transporters present at the CNS barriers on CSF or  
198 parenchyma drugs exposure is currently unclear. Anyhow, the effect of SNPs in the HNF4 $\alpha$  gene is  
199 an interesting finding although multiple comparison may probably explain this results since after  
200 Bonferroni correction it did not retain statistical significancy. This intra nuclear factor has been  
201 described to regulate (along with PXR and CAR) several pathways and specifically the ones leading  
202 to the expression of OAT1, OAT2 and OCT1.<sup>9</sup> OAT1 is present at choroid plexus and has the  
203 potential to regulate the passage of drugs at the blood CSF barrier<sup>10</sup> and being raltegravir substrate  
204 of this transporter a possible mechanism could be foreseen. Nevertheless with a limited samples  
205 size and with ABCB1 polymorphisms not in Hardy-Weinberg equilibrium (possibly representing  
206 population selection bias) we are not able to show clear effect of the studied SNPs. Furthermore the  
207 co-administered drugs may potentially modulate drug transport at the BBB: while we found no  
208 effect of protease inhibitors on raltegravir CSF penetration we had insufficient patients groups to  
209 analyse other drugs influence (NNRTIs, NRTIs).

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

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219 **Funding:** This work was supported by internal funding.

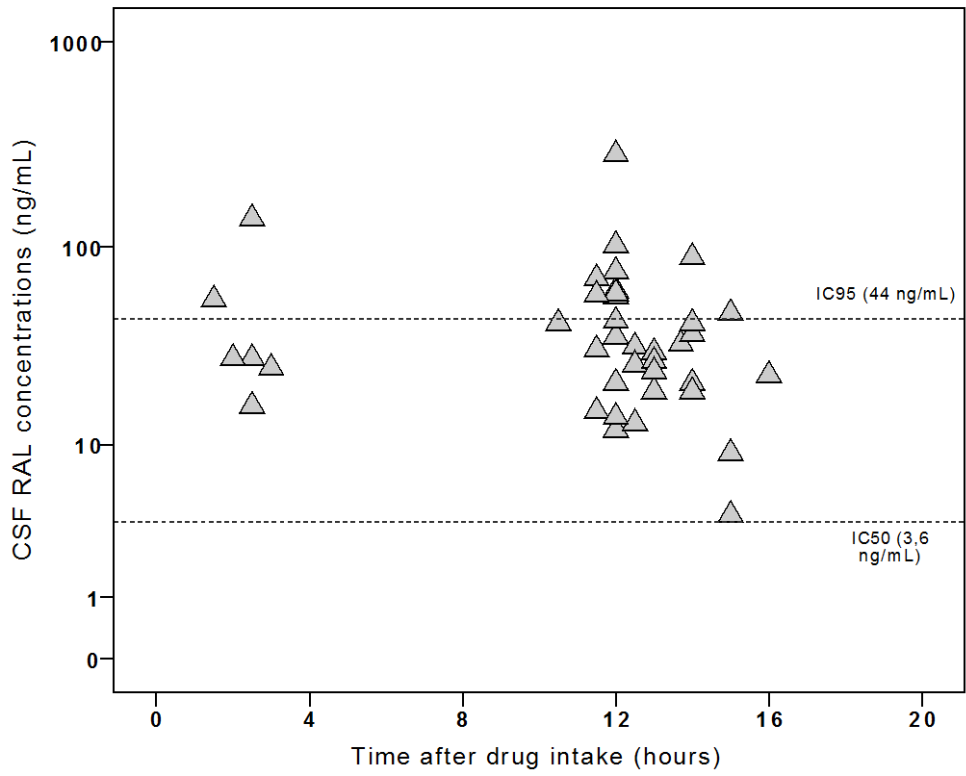
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222 **Transparency declaration:** A. Calcagno has received travel grants or speaker's honoraria from  
223 Abbott, Bristol-Myers Squibb (BMS), Merck Sharp & Dohme (MSD) and Janssen-Cilag; he is  
224 currently receiving a research grant from Gilead. S. Bonora has received travel grants and  
225 consultancy fees from Abbott, Boehringer-Ingelheim, BMS, Gilead-Sciences, GSK, MSD, Pfizer  
226 and Janssen-Cilag; he is currently receiving a research grant from BMS. G. Di Perri has received  
227 grants, travel grants and consultancy fees from Abbott, Boehringer-Ingelheim, BMS, Gilead-  
228 Sciences, GSK, MSD, Pfizer, Roche and Tibotec (Johnson & Johnson). Other authors have no  
229 potential conflict of interest to declare.

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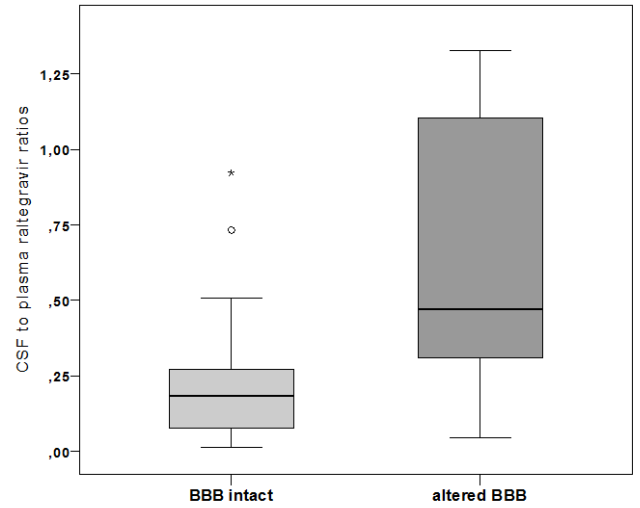
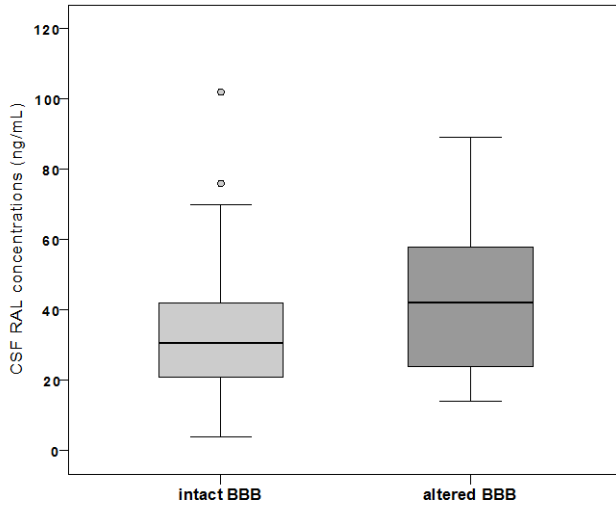
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**Figure 1.** Raltegravir cerebrospinal fluid concentrations ( $\text{Log}_{10}$  ng/mL) according to time after drug intake (hours). Dotted lines represent  $\text{IC}_{50}$  and  $\text{IC}_{95}$  (in ng/mL).



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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
<b>ABCB1</b>						
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	-		-	
<b>SLCO1A2</b>						
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	-		-	
<b>ABCC2</b>						
-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	
<b>SLC22A6</b>						
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	
<b>HNF4α</b>						
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	-		-	

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**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