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E138A in HIV-1 reverse transcriptase is more common in subtype C than B: Implications for rilpivirine use in resource-limited settings

Nicolas Sluis-Cremer[a,*], **Michael R. Jordan**[a,b], **Kelly Huber**[a], **Carole L. Wallis**[d], **Silvia Bertagnolio**[e], **John W. Mellors**[a], **Neil T. Parkin**[f], and **P. Richard Harrigan**[g]

[a]University of Pittsburgh School of Medicine, Department of Medicine, Division of Infectious Diseases, Pittsburgh, PA, USA [b]Division of Geographic Medicine and Infectious Disease, Tufts Medical Center, Boston, USA [c]Department of Public Health and Community Medicine, Tufts University School of Medicine, Boston, MA, USA [d]Lancet Laboratories, Johannesburg, South Africa [e]HIV Department, World Health Organization, Geneva, Switzerland [f]Data First Consulting, Belmont, CA, USA [g]BC Centre for Excellence in HIV/AIDS, Vancouver, Canada

Abstract

The nonnucleoside reverse transcriptase (RT) inhibitor rilpivirine (RPV) has been co-formulated with emtricitabine and tenofovir disoproxil fumarate for initial therapy of HIV-1-infected individuals. RPV, formulated as a long-acting nanosuspension, will also be assessed for its ability to prevent HIV-1 infection in resource limited settings. In this study, we determined whether any pre-existing genetic differences occurred among different HIV-1 subtypes at residues in RT associated with decreased virologic response to RPV. We found that the E138A substitution occurs more frequently in subtype C (range: 5.9–7.5%) than B (range: 0–2.3%) sequences from both treatment-naïve and -experienced individuals ($p<0.01$) in 4 independent genotype databases. In one of the databases (Stanford University), E138K and E138Q were also more common in RTI-experienced subtype C sequences (1.0% and 1.1%, respectively) than in subtype B sequences (0.3% and 0.6%, respectively). E138A/K/Q in subtype C decreased RPV susceptibility 2.9-, 5.8-, and 5.4-fold, respectively. Taken together, these data suggest that E138A could impact treatment or prevention strategies that include RPV in geographic areas where subtype C infection is prevalent.

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[*]Corresponding author: Nicolas Sluis-Cremer, University of Pittsburgh School of Medicine, Department of Medicine, Division of Infectious Diseases, S817 Scaife Hall, 3550 Terrace Street, Pittsburgh, PA, 15261, USA; Tel: 1-412-648-8457; Fax: 1-412-648-8521; nps2@pitt.edu.

Conflicts of interest
None to declare

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Keywords

HIV-1; reverse transcriptase; NNRTI; rilpivirine; E138A; resistance

Nonnucleoside reverse transcriptase (RT) inhibitors (NNRTIs) are important components of antiretroviral therapies used for the treatment of HIV-1 infection. Rilpivirine (RPV) is a second generation diarylpyrimidine NNRTI (Azijn et al., 2010), that has been co-formulated with the nucleoside RT inhibitors emtricitabine and tenofovir disoproxil fumarate for initial therapy of HIV-1-infected individuals. A long-acting nanosuspension formulation for RPV (RPV-LA) has also been developed (Baert et al., 2009), and is currently being assessed in an ongoing phase IIb clinical study to determine whether it can, in combination with the integrase inhibitor GSK1265744, maintain virologic suppression in infected individuals (ClinicalTrials.gov Identifier: NCT01641809). Additionally, RPV-LA will be studied in the pre-exposure prophylaxis (PrEP) setting because it has the potential to reduce reliance on daily adherence.

Although RPV has been reported to have higher *in vitro* genetic barrier to resistance (Azijn et al., 2010), at least 17 single substitutions in HIV-1 RT (L100I, K101E/P, E138A/G/K/Q/R, V179L, Y181C/I/V, Y188L, H221Y, F227C, and M230I/L) have been associated with a decreased virologic response to this NNRTI (Anta et al., 2013). Unfortunately most HIV-1 drug resistance research has focused predominantly on subtype B viruses, even though non-subtype B strains are responsible for the majority of global infections. Specifically, HIV-1 subtype C, which predominates in Southern and Eastern Africa, India and Nepal, is responsible for > 50% of all infections globally (Lihana et al., 2012). Importantly, recent studies have documented increases in the prevalence of drug resistance, especially NNRTI resistance, among treatment-naïve individuals in sub-Saharan Africa since the inception of rollout of antiretroviral therapy (Gupta et al., 2012; Price et al., 2011). There is also increasing evidence that naturally occurring genetic differences in different HIV-1 subtypes can impact antiretroviral drug susceptibility and drug resistance. For example, the V106M RT substitution, which confers resistance to the NNRTIs efavirenz (EFV) and nevirapine (NVP), has been reported more frequently in subtype C viruses than in subtype B (Brenner et al., 2003). Along these lines, we were interested in determining whether other pre-existing genetic differences occurred among different HIV-1 subtypes at residues associated with decreased virologic response to RPV.

We assessed sequences from RT inhibitor (RTI)-naïve and -experienced individuals in the Stanford University HIV Drug Resistance database (Rhee et al., 2006) and in two independent clinical databases. One of the clinical databases is located in Vancouver, Canada at the BC Centre for Excellence in HIV/AIDS (Gill et al., 2010), and the other in Johannesburg, South Africa at Lancet Laboratories. RT sequences were also analyzed from specimens gathered during surveillance of transmitted drug resistance (TDR) in 23 countries in Africa, Asia, and Central America that were generated by World Health Organization (WHO)-designated genotyping laboratories (http://www.who.int/hiv/pub/drugresistance/report2012/en/). The HIV-infected individuals included in the WHO TDR surveys were likely recently infected and thus not previously treated with antiretroviral drugs. We found

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that the E138A substitution in HIV-1 RT occurs more frequently in subtype C than B sequences from both treatment-naïve and -experienced individuals (Table 1). In the Stanford University database, E138A was present in 350/17481 (2.0%) subtype B sequences and in 415/6795 (6.1%) subtype C sequences from RTI-naïve HIV-1-infected individuals ($p<0.0001$). In the BC database, E138A was present in 76/3320 (2.3%) subtype B sequences compared to 6/101 (5.9%) subtype C sequences ($p=0.03$). In the WHO TDR surveys, E138A was not observed in subtype B sequences, but was present in 97/1296 (7.5%) subtype C sequences ($p<0.01$). Of note, in the Stanford University database E138A was not more common in treatment-naïve sequences of other subtypes, including subtypes A (3.2%), D (2.3%), F (3.6%), G (1.7%), CRF01 (0.4%) or CRF02 (2.3%). However, in the WHO TDR surveys, E138A was also more frequently observed in subtype A (6.0 %; $p = 0.02$), but not in subtypes D (0 %), G (5.9 %; $p = 0.22$), CRF01 (0.4 %), CRF02 (2.0 %), CRF06 (0 %), CRF07 (0 %), CRF08 (2.6 %) and CRF11 (0 %) (Supplementary Table 1). In contrast to E138A, the frequencies of other substitutions at codon 138 (i.e., E138G/K/Q) were similar in both subtypes B and C. E138A was also more common in subtype C than B sequences from RTI-experienced HIV-1-infected individuals in the Stanford University and BC Centre databases ($p < 0.01$ in both databases) (Table 1), but its frequency was not higher in either subtype B or C sequences from individuals who had NRTI, but not NNRTI, containing regimens or those who received both NRTI- and NNRTI containing regimens (Table 1). Taken together, these data indicate that E138A is polymorphic and does not appear to be strongly selected by prior RTI exposure. In the Stanford University HIV Database, E138K and E138Q were also more common in subtype C than B isolates from RTI-experienced individuals, although their overall frequencies (1.0% and 1.1%, respectively) were lower than E138A (6.1%). Consistent with these findings, an analysis of 2578 sequences (majority subtype C) from both naïve and RTI-experienced (but with no prior exposure to RPV or etravirine (ETR)) HIV-1-infected individuals in the Lancet Laboratories (South African) database revealed that 206 (8%), 43 (1.7%), 29 (1.1%) and 23 (0.9%) harbored the E138A, G, K or Q mutations, respectively (data not shown).

Prior studies have assessed the impact of substitutions at codon 138 in RT on NNRTI susceptibility of subtype B, but not subtype C HIV-1 (Azjin et al., 2010; Tambuyzer et al., 2011). Therefore, we introduced the E138A, E138G, E138K, E138Q and E138R mutations into subtype C RT, as described previously (Brehm et al., 2012), and assessed virus susceptibility to RPV, ETR, EFV and NVP in P4/R5 cells (Table 2). For comparison, we also phenotyped subtype B virus (LAI strain) containing the same mutations. The E138A mutation reduced susceptibility to RPV by 5.6-fold in HIV-1 subtype B and by 2.9-fold in HIV-1 subtype C, respectively. E138A was also found to reduce susceptibility to ETR, but did not significantly reduce susceptibility to NVP or EFV in either subtype B or C. The E138G/K/Q/R mutations in HIV-1 subtype B reduced susceptibility to RPV and ETR but, with the exception of E138Q which decreased susceptibility to NVP 3.0-fold, had no impact on susceptibility to either NVP or EFV. In subtype C RT, the E138G/K/Q/R mutations reduced susceptibility to RPV and ETR (range: 2.7–6.8-fold). Interestingly, the E138K/Q/R mutations in HIV-1 subtype C had noticeable effects on NVP and EFV susceptibility (2.3 to 7.1-fold).

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Collectively, our data shows that the E138A mutation in HIV-1 RT is ~3-fold more common in naïve- and RTI-experienced isolates from individuals infected with subtype C, compared to those infected with other subtypes, in 4 independent datasets. In the context of subtype C RT, the E138A substitution reduces virus susceptibility to RPV. The biological cutoff (BCO) for RPV has been set at 2.0, and a virus, such as subtype C HIV-1 containing E138, with a fold-change in resistance above the cutoff is considered resistant. As such, these findings suggest the possibility of higher risk of virologic failure of RPV-based therapy in geographic regions such as sub-Saharan African in which HIV-1 subtype C infections predominate. The higher frequency of E138A in subtype C may also compromise the efficacy of RPV-LA as a PrEP agent in sub-Saharan Africa, although additional studies are warranted to assess this possibility.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations used

RT	reverse transcriptase
NNRTI	nonnucleoside reverse transcriptase inhibitor
RPV	rilpivirine
ETR	etravirine
EV	efavirenz
NVP	nevirapine
PrEP	pre-exposure prophylaxis

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Highlights

- We looked for genetic differences at RPV resistance codons among HIV-1 subtypes
- E138A was found to occur more frequently in subtype C than B HIV-1 sequences
- E138A could impact RPV therapy in resource limited settings

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Table 1

Frequency of substitutions at codon 138 in HIV-1 RT in isolates from RTI-naïve and -experienced individuals.

	Stanford HIV Database			BC Centre Database			WHO TDR Surveys		
	Subtype B	Subtype C	p-value[a]	Subtype B	Subtype C	p-value[a]	Subtype B	Subtype C	p-value[a]
Treatment-naïve									
138A	350/17481 (2.0 %)	415/6795 (6.1 %)	< 0.01	76/3320 (2.3 %)	6/101 (5.9 %)	0.03	0/132 (0%)	97/1296 (7.5%)	< 0.01
138G	35/17481 (0.2 %)	20/6795 (0.3 %)	0.18	3/3320 (0.09 %)	1/101 (1 %)	0.12	0/132 (0%)	3/1296 (0.23%)	0.58
138K	17/17481 (0.1 %)	13/6795 (0.2 %)	0.07	1/3320 (0.03 %)	1/101 (1 %)	0.06	0/132 (0%)	2/1296 (0.15%)	0.65
138Q	0/17481 (0.0 %)	0/6795 (0 %)	N/A	0/3320 (0 %)	0/101 (0 %)	N/A	0/132 (0%)	2/1296 (0.15%)	0.65
RTI-experienced									
138A	402/20112 (2.0 %)	210/3439 (6.1 %)	< 0.01	168/6593 (2.5 %)	13/222 (5.9 %)	< 0.01			
138G	80/20112 (0.4 %)	14/3439 (0.4 %)	0.88	9/6593 (0.1 %)	0/222 (0.0 %)	1.0			
138K	60/20112 (0.3 %)	34/3439 (1.0 %)	< 0.01	14/6593 (0.2 %)	2/222 (0.9 %)	0.09			
138Q	121/20112 (0.6 %)	38/3439 (1.1 %)	< 0.01	7/6593 (0.1 %)	0/222 (0.0 %)	1.0			
NRTI- but not NNRTI-experienced									
138A	83/3966 (2.1 %)	24/288 (8.4 %)	< 0.01	118/4521 (2.6 %)	6/120 (5 %)	0.14			
138G	4/3966 (0.1 %)	2/288 (0.7 %)	0.08	7/4521 (0.2 %)	0/120 (0.0 %)	1.0			
138K	8/3966 (0.2 %)	3/288 (1.1 %)	0.04	9/4521 (0.2 %)	1/120 (0.8 %)	0.23			
138Q	4/3966 (0.1 %)	0/288 (0 %)	0.59	6/4521 (0.1 %)	0/120 (0.0 %)	1.0			
NRTI- and NNRTI-experienced									
138A	113/6297 (1.8 %)	112/1927 (5.8 %)	< 0.01	50/2069 (2.4%)	7/102 (6.9%)	0.02			
138G	38/6297 (0.6 %)	6/1927 (0.3 %)	0.18	2/2069 (0.1%)	0/102 (0.0%)	1.0			
138K	19/6297 (0.3 %)	12/1927 (0.6 %)	0.07	5/2069 (0.2%)	1/102 (1%)	0.25			
138Q	57/6297 (0.9 %)	17/1927 (0.9 %)	0.93	1/2069 (0.0%)	0/102 (0.0%)	1.0			

[a]Statistical analyses of the prevalence of mutations at codon 138 in sequences from individuals infected with subtype B versus those infected with subtype C virus were performed using a Chi-square test.

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Table 2

NNRTI susceptibility of recombinant HIV-1 subtype B and C containing mutations at codon 138 in RT.

Virus	RPV		ETR		NVP		EFV	
	EC_{50} (nM)	Fold-R[a] (p-value)[b]	EC_{50} (nM)	Fold-R (p- value)[a]	EC_{50} (nM)	Fold-R (p-value)[a]	EC_{50} (nM)	Fold-R (p- value)[a]
Subtype B								
WT	0.3±0.1	-	1.3±0.2	-	280±10	-	2.5±0.9	-
E138A	1.5±0.2	5.6 (0.03)	2.9±0.5	2.2 (0.05)	230±9	0.8 (>0.05)	1.3±0.5	0.5 (>0.05)
E138G	0.7±0.4	2.5 (0.05)	3.2±0.2	2.5 (0.05)	360±9	1.3 (>0.05)	1.3±0.4	0.2 (0.05)
E138K	0.8±0.2	3.0 (0.04)	2.8±0.3	2.2 (0.05)	120±6	0.4 (>0.05)	1.2±0.4	0.5 (0.03)
E138Q	1.3±0.1	4.8 (0.04)	4.0±0.1	3.1 (0.01)	840±40	3.0 (0.05)	2.6±0.3	1.0 (>0.05)
E138R	1.6±0.4	6.0 (0.05)	5.3±1.6	4.1 (0.04)	490±140	1.8 (>0.05)	3.2±0.4	1.3 (>0.05)
Subtype C								
WT	2.2±0.6	-	0.7±0.3	-	75±15	-	0.6±0.1	-
E138A	7.3±1.4	3.3 (0.03)	2.0±0.4	2.9 (0.01)	90±20	1.2 (>0.05)	0.9±0.1	1.5 (0.03)
E138G	6.5±1.9	3.0 (0.03)	1.9±0.4	2.7 (0.01)	140±10	1.9 (0.02)	0.8±0.3	1.3 (>0.05)
E138K	11.8±3.6	5.4 (0.05)	3.9±0.7	5.8 (0.002)	170±20	2.3 (0.03)	2.1±0.4	3.6 (0.01)
E138Q	12.5±4.8	5.7 (0.05)	3.4±1.6	5.0 (0.02)	530±10	7.1 (0.01)	2.7±0.4	4.6 (0.008)
E138R	10.4±3.5	4.8 (0.03)	4.7±1.3	6.8 (0.02)	320±44	4.2 (0.04)	1.9±0.4	3.3 (0.01)

[a] Fold-resistance = $EC_{50}^{Mutant}/EC_{50}^{WT}$ for both subtype B and C HIV-1.

[b] A p-value was calculated to determine whether the fold-change in EC50 between the WT and mutant viruses for each subtype, were statistically significant. This value was determined as follows: the concentrations of drug required to inhibit viral replication by 50% (EC_{50}) from 3–6 independent experiments were $\log_{10}$ transformed and compared for statistically significant differences (p-value < 0.05) using the two-sample Student's paired *t* test. The p-value