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# Mutation V111I in HIV-2 Reverse Transcriptase Increases the Fitness of the Nucleoside Analogue-Resistant K65R and Q151M Viruses

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## ABSTRACT

Infection with HIV-2 can ultimately lead to AIDS, although disease progression is much slower than with HIV-1. HIV-2 patients are mostly treated with a combination of nucleoside reverse transcriptase (RT) inhibitors (NRTIs) and protease inhibitors designed for HIV-1. Many studies have described the development of HIV-1 resistance to NRTIs and identified mutations in the polymerase domain of RT. Recent studies have shown that mutations in the connection and RNase H domains of HIV-1 RT may also contribute to resistance. However, only limited information exists regarding the resistance of HIV-2 to NRTIs. In this study, therefore, we analyzed the polymerase, connection, and RNase H domains of RT in HIV-2 patients failing NRTI-containing therapies. Besides the key resistance mutations K65R, Q151M, and M184V, we identified a novel mutation, V111I, in the polymerase domain. This mutation was significantly associated with mutations K65R and Q151M. Sequencing of the connection and RNase H domains of the HIV-2 patients did not reveal any of the mutations that were reported to contribute to NRTI resistance in HIV-1. We show that V111I does not strongly affect drug susceptibility but increases the replication capacity of the K65R and Q151M viruses. Biochemical assays demonstrate that V111I restores the polymerization defects of the K65R and Q151M viruses but negatively affects the fidelity of the HIV-2 RT enzyme. Molecular dynamics simulations were performed to analyze the structural changes mediated by V111I. This showed that V111I changed the flexibility of the 110-to-115 loop region, which may affect deoxynucleoside triphosphate (dNTP) binding and polymerase activity.

## IMPORTANCE

Mutation V111I in the HIV-2 reverse transcriptase enzyme was identified in patients failing therapies containing nucleoside analogues. We show that the V111I change does not strongly affect the sensitivity of HIV-2 to nucleoside analogues but increases the fitness of viruses with drug resistance mutations K65R and Q151M.

Human immunodeficiency virus type 2 (HIV-2) differs from HIV-1 by its lower pathogenicity and lower infectivity rate (1, 2). This has resulted in a more restricted spread of HIV-2, mainly to parts of West Africa (3). Disease progression is slower than in HIV-1 infection; however, HIV-2-infected patients can ultimately develop AIDS. HIV-2 patients are treated with antiretroviral drugs designed for HIV-1, although the sequences of HIV-1 and HIV-2 differ by about 60% at the nucleotide level. The reverse transcriptase (RT) enzymes of the two viruses diverge by approximately 40% at the amino acid level but are similar in overall structure and function. Both are heterodimers composed of a large catalytic subunit and a small subunit for structural support. The catalytic subunit is composed of two domains: the polymerase domain, containing the fingers, palm, thumb, and connection subdomains, and the RNase H domain (4). RT is crucial in the retroviral life cycle, as it converts the single-stranded RNA genome into a double-stranded DNA that becomes integrated into the genome of the host cell. Reverse transcription of both HIV-1 and HIV-2 can be inhibited by nucleoside/nucleotide RT inhibitors (NRTIs). When incorporated into the nascent viral DNA, these nucleoside analogues act as chain terminators (5, 6). However, HIV-2 was found to be intrinsically resistant to nonnucleoside RT inhibitors (NNRTIs) (4, 7). In addition, HIV-2 is resistant to the fusion inhibitor enfuvirtide (8) and exhibits reduced susceptibility to some of the protease inhibitors (9, 10). This limits

the therapeutic possibilities for HIV-2 patients, and most patients, therefore, are on regimens containing two NRTIs with a protease inhibitor. Due to the low number of patients and the limited geographical spread of HIV-2, little information is available concerning the efficacy of antiretroviral therapy and the emergence of drug resistance.

Treatment of HIV with NRTIs can eventually result in the selection of viruses with decreased susceptibility to NRTIs. This means that specific mutations in RT enhance the ability to select normal deoxynucleoside triphosphates (dNTPs) over the analogues. Two primary mechanisms have been identified by which

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resistance is mediated in HIV-1 (for a review, see reference 11). One mechanism is exclusion, in which the mutant RT has a reduced ability to bind and incorporate the analogue. Mutations associated with this mechanism in HIV-1 are K65R, Q151M, and M184V. The second mechanism is excision of the analogue by ATP-mediated pyrophosphorolysis. In this mechanism, the analogue is still efficiently incorporated but is removed from the end of the primer using ATP as a pyrophosphate donor. Mutations associated with this mechanism in HIV-1 are collectively called thymidine analogue mutations (TAMs). Several studies suggest that HIV-1 and HIV-2 evolve NRTI resistance by different mutational pathways. Although relying on limited amounts of genotypic data for HIV-2, three key resistance mutations, K65R, Q151M, and M184V, were reported to be associated with drug resistance (12–15). The K65R and Q151M substitutions are more common in HIV-2 than in HIV-1 and often appear together with M184V in patients who received NRTI therapy. This suggests that HIV-2 RT favors the exclusion pathway for resistance to NRTI. In contrast, the TAMs that provide the principal route to NRTI resistance in HIV-1 by the excision pathway are rarely observed in HIV-2. This difference between HIV-1 and HIV-2 may be explained by the reduced ability of the HIV-2 RT enzyme to carry out the excision reaction using ATP-mediated pyrophosphorolysis (16, 17). The individual contributions of the key resistance mutations K65R, Q151M, and M184V in HIV-2 were addressed using site-directed mutants in cell culture-based phenotypic assays (18). This study showed that the combination of the three mutations is sufficient for classwide NRTI resistance. Recently, a rule set for HIV-2 drug resistance interpretation was defined, and an automated Internet tool was developed to support resistance analyses (19).

However, the roles of several mutations in the polymerase domain of RT remain unclear. For instance, mutation V111I was reported to be coselected with mutation Q151M in HIV-2 patients failing therapy (14, 15, 20). A study using patient-derived HIV-2 isolates suggested that Q151M alone confers resistance only to stavudine (d4T) and abacavir (ABC), whereas resistance to other NRTIs involved the coselection of V111I (20). In contrast, another study showed that mutation Q151M alone is sufficient to produce high-level zidovudine (AZT) resistance, and therefore, V111I was not required for broad-spectrum NRTI resistance (18). Recent studies on HIV-1 suggest that mutations in the connection and RNase H domains of RT may contribute to NRTI and NNRTI resistance (for a review, see reference 21). In this study, we further explore the roles of additional mutations in the polymerase, connection, and RNase H domains of HIV-2 RT. Therefore, the complete RT genes of 54 HIV-2 patient isolates were sequenced. Besides the key resistance mutations K65R, Q151M, and M184V, we identified mutation V111I in the polymerase domain. This mutation was significantly associated with mutations K65R and Q151M. In the connection and RNase H domains, two polymorphisms (V357I and K467V) were found with increased frequency in patients failing therapy. However, none of the connection subdomain mutations reported for HIV-1 (for a review, see reference 21) were found in HIV-2 patients. We show that V111I does not strongly affect drug susceptibility but increases the replication capacity of the K65R and Q151M viruses. Molecular dynamics simulations were performed to analyze the structural changes mediated by V111I. Biochemical assays show that V111I restores the

polymerization defects of the K65R and Q151M viruses but negatively affects the fidelity of the HIV-2 RT enzyme.

## MATERIALS AND METHODS

**Sequencing of HIV-2 patient samples.** Plasma samples from HIV-2 subtype A patients in the French National HIV-2 cohort were collected. The study was approved by the institutional review board of the French Agence Nationale de Recherche sur le SIDA et les Hépatites Virales (ANRS) CO5 HIV-2 Cohort. Written informed consent was obtained from each patient at the time of inclusion in the French ANRS CO5 HIV-2 Cohort. Plasma HIV-2 RNA was quantified using real-time PCR (22). Plasma RNA was extracted using a Qiagen viral RNA minikit. The RT, connection, and RNase H domains were amplified using the RTC (5'-ATGACAGGGGATACCCCAATCAACATTTTG-3') and Pol D (5'-CTCTTTTAAAATTCATGCAATGAACTGCC-3') primers for the real-time PCR. Nested PCRs were performed using RTC/RT4 (5'-TCCCCAAATGACTAGTGCTTCTTTTCCTA-3'), RT3 (5'-GAGGCACTAAAAGAGATCTGTGAAAAATGG-3')/RT2 (5'-GAAGTCCCAGTCTGGGATCCATGTCACCTGCCA-3'), and RT4S (5'-AAAAGAAGCACTAGTCATTTGGGG-3')/Pol D primers. The nested-PCR products were sequenced using ABI Technologies RTC, RT4, RT3, RT2, RT4S, and Pol D primers. We previously showed that in 50% of the samples that had a viral load below 250 copies/ml, amplification of the RT gene failed and sequencing was not possible (2). In the available plasma samples, amplification of the complete RT gene was successful in 19 out of 30 antiretroviral (ARV)-naive patients, and 35 out of 45 ARV-treated HIV-2-infected patients who received NRTI-containing regimens were studied. At the time of virological failure, the patients were on regimens containing the NRTIs AZT (55%), didanosine (ddI) (18%), d4T (21%), lamivudine (3TC) (73%), ABC (18%), and tenofovir (TDF) (24%).

**DNA constructs.** The HIV-2 group A infectious molecular clone pROD9 (a kind gift from Robert Smith) was generated by Michael Emerman, Fred Hutchinson Cancer Research Center, Seattle, WA. For mutation of the RT gene, we used the construct pBS-RT2, which contains the XhoI-AvrII fragment of pROD9 (positions 2979 to 3861) cloned into pBluescript (Agilent Technologies). The K65R, M184V, Q151M, and V111I mutations were introduced using the QuikChange Lightning site-directed mutagenesis kit (Agilent Technologies) according to the manufacturer's protocol. The substitution was verified by sequencing analysis and cloned into the pROD9 construct using the XhoI-AvrII restriction sites.

**Cells and viruses.** 293T and TZM-bl cells were grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS) at 37°C and 5% CO<sub>2</sub>. For generation of the HIV-2 stocks, 293T cells were transiently transfected using the calcium phosphate method (CalPhos kit; Clontech) according to the manufacturer's protocol. Three days after transfection, the culture medium was centrifuged at 1,600 rpm for 10 min to remove cells and subsequently filtered through a 0.45- $\mu$ m-pore-size filter. The virions were pelleted by centrifugation at 25,000 rpm for 60 min in a Beckman SW28 rotor. The virions were resuspended in phosphate-buffered saline, and aliquots were stored at -80°C.

SupT1 T cells were grown in RPMI 1640 medium supplemented with 10% FCS at 37°C and 5% CO<sub>2</sub>. The replication capacity of the viruses was studied in SupT1 cells. Therefore,  $0.5 \times 10^6$  cells were seeded in a 25-cm<sup>2</sup> culture flask containing 5 ml of culture medium.

**Titration of virus stocks.** Titration of the HIV-2 stocks was performed using a 50% tissue culture infective dose (TCID<sub>50</sub>) assay in TZM-bl cells. The single-round infection assay was performed in white 96-well flat-bottom culture plates (Costar) seeded with 10,000 cells in 100  $\mu$ l culture medium. The cells were incubated overnight with RT inhibitors prior to the addition of 100  $\mu$ l of virus serially diluted in culture medium, in quadruplicate. The cells were incubated for 48 h. Then, the medium was removed, and the wells were washed with phosphate-buffered saline. Finally, 25  $\mu$ l of D-luciferin (Duchefa Biochemie) reagent was added to the

wells, and after a 5-min incubation in the dark, the luminescence was measured using a luminometer (CentroPRO; Berthold Technologies).

**Drug susceptibility assays.** Virus stocks were titrated, and equal amounts of infectious virus were used to infect TZM-bl cells in the presence or absence of RT inhibitors. Fourfold dilutions were used for all RTIs, and the range of dilutions used for each drug was a function of the 50% inhibitory concentration ( $IC_{50}$ ) of the respective compound. After 3 days, luciferase expression was measured using a luminometer. The results were expressed as  $IC_{50}$ s, defined as the concentration of compound ( $\mu\text{mol/liter}$ ) achieving 50% inhibition of the virus-induced luciferase expression compared to the untreated virus-infected control cells. The ratio between the mutant and wild-type virus  $IC_{50}$ s gives the fold change value, as an indicator of the level of resistance mediated by the substitution. The RT inhibitors AZT, ddI, d4T, 3TC, emtricitabine (FTC), ABC, and TDF were obtained through the NIH AIDS Research and Reference Reagent Program.

**Competition experiments.** Pairwise competition experiments were performed at least in triplicate from independent plasmid isolations and virus stocks to determine the relative fitness of the viruses. Cells ( $0.5 \times 10^6$ ) were infected with equal amounts (50 times the  $TCID_{50}$ ) of the two different viruses. Virus replication was monitored by the microscopic detection of syncytia. At peak infection, a small sample (10  $\mu\text{l}$ ) of cell-free supernatant was passaged onto fresh cells to continue the competition. Cells were harvested at several time points, total cellular DNA was isolated, and the frequencies of the two viruses were determined by sequencing. The relative peak heights at the mutated position were calculated from Sanger electropherograms using a plug-in (developed by Patrick Dekker) for the CLCbio workbench.

**Preparation of protein structures for simulation.** In order to perform molecular dynamics simulations, we required models of the structures of both the wild-type and V111I mutant HIV-2 RTs. The only available crystal structure of HIV-2 RT (Protein Data Bank [PDB] code 1MU2) is incomplete and is missing several key loops, including that involved in dNTP binding (23). In order to build a complete protein structure for simulation, we created a homology model using the 1MU2 structure in conjunction with a complete HIV-1 RT structure (PDB code 1HQU) as templates (24). Both the creation of the wild-type homology model and subsequent incorporation of the V111 mutation were carried out using the Modeller software package (25). The regions of the HIV-2 RT structure completed through this modeling process are p68 residues 1 and 2, 68 to 71, 133 to 141, and 357 to 358 and p51 residues 65 to 68, 92 to 94, and 212 to 228. In each case, the final model contains 555 residues in the first (p68) chain and 431 in the second (p51) chain for a total of 986 residues. The system was prepared for simulation using the tools provided by the GROMACS 4.6.0 package (26), with parameters taken from the AMBER99SB-ILDN force field (27). Each system was solvated using a cubic box of single-point charge (SPC) water molecules providing a buffer of at least 14 Å distance around the protein (28). The systems were neutralized by the addition of sodium ions.

**Molecular dynamics simulation protocol.** All molecular dynamics simulations during this study were performed using GROMACS 4.6.0 (26). The initial structures were energy minimized for 5,000 steps using the steepest descent algorithm in order to remove any steric clashes or strained bond angles. After minimization, the quality of the models was assessed using the PSVS server ([http://psvs-1\\_5-dev.nesg.org/](http://psvs-1_5-dev.nesg.org/)). Both starting models were found to be of good quality using all metrics provided by the server, with less than 1% of the residues having phi-psi angles in the disallowed region of the Ramachandran plot. Five replica simulations were created for each sequence, differing only in the random initial velocities assigned to each atom. Equilibration of the temperature and pressure of the system was conducted in two stages, the first in the isothermal-isovolumetric (NVT) ensemble and the second in the isothermal-isobaric (NPT) ensemble. Both of these steps lasted 100 ps and used the modified Berendsen thermostat (29) to maintain a temperature of 300 K. In the NPT stage, the pressure was controlled using a Parinello-Rahman

barostat with a target pressure of  $10^5$  Pa (30). Throughout this equilibration phase, positional restraints were applied to the heavy atoms in the protein to prevent unwanted motion from occurring before equilibrium was reached. Positional restraints on the protein were then released, and 5 ns of unrestrained dynamics was run (all other simulation conditions were identical to those in the NPT equilibration runs). After this point, the global root mean square deviation (RMSD) of the structure relative to the crystal structure was calculated to ensure that equilibration was complete. The track of the RMSD over time plateaued after approximately 2 ns for all simulations (data not shown). Coordinates from all simulations were sampled once every 10 ps, and data analysis was carried out using GROMACS and VMD (31). All protein images were visualized in VMD.

**Virion-derived RT.** 293T cells were transiently transfected as described above. Three days after transfection, the culture medium was centrifuged at 1,600 rpm for 10 min to remove the cells and filtered through a 0.45- $\mu\text{m}$ -pore-size filter. The virions were pelleted by centrifugation at 25,000 rpm for 60 min in a Beckman SW28 rotor. The virions were resuspended and pooled in 100  $\mu\text{l}$  of NP buffer (50 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5% NP-40), and aliquots were stored at  $-20^\circ\text{C}$ .

**Polymerase assays.** The HIV-2 molecular clone ROD9 and the HIV-1 molecular clone pNL4.3 were used as templates for the PCR amplification and subsequent *in vitro* transcription. Positions +1 to +368 of the HIV-1 genome were amplified using a forward primer containing the T7 RNA polymerase promoter sequence (5'-TAATACGACTCACTATAGGGTCTCTCTGGTTAGACCAG-3'; italics indicate the T7 sequence) and reverse primer (5'-CATCTCTCTCCTTCTAGCCTC-3'). Positions +1060 to +1396 of the HIV-2 genome were amplified using forward primer (5'-T AATACGACTCACTATAGGTCATTTCGGTGTTCACCTG-3') and reverse primer (5'-CATCTCCCACAATCTTCTACC-3'). *In vitro* RNA transcription was mediated using the T-7 Megascript kit (Ambion), and RNA was purified using the Megaclear kit (Ambion) according to the manufacturer's protocol.

Minus-strand DNA synthesis was initiated on the RNA templates using a 21-nucleotide (nt) DNA primer complementary to the 18-nt primer binding site (PBS) sequence with 3 additional nucleotides at its 5' end (HIV-1, 5'-CAAGTCCCTGTTCGGGCGCCA-3', and HIV-2, 5'-CAAGTCCCTGTTCAGGCGCCA-3'). The DNA primer was 5' end labeled with [ $\gamma$ - $^{32}\text{P}$ ]ATP and T4 polynucleotide kinase (NEB) and purified using NucAway columns (Ambion). For annealing, 50 ng of RNA template was incubated with 10 ng of labeled DNA primer in 15  $\mu\text{l}$  annealing buffer (83 mM Tris-HCl, pH 7.5, 125 mM KCl) for 10 min at  $65^\circ\text{C}$ , followed by snap cooling on ice. Polymerization was initiated by the addition of equal amounts of virus lysate (based on the  $TCID_{50}$ ) in 40  $\mu\text{l}$  of RT buffer (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM dithiothreitol [DTT], 10  $\mu\text{M}$  dNTPs, 3 mM  $\text{MgCl}_2$ ). The reaction mixtures were incubated for 30, 60, and 120 min at  $37^\circ\text{C}$  and then stopped by the addition of formamide loading buffer and analyzed on 10% denaturing sequencing gels. The products were quantified using the Typhoon imaging system (GE Healthcare).

**Fidelity assays.** The abilities of the wild-type and V111I RTs to incorporate a single correct or incorrect nucleotide were measured using the HIV-2 RNA template and DNA primer complementary to the PBS (see above). The DNA primer was 5' end labeled with [ $\gamma$ - $^{32}\text{P}$ ]ATP and T4 polynucleotide kinase (NEB) and purified from a denaturing 10% gel. For annealing, 50 ng of RNA template was incubated with 10 ng of labeled DNA primer in 15  $\mu\text{l}$  annealing buffer (83 mM Tris-HCl, pH 7.5, 125 mM KCl) for 10 min at  $65^\circ\text{C}$ , followed by snap cooling on ice. The primer-template complex was incubated with equal amounts of virus lysate (based on the  $TCID_{50}$ ) in 10  $\mu\text{l}$  of RT buffer (50 mM Tris-HCl, pH 7.5, 50 mM KCl, 10 mM DTT, 3 mM  $\text{MgCl}_2$ , and increasing concentrations of either dATP or dTTP) for 5 min at  $37^\circ\text{C}$ . The reactions were stopped by the addition of formamide loading buffer and analyzed on 12% denaturing sequencing gels. The products were quantified using the Typhoon imaging system (GE Healthcare).

**Statistical analysis.** The proportions of sequences containing a particular mutation were compared between groups using Fisher's exact test.  $IC_{50}$

**TABLE 1** Characteristics of the 54 HIV-2-infected patients

Characteristic <sup>a</sup>	Value
Women [ <i>n</i> (%)]	28 (52)
Age [median yr (IQR)]	45 (37–53)
ARV treatment [ <i>n</i> (%)]	
No	19 (35)
Yes	35 (65)
ARV-naive patients	
HIV-2 RNA load [median log <sub>10</sub> copies/ml (IQR) in patients with detectable viremia (>100 copies/ml) ( <i>n</i> = 19)]	4.00 (3.51–4.19)
ARV-treated patients	
HIV-2 RNA load [median log <sub>10</sub> copies/ml (IQR) in patients with detectable viremia (>100 copies/ml) ( <i>n</i> = 35)]	3.98 (3.47–4.59)
Duration of NRTI exposure [median mo (IQR)]	40 (6–184)
ARV treatment at time of sampling [ <i>n</i> (%)]	
2 NRTIs + 1 PI/r	19
3 NRTIs	10
3 NRTIs + 1 PI/r	6

<sup>a</sup> IQR, interquartile range; PI/r, protease inhibitor boosted with ritonavir.

values were compared using a *t* test. Because *P* values were not corrected for multiple comparisons, findings of statistical significance should be considered provisional. Statistical analyses were performed using R version 3.0.3.

**Nucleotide sequence accession numbers.** The HIV-2 RT and RNase H sequences were deposited in GenBank with accession numbers [KM208882](#) to [KM208955](#).

## RESULTS

**Identification of mutation V111I in HIV-2 patients.** To identify mutations in the polymerase, connection, and RNase H domains of HIV-2 RT that mediate resistance to NRTIs, we sequenced the complete RT genes of patients in the French National HIV-2 cohort. The complete RT sequences of 19 ARV-naive and 35 ARV-treated patients failing an NRTI-containing regimen were analyzed. The patients were all infected with HIV-2 subtype A. The ARV-treated patients received therapies with 2 or 3 NRTIs with or without a protease inhibitor. The patient characteristics and treatment regimen are summarized in [Table 1](#). The key resistance mutation K65R was found in 40%, M184V in 51%, and Q151M in 31% of the ARV-treated patients, and these mutations were highly associated with virological failure ([Table 2](#)). In addition, mutation V111I in the polymerase domain was found in 43% of the viruses retrieved from ARV-treated patients, whereas the mutation was not detected in viruses from ARV-naive patients. Analysis of the connection and RNase H domains identified two polymorphisms, V356I and I467V, that were found with increased frequency in patients failing therapy ([Table 2](#)). No other significant changes were identified in the polymerase, connection, or RNase H domain of the HIV-2 RT enzyme. Further analysis of mutation V111I showed that the mutation is significantly associated with key resistance mutations K65R and Q151M, but not with mutation M184V ([Table 3](#)). No differences were observed between the patients harboring viruses with or without V111I with regard to the number or type of NRTIs they received or the number of months on treatment. Combinations of the key resistance mutations (K65R and M184V, K65R and Q151M, and Q151M and

**TABLE 2** Changes in the RT sequences of HIV-2 subtype A viruses found with increased frequency in patients failing therapy

Mutation	HIV-2 patients [no. (%)] positive		Significance ( <i>P</i> value <sup>a</sup> )
	Naïve ( <i>n</i> = 19)	Treated ( <i>n</i> = 35)	
Polymerase domain			
M184V	0 (0)	18 (51)	0.0001
V111I	0 (0)	15 (43)	0.0004
K65R	0 (0)	14 (40)	0.0009
Q151M	0 (0)	10 (29)	0.0097
Connection domain			
V356I <sup>b</sup>	7 (47)	24 (77)	0.0497
RNaseH			
I467V <sup>c</sup>	8 (58)	25 (93)	0.0197

<sup>a</sup> *P* values of <0.05 are considered significant.

<sup>b</sup> Based on 15 naïve and 31 treated patients.

<sup>c</sup> Based on 12 naïve and 27 treated patients.

M184V) were found in 9%, 6%, and 9% of the ARV-treated patients, respectively. The triple mutant (K65R, Q151M, and M184V) was found in 9% of the patients. No significant association was found between mutation V111I and these combination mutants.

**Drug susceptibilities of V111I viruses.** The frequency at which V111I in HIV-2 RT is selected in patients failing NRTI-based therapies suggests that it is involved in escape from drug therapy. To determine its role, we introduced mutation V111I and the key resistance mutations K65R, Q151M, and M184V into the HIV-2 subtype A molecular clone pROD9 by site-directed mutagenesis. Subsequently, we examined the individual and combined effects of V111I and these key resistance mutations on NRTI sensitivity ([Table 4](#)). Interestingly, no significant effects of mutation V111I on NRTI sensitivity were measured. Mutation Q151M mediated approximately 3-fold resistance to d4T, ABC, and ddI and a high level of resistance to AZT (50-fold). Strikingly, resistance to ABC and ddI was lost in the Q151M V111I combination. Mutation M184V was found to confer high-level resistance to 3TC (>500-fold), also in combination with V111I. K65R was found to confer modest levels of resistance to TDF and ddI and high-level resistance to 3TC (>100-fold). Similar resistance levels were measured for the K65R V111I combination, which in addition resulted in a 2.5-fold increase in resistance to d4T compared to the wild-type virus. These combined results suggest that mutation V111I does not strongly contribute to NRTI resistance, as only a modest 3-fold resistance to d4T was measured in combination with K65R.

**Fitness of V111I viruses.** Pairwise competition experiments were performed to determine the relative fitness of the V111I vi-

**TABLE 3** Association between position 111 and key resistance mutations found in HIV-2 subtype A patients failing therapy

Mutation	Association (no. of patients)		<i>P</i> value <sup>a</sup>
	V111	I111	
K65R	14	86	0.0001
Q151M	27	73	0.0271
M184V	61	39	0.7380

<sup>a</sup> *P* values of <0.05 are considered significant.

TABLE 4 Sensitivities of HIV-2 wild-type and mutant viruses to NRTIs

NRTI	IC <sub>50</sub> (μmol/liter) <sup>a</sup>							
	Wild type	Q151M	M184V	K65R	V111I	Q151M V111I	M184V V111I	K65R V111I
AZT	0.05 ± 0.02	<b>2.54 ± 1.25</b> ( <i>P</i> = 0.014)	0.07 ± 0.02 ( <i>P</i> = 0.085)	0.27 ± 0.21 ( <i>P</i> = 0.192)	0.06 ± 0.01 ( <i>P</i> = 0.093)	<b>1.41 ± 0.91</b> ( <i>P</i> = 0.029)	0.10 ± 0.04 ( <i>P</i> = 0.238)	0.24 ± 0.05 ( <i>P</i> = 0.091)
TDF	0.59 ± 0.26	0.66 ± 0.27 ( <i>P</i> = 0.731)	0.58 ± 0.18 ( <i>P</i> = 0.940)	<b>4.42 ± 0.46</b> ( <i>P</i> = 0.001)	0.84 ± 0.12 ( <i>P</i> = 0.119)	1.15 ± 0.89 ( <i>P</i> = 0.243)	0.83 ± 0.51 ( <i>P</i> = 0.391)	<b>3.79 ± 0.92</b> ( <i>P</i> = 0.027)
d4T	2.22 ± 0.67	<b>6.52 ± 1.87</b> ( <i>P</i> = 0.016)	1.79 ± 0.76 ( <i>P</i> = 0.386)	3.36 ± 0.44 ( <i>P</i> = 0.052)	1.78 ± 0.53 ( <i>P</i> = 0.246)	<b>6.74 ± 2.03</b> ( <i>P</i> = 0.006)	2.31 ± 0.84 ( <i>P</i> = 0.870)	<b>5.87 ± 0.67</b> ( <i>P</i> = 0.046)
ABC	2.16 ± 0.82	<b>6.37 ± 2.05</b> ( <i>P</i> = 0.019)	2.69 ± 1.13 ( <i>P</i> = 0.495)	4.23 ± 1.76 ( <i>P</i> = 0.097)	0.84 ± 0.26 ( <i>P</i> = 0.058)	1.04 ± 0.10 ( <i>P</i> = 0.091)	3.06 ± 1.24 ( <i>P</i> = 0.355)	2.52 ± 1.06 ( <i>P</i> = 0.661)
ddI	2.74 ± 1.45	<b>8.73 ± 2.11</b> ( <i>P</i> = 0.005)	3.88 ± 0.45 ( <i>P</i> = 0.140)	<b>7.45 ± 1.62</b> ( <i>P</i> = 0.003)	1.55 ± 0.60 ( <i>P</i> = 0.116)	3.76 ± 1.76 ( <i>P</i> = 0.443)	2.75 ± 0.63 ( <i>P</i> = 0.982)	<b>8.55 ± 3.74</b> ( <i>P</i> = 0.047)
ddC <sup>b</sup>	0.09 ± 0.02	0.40 ± 0.13 ( <i>P</i> = 0.055)	0.12 ± 0.07 ( <i>P</i> = 0.606)	1.01 ± 0.46 ( <i>P</i> = 0.074)	0.08 ± 0.02 ( <i>P</i> = 0.211)	0.34 ± 0.14 ( <i>P</i> = 0.089)	0.16 ± 0.06 ( <i>P</i> = 0.208)	0.61 ± 0.10 ( <i>P</i> = 0.052)
3TC	0.46 ± 0.19	0.95 ± 0.57 ( <i>P</i> = 0.266)	<b>&gt;500</b> ( <i>P</i> < 0.05)	<b>&gt;100</b> ( <i>P</i> < 0.05)	0.23 ± 0.08 ( <i>P</i> = 0.056)	0.52 ± 0.34 ( <i>P</i> = 0.800)	<b>&gt;500</b> ( <i>P</i> < 0.05)	<b>&gt;100</b> ( <i>P</i> < 0.05)

<sup>a</sup> The IC<sub>50</sub> values are the means of at least 3 independent experiments ± standard deviations. The *P* values were calculated using the *t* test, in which we compared the IC<sub>50</sub> values for a particular mutation with the IC<sub>50</sub> obtained using the wild-type virus. The statistical analysis was performed in R version 3.0.3. The values in boldface differ significantly from the IC<sub>50</sub> measured for the wild-type virus.

<sup>b</sup> ddC, zalcitabine.

rus. Therefore, the SupT1 T-cell line was infected with equal amounts of two viruses. During prolonged culturing of the mixtures, the HIV-2 strain with the best fitness outcompetes the other virus. The frequencies of the two viruses were assessed at several time points by population sequencing of the proviral polymerase gene. Representative competitions between the wild-type ROD9 virus and V111I, K65R and K65R V111I, Q151M and Q151M V111I, and M184V and M184 V111I were performed (Fig. 1). Each competition experiment was initiated at least three times

with independent virus stocks and produced the same result. The experiments showed that mutation V111I resulted in a reduced replication capacity compared to the wild-type virus (Fig. 1A). Reduced fitness was also measured for the V111I M184V virus compared to the M184V virus (Fig. 1B). However, mutation V111I was found to increase the replication capacity of the K65R and Q151M viruses (Fig. 1C and D).

**Structural changes mediated by V111I.** Position 111 is located in a loop comprised of residues 110 to 115 (Fig. 2A) in the palm

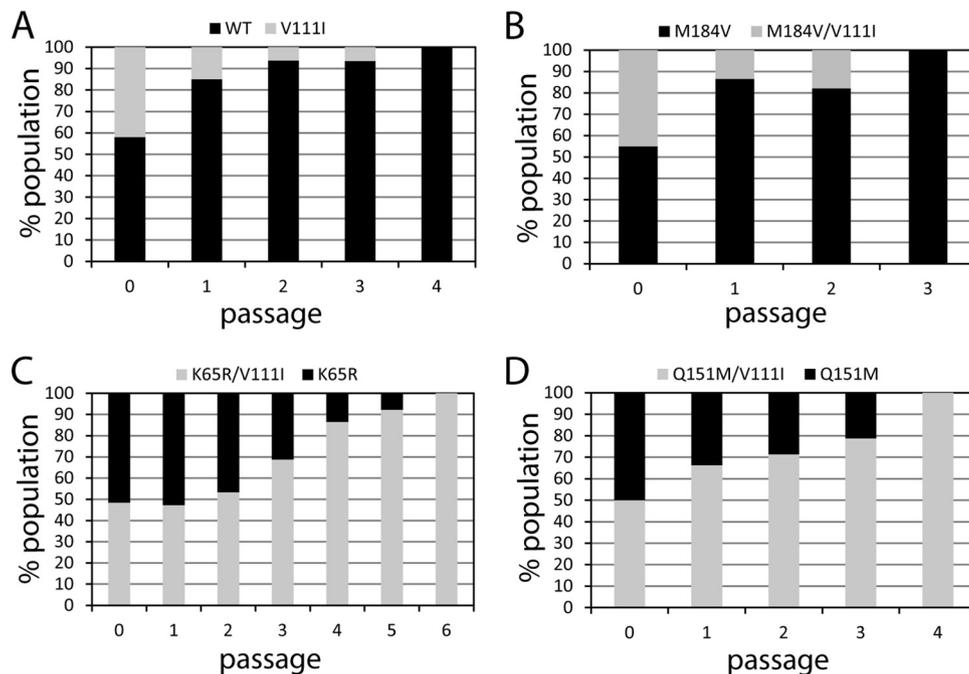
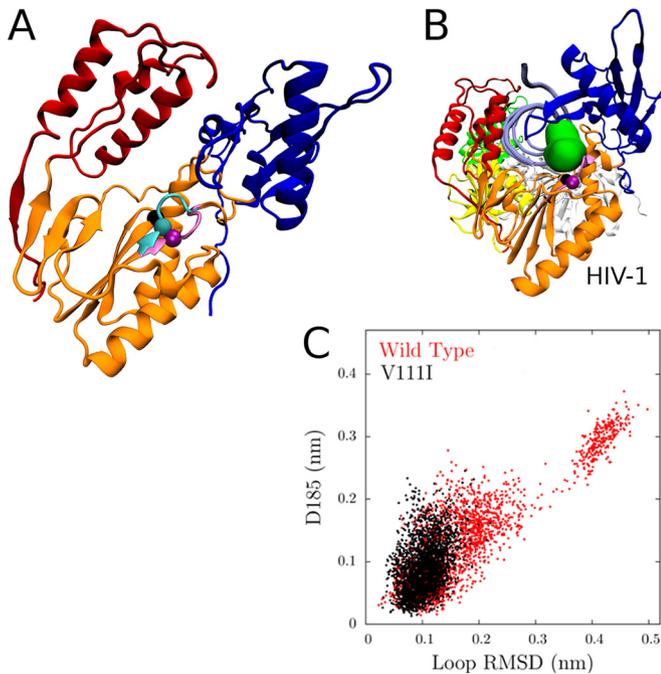


FIG 1 Relative fitness of wild-type (WT) and mutant viruses. The SupT1 T-cell line was infected with equal amounts of two viruses, and the frequencies of the viral genotypes were assessed at each passage by population sequencing of the RT gene. The percentage of each virus in the population was calculated from the Sanger sequencing electropherograms. Shown are pairwise competition experiments between the wild-type and V111I (A), M184V and M184V V111I (B), K65R and K65R V111I (C), and Q151M and Q151M V111I (D) viruses. All experiments were repeated at least three times, and representative experiments are shown.



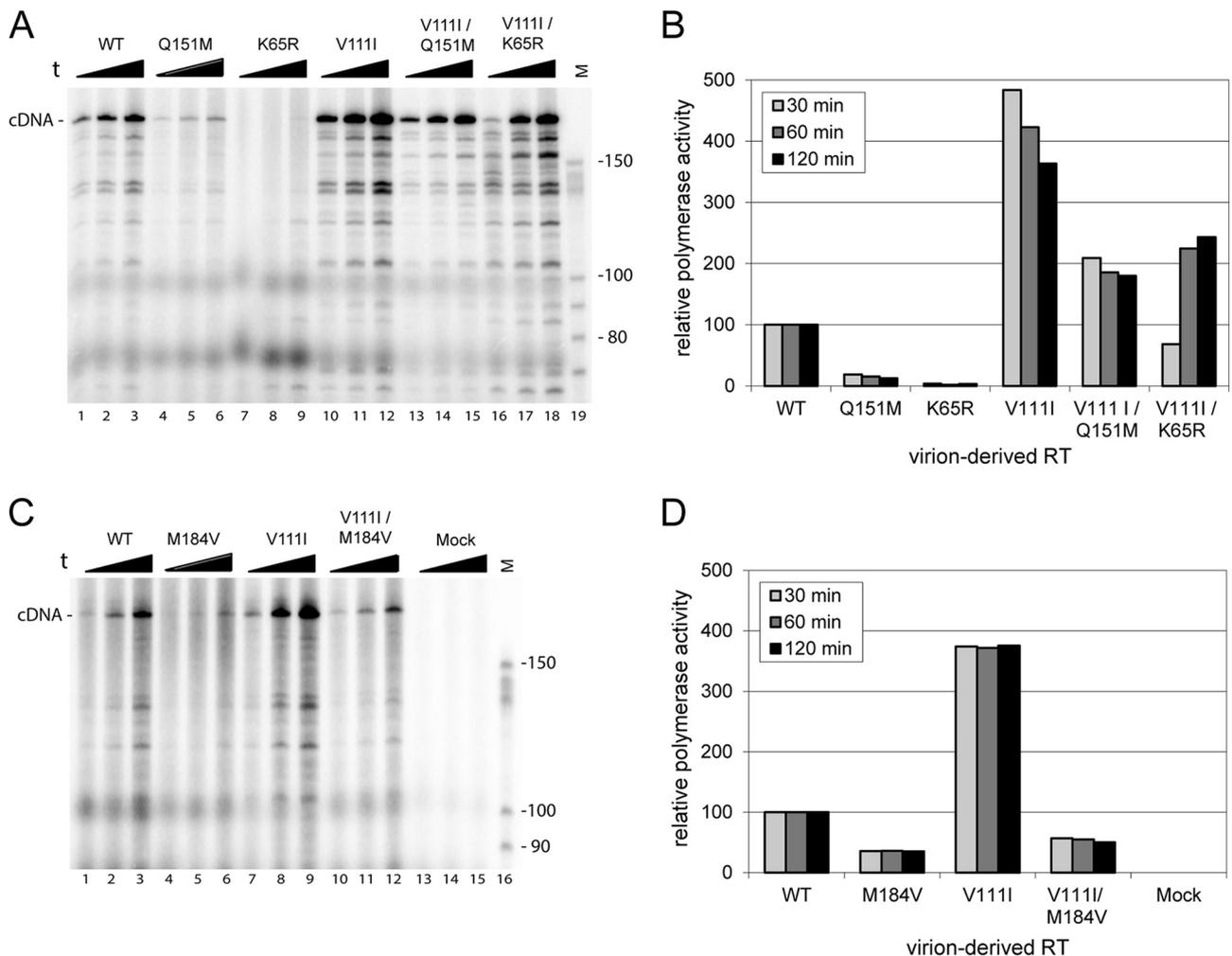
**FIG 2** Molecular dynamics simulations of the wild-type and V111I HIV-2 RT enzymes. (A) Crystal structure of the polymerase domain of HIV-2 RT, with the palm (yellow), thumb (red), and fingers (blue) subdomains. The position of the 110-to-115 loop is shown in pink. The second loop conformation, observed only during simulations of the wild-type RT, is shown in cyan. The location of residue 111 within the loop is indicated by a sphere (placed on the carbon alpha atom). The location of residue D185 in the polymerase active site is marked by a black sphere. (B) Structure of HIV-1 RT bound to DNA/dATP (PDB code 3KK2) indicating the location of the dNTP binding pocket relative to the polymerase active site (which is highly conserved between HIV-1 and HIV-2). The subdomain coloring and orientation are the same as for HIV-2 RT; DNA is shown in light blue, and dATP in green. (C) The RMSD of the 110-to-115 loop plotted against the RMSD of residue D185 in the polymerase active site over the simulations (relative to the position in the HIV-2 crystal structure). Two conformations of the 110-to-115 loop are observed for wild-type RT (red), whereas only a single cluster of values is observed for V111I RT (black). The flexibility of the loop is associated with displacement of catalytic residue D185 in the wild-type simulations.

region of the HIV-2 RT enzyme, very near the polymerase active site (residues D110, D185, and D186). Unlike K65R and Q151M, mutation V111I is not within the dNTP binding pocket and does not interact with the template/primer directly. Consequently, any impact upon RT activity and selectivity must be mediated through changes in conformation or dynamics in adjacent regions of the RT structure. This makes it an ideal candidate for investigation through molecular dynamics simulations. Models of the wild-type and V111I RTs were constructed using the Modeler package (25) and the only available crystal structure of HIV-2 RT (PDB code 1MU2) (23). Five simulations producing 5 ns of sampled trajectory were performed, and changes in the palm domain were analyzed. During most of the simulation time, both RT enzymes were close to the average structure (data not shown). However, during simulation of the wild-type RT, two different conformations of the 110-to-115 loop were sampled, whereas only one conformation was found for V111I RT. The two loop conformations are depicted in Fig. 2A. These results suggest that replacement of valine at position 111 with the bulkier isoleucine reduces the flexibility of the loop.

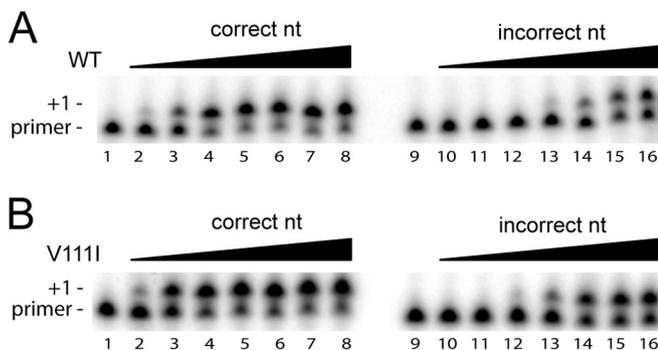
As the structure of the nucleic-acid-bound HIV-2 RT is not available, the simulated structures were aligned to the closely related HIV-1 RT bound to a DNA-DNA template-primer complex (PDB code 3KK2) (32) (Fig. 2B). The conformation of the 110-to-115 loop in this HIV-1 crystal structure is similar to that in the HIV-2 crystal structure (Fig. 2A and B, pink loop). The loop is located near the polymerase active site and the dNTP binding pocket. Interestingly, the second loop conformation found for HIV-2 RT (Fig. 2A, cyan loop) may block the dNTP binding site. This loop conformation was not observed for V111I RT, suggesting that the dNTP binding pocket may be more accessible in that mutant. We also observed that the flexibility of the loop in wild-type simulations is accompanied by motion of polymerase active site residue D185 away from the position observed in the HIV-2 crystal structure (Fig. 2C). This offers a second potential mechanism that could modulate the polymerase activity of the V111I RT enzyme. Changes in the flexibility of the 110-to-115 loop thus may affect the dNTP binding and catalytic activity of the HIV-2 V111I RT enzyme.

**Effect of V111I on polymerase activity.** To characterize the effects of V111I on polymerase function, the activities of the virion-derived RT enzymes were analyzed (Fig. 3). Therefore, viral lysates were generated, concentrated, and normalized for the amount of infectious virus. We used an RNA template encompassing the HIV-1 PBS region and a labeled DNA primer complementary to the PBS. Minus-strand DNA synthesis was initiated by the addition of virion-derived RT and dNTPs. Extension of the 21-nt primer resulted in increasing amounts of the 202-nt labeled cDNA product over time for the wild-type RT (Fig. 3A, lanes 1 to 3). The amounts of cDNA products generated by the different virion-derived RTs were quantified and are plotted in Fig. 3B and D. The Q151M (Fig. 3A, lanes 4 to 6) and K65R (lanes 7 to 9) mutations were found to reduce the polymerase activity of the HIV-2 RT enzyme. Surprisingly, V111I was found to increase the polymerase activity approximately 4-fold (lanes 10 to 12) and restored the reduced polymerase activity mediated by mutations Q151M and K65R (lanes 13 to 15 and 17 to 19). The polymerase activity measured for Q151M V111I and K65R V111I was increased 2-fold compared to the wild type. Mutation M184V was also found to reduce polymerase activity (Fig. 3C, lanes 4 and 5); however, V111I was unable to completely restore this defect (lanes 10 to 12). Similar results were obtained using an HIV-2 RNA template and primer complementary to the HIV-2 PBS (results not shown).

**Effect of V111I on polymerase fidelity.** Molecular dynamics simulations suggest that substitution V111I may affect nucleotide binding. To investigate the putative effects of V111I on RT fidelity, we performed single-nucleotide incorporation assays to measure site-specific misincorporation (Fig. 4). The lack of proofreading activity enables the analysis of fidelity without the interference of exonuclease activity. We used an RNA template encompassing the HIV-2 PBS region and a labeled DNA primer complementary to the PBS. Virion-derived RT was added and incubated with increasing concentrations of dATP as the correct nucleotide or dTTP as the incorrect nucleotide. The kinetics of misincorporation were measured as a function of increasing dNTP concentration, and the Michaelis constant ( $K_m$ ) and maximum velocity ( $V_{max}$ ) for dNTP incorporation were calculated. The insertion efficiency for the incorrect versus the correct nucleotide provides the frequency of misincorporation (Table 5). We measured an



**FIG 3** Polymerase activities of wild-type and mutant virus-derived HIV-2 RT enzymes. (A) The polymerase activities of the wild-type, Q151M, K65R, V111I, Q151M V111I, and K65R V111I HIV-2 RT enzymes were determined using an HIV-1 RNA template and an end-labeled DNA primer complementary to the PBS. The primer was heat annealed onto the template and extended by the addition of virion-derived RT and dNTPs. Formation of the 202-nt cDNA product (cDNA) was monitored after 30, 60, and 120 min of incubation time (t). Increasing incubation time is marked by a triangle. A DNA size marker (M) was loaded as a reference (nt). Similar results were obtained in at least three independent experiments. (B) The amount of cDNA production was quantified, and the polymerase activity of the wild-type virus-derived RT was set at 100%. (C) Polymerase activities of wild-type M184V, V111I, and M184V V111I virus-derived RT enzymes. (D) Quantification of the cDNA products, with the activity of wild-type RT set at 100%. Representative gels are shown, and similar results were obtained in at least two independent experiments and were repeated using HIV-2 RNA templates.



**FIG 4** Fidelity of the wild-type and V111I HIV-2 RT enzymes. (A) Single-nucleotide incorporation assays were performed using an RNA template encompassing the HIV-2 PBS region and a labeled DNA primer complementary to the PBS. Virion-derived wild-type or V111I RT was added and incubated with increasing concentrations of dATP as the correct nucleotide or dTTP as the incorrect nucleotide (marked by triangles). Single-nucleotide incorporation was quantified in three independent experiments (Table 5).

approximately 9-fold increase in the frequency of misincorporation for mutant V111I. The increase from  $9 \times 10^{-4}$  for the wild type to  $80 \times 10^{-4}$  for V111I was mostly due to a change in  $K_m$  rather than  $V_{max}$ . These results indicate that mutation V111I reduces the fidelity of the HIV-2 RT enzyme.

## DISCUSSION

Analysis of complete HIV-2 RT sequences showed that mutations K65R, Q151M, and M184V are frequently selected in French HIV-2 patients failing NRTI-containing therapies. These mutations were found to reduce the incorporation of nucleotide analogues during reverse transcription in both HIV-1 (33) and HIV-2 (18). Consistent with previous studies, TAMs, which provide the principal route to NRTI resistance in HIV-1 by the excision pathway, were observed only at very low frequencies in the HIV-2 patients. It has been suggested that the mechanisms responsible for NRTI resistance are different for HIV-1 and HIV-2 (16, 17).

TABLE 5 Kinetics of single-nucleotide incorporation for the HIV-2 wild-type and V111I RT enzymes<sup>a</sup>

HIV-2 RT	dNTP	$V_{\max}$ (%/min)	$K_m$ ( $\mu$ M)	$F_{\text{ins}}^b$
WT <sup>c</sup>	Correct	13.68 $\pm$ 0.14	0.04 $\pm$ 0.00	1
	Incorrect	10.26 $\pm$ 0.06	34.01 $\pm$ 2.45	8.94 $\times 10^{-4}$
V111I	Correct	12.78 $\pm$ 0.45	0.04 $\pm$ 0.00	1
	Incorrect	10.82 $\pm$ 0.65	4.24 $\pm$ 0.10	79.8 $\times 10^{-4}$

<sup>a</sup> The Michaelis constant ( $K_m$ ) and maximum velocity ( $V_{\max}$ ) for the correct and incorrect dNTPs were determined from graphs in which the rate of primer elongation (percent per minute) was plotted as a function of the dNTP concentration. The results ( $\pm$  standard deviation) from 3 independent experiments were quantified.

<sup>b</sup> The ratio of the incorporation efficiency of the incorrect versus the correct dNTP provides the frequency of misinsertion ( $F_{\text{ins}}$ ) (arbitrarily set at 1.0 for the correct nucleotide).

<sup>c</sup> WT, wild type.

Diminished incorporation may play a more important role in HIV-2 than selective excision of nucleotide analogues. In regard to the K65R mutation, several clinical studies reported a high frequency of the mutation in NRTI-treated patients infected with HIV-2 (13, 14), whereas in other studies K65R was rarely observed (34–36). In addition, *in vitro*, the selection of K65R in HIV-2 is not common (37, 38). In this study, K65R was found in 40% of the treated patients. The reasons for these differences in prevalence are unclear, but they may reflect differences in the treatment regimen. In this study, the HIV-2 patients were on treatment regimens containing 3TC (73%) and TDF (24%), which are known to select for the K65R mutation. More studies are needed to uncover the mechanism of K65R selection.

Besides these key resistance mutations, mutation V111I was identified in the polymerase domain. Furthermore, polymorphism V356I in the connection subdomain and I467V in the RNase H domain were found with increased frequency in patients failing therapy. Several polymorphisms in the connection subdomain of HIV-1 were previously found to contribute to resistance (39, 40). However, none of the connection subdomain mutations found to contribute to NRTI resistance in HIV-1 (for a review, see reference 21) were found in HIV-2 patients. In HIV-1, the mutations in the connection subdomain are selected together with TAMs. The absence of TAMs in HIV-2 patients failing therapy may restrict the selection of connection subdomain mutations. The potential roles of polymorphisms V356I and I467V in NRTI resistance in HIV-2 remain to be determined. The selection of V111I in the polymerase domain of RT was found to be associated with key resistance mutations K65R and Q151M. Analysis of 1,017 HIV-2 subtype A sequences deposited in the HIV Sequence Database in Los Alamos showed that mutation V111I is present in 11% of the sequences without key resistance mutation K65R, Q151M, or M184V. However, V111I is found in 55% of the sequences containing Q151M and in 81% of the sequences containing K65R, whereas V111I is present in only 20% of the sequences with M184V. This analysis thus also suggests an association between mutation V111I and mutations K65R and Q151M. In HIV-1, the amino acid present at position 111 is also a valine. In the Stanford database, a valine-to-isoleucine substitution at position 111 is found in approximately 1% of the HIV-1 isolates and appears to be linked to NNRTI treatment. It is currently unknown if V111I plays a role in the development of drug resistance in HIV-1.

In HIV-2 patients failing therapy, V111I was previously reported to be coselected with mutation Q151M (14, 15, 20). Mutation Q151M alone was suggested to confer resistance to d4T and ABC, whereas resistance to other NRTIs would involve the coselection of V111I (20). However, a previous *in vitro* study demonstrated that Q151M mediates resistance to AZT, d4T, and ddI (18). Resistance to these NRTIs was also measured in our study for Q151M. However, we also measured a 3-fold increase in the IC<sub>50</sub> for ABC, whereas the 2-fold increase in the IC<sub>50</sub> in the previous study did not reach statistical significance. Mutation M184V provides high-level resistance to 3TC, and K65R provides resistance to ddI, TDF, and 3TC. In the previous study (18), no increase in the IC<sub>50</sub> for TDF was measured for mutation K65R. This could be due to differences in the assay used to determine drug susceptibility. Although there was no evidence for ABC resistance mediated by K65R in these *in vitro* studies, *in vivo*, the mutation was reported to be selected in HIV-2-infected patients experiencing virological failure while receiving an ABC-containing regimen (14, 15, 20). In both *in vitro* studies, mutation K65R was introduced in the HIV-2 ROD9 background, which suggests that variations or mutations present in the patient isolates contribute to resistance mediated by the K65R mutation. We show that mutation V111I alone does not affect drug susceptibility. In combination with K65R, a modest 2.5-fold increase in resistance to d4T was measured. Furthermore, V111I was found to reduce the resistance mediated by Q151M to ddI (2.3-fold) and ABC (6.1-fold).

As mutation V111I was not found to strongly contribute to NRTI resistance, we hypothesized that it may play a role in viral fitness by enhancing the replication capacity of the resistant viruses. In HIV-1, the mutations K65R, Q151M, and M184V were found to result in replication defects. Mutation K65R reduces the replication capacity of HIV-1 in T-cell lines and monocytes (41, 42), whereas mutation M184V results in reduced fitness in monocytes (43, 44). Mutation Q151M does not seem to reduce the HIV-1 replication capacity (45, 46). However, the acquisition of four additional mutations (A62V, V75I, F77L, and F116Y) in the Q151M complex provides multidrug resistance and increases the replication capacity of the Q151M virus (45). We therefore studied the relative fitness of the HIV-2 V111I viruses in pairwise competition assays (Fig. 1). Mutation V111I reduced the fitness of the wild-type and M184V viruses. Interestingly, V111I was found to increase the replication capacity of the K65R and Q151M viruses. These results are consistent with the fact that a strong association was found between V111I and mutations K65R and Q151M in ARV-treated HIV-2 patients failing therapy, whereas no association was found with mutation M184V (Table 3). Mutation V111I in HIV-2 thus enhances the replication capacity of the K65R and Q151M viruses, like the additional mutations in the HIV-1 Q151M complex. No compensatory mutations in HIV-1 have been reported that restore the replication defect of the K65R virus.

Residue 111 in the HIV-2 RT enzyme is located near the polymerase active site in a loop comprised of residues 110 to 115 (Fig. 2). Molecular dynamics simulations indicated that this loop is highly flexible in wild-type RT, whereas its motion is constrained by the replacement of valine with the bulkier isoleucine in the V111I mutant. Residue 111 is not located within the dNTP binding pocket. However, using the dNTP/DNA-bound HIV-1 RT as a model, we hypothesized that V111I does affect dNTP binding. The highly flexible loop of the wild-type RT can adopt a conformation that may block the dNTP binding pocket. This conformation was

not observed for V111I RT, suggesting that the dNTP binding pocket of the mutant is more accessible. Moreover, the flexibility of the loop in wild-type simulations was accompanied by motion of polymerase active site residue D185 away from the position observed in the crystal structure of HIV-2 RT. The position of D185 in the HIV-2 crystal structure is similar to that in the dNTP/DNA-bound HIV-1 RT and likely represents the catalytically competent conformation of the polymerase active site. Movement of D185 away from this position may be expected to reduce the polymerase activity of the enzyme. This change in the position of D185 was not observed during simulations of the V111I RT enzyme. The reduced flexibility of the 110-to-115 loop in V111I RT thus may affect both dNTP binding and polymerase activity. In agreement with this, both enhanced polymerase activity and reduced fidelity were observed for the HIV-2 V111I RT enzyme in the biochemical assays. Finally, V111I may affect the divalent metal binding at the dNTP binding pocket. Complete insight into the structural changes induced by V111I would therefore require modeling of not only dNTP and nucleic acid, but also the metal ions chelated at the active site.

Polymerase assays were performed to study the polymerase activity of the V111I RT enzyme and that of RT enzymes containing the key resistance mutations. In HIV-1, mutations M184V, K65R, and Q151M were shown to reduce the polymerase activity of the RT enzyme and increase the fidelity of reverse transcription (41, 47–51). A previous study showed that Q151M in the HIV-2 RT enzyme also results in reduced polymerase activity (16). We demonstrated that Q151M, K65R, and M184V severely reduce the polymerase activity of the HIV-2 RT enzyme. Interestingly, mutation V111I resulted in a 4-fold increase in polymerase activity. V111I was able to restore the polymerase activity of K65R and Q151M mutants and partially restore that of M184V. We subsequently studied the fidelity of *in vitro* DNA synthesis mediated by the wild-type and V111I HIV-2 enzymes. The abilities of the RT enzymes to incorporate one correct or incorrect nucleotide were measured. The results indicated that the V111I mutant displayed an approximately 9-fold-increased frequency of misinsertion compared to the wild-type enzyme. This is largely attributable to a decrease in  $K_m$  rather than a significant change in  $V_{max}$  for V111I RT. These results are in agreement with the molecular dynamics simulations, which suggested that V111I results in a continuously accessible nucleotide binding site and a catalytically competent conformation of the HIV-2 RT enzyme.

Mutation V111I thus increases the polymerase activity but reduces the fidelity of the HIV-2 RT enzyme. This may result in a higher error rate during reverse transcription of the viral genome, which can be deleterious to virus replication. In agreement with this hypothesis, the V111I virus has a reduced replication capacity compared to the wild type. However, V111I was found to restore the polymerase activity of K65R and Q151M mutants and increase the replication capacity of these viruses. The partial restoration of the polymerase activity of the M184V mutant did not increase virus fitness, in agreement with the observation that M184V V111I is not selected in HIV-2 patients failing therapy. In HIV-1, mutations K65R and Q151M were found to increase the fidelity of the RT enzyme, resulting in reduced incorporation of nucleotide analogues. Therefore, the reduced fidelity mediated by V111I in HIV-2 RT may be expected to reduce the resistance of the K65R and Q151M viruses. Consistent with this, V111I was found to reduce the resistance to ddI and ABC mediated by mutation Q151M. These re-

sults emphasize the complex nature of HIV-2 resistance to NRTIs. The frequent selection of V111I in clinical samples suggests that it contributes to an optimal balance between replication efficiency and drug resistance in the K65R and Q151M viruses.

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