Genetic Changes in HIV-1 Gag-Protease Associated with Protease Inhibitor-Based Therapy Failure in Pediatric Patients

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Abstract

Studies have shown a low frequency of HIV-1 protease drug resistance mutations in patients failing protease inhibitor (PI)-based therapy. Recent studies have identified mutations in Gag as an alternate pathway for PI drug resistance in subtype B viruses. We therefore genotyped the Gag and protease genes from 20 HIV-1 subtype C-infected pediatric patients failing a PI-based regimen. Major protease resistance mutations (M46I, I54V, and V82A) were identified in eight (40%) patients, as well as Gag cleavage site (CS) mutations (at codons 373, 374, 378, 428, 431, 449, 451, and 453) in nine (45%) patients. Four of these Gag CS mutations occurred in the absence of major protease mutations at PI failure. In addition, amino acid changes were noted at Gag non-CS with some predicted to be under HLA/KIR immune-mediated pressure and/or drug selection pressure. Changes in Gag during PI failure therefore warrant further investigation of the Gag gene and its role in PI failure in HIV-1 subtype C infection.

Introduction

SOUTH AFRICA HAS AN ESTIMATED 410,000 children infected with HIV-1 of whom 58% were eligible and receiving antiretroviral treatment by the end of 2012.¹ The national treatment guidelines follow World Health Organization recommendations to treat HIV-infected infants <3 years of age with a protease inhibitor (PI)-based regimen. This is due to the high rates of nonnucleoside reverse transcriptase inhibitor (NNRTI) resistance in infants treated for prevention of mother-to-child transmission (PMTCT) and the effectiveness of using a first-line PI-based regimen to reduce mortality and disease progression in children.^{2,3}

Lopinavir coformulated with ritonavir (LPV/r) is used as part of a triple antiretroviral treatment regimen for infants >6 months of age. Prior to 2008, for infants <6 months of age or children receiving rifampicin for cotreatment of tuberculosis, ritonavir (RTV) alone was used. LPV/r was subsequently licensed for use in infants <6 months, and the boosting of LPV/r with additional RTV or double-dosing of LPV/r when using rifampicin was introduced in 2008.⁴⁻⁶

PI drug resistance is mediated by an accumulation of major mutations in the protease gene resulting in reduced binding of the inhibitor to the protease (PR) enzyme and high level resistance.⁷ In addition, minor PR mutations, which have little or no effect on PI susceptibility, contribute to reduced susceptibility in combination with major mutations.⁸ Studies have shown that mutations in Gag cleavage sites (CS) also contribute to PI resistance development.⁹⁻¹² HIV-1 PR recognizes and cleaves the Gag and Gag Pol polypeptides to release the structural components: matrix (MA), capsid (CA), p6, nucleocapsid (NC), enzymes protease, reverse transcriptase, RNase H, integrase, the small polypeptides p1 and p2, as well as transframe protein (TFP).¹³ Cleavage sites in Gag comprise five amino acids flanking either side of the scissile bond, with position 1 (P1) located immediately upstream and position 1 prime (P1') located immediately downstream of the cleavage point.¹⁴ Gag CS mutations such as A431V (CS NC/p1), L449P, and P453L (CS p1/p6^{Gag}) have been shown to coevolve with the HIV-1 PR gene under drug selection pressure.⁹⁻¹¹ While these sites are the most commonly described, substitutions at all CS have been

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observed at PI failure.^{9,10,15} In addition, studies have shown that certain non-CS mutations in Gag can contribute to reduced susceptibility to PIs in the presence of major PR mutations *in vitro*.^{16,17} However, the mechanism of resistance is not well understood since these mutations occur outside of the functionally related regions.

There are limited genetic data on pediatric patients infected with HIV-1 subtype C failing PI-based therapy, with no published data on Gag. Given the large numbers of children initiating LPV/r-based regimens, identifying reasons for therapy failure is increasingly important. Recent studies in HIV-1 subtype C-infected children and adults have shown a lack of PR mutations at failure, possibly suggesting alternate routes of PI resistance.^{18,19} In this study, the PR and Gag genes of pediatric patients were sequenced at baseline and at the time of virological failure to identify changes associated with PI failure, taking into account both drug and immune selection pressures.

Materials and Methods

Study cohort

A retrospective cohort study was undertaken on 20 matched baseline and posttreatment plasma samples from children less than 24 months of age and failing PI-based therapy. These children were initiated on treatment and followed as part of the prerandomization screening phase of a randomized clinical trial between 2005 and 2009. Because these children failed their initial LPV/r-based therapy they were not eligible for randomization as part of the trial that was designed to assess the feasibility of switching to an NNRTI-based regimen following 1 year of suppressive PI-based therapy.^{20–22} Children over 6 months of age were treated with lamivudine (3TC), stavudine (d4T), and zidovudine (ZDV)-boosted lopinavir (LPV/r). Those younger than 6 months of age and those receiving rifampicin-based regimens for treatment of tuberculosis or Bacille Calmette-Guérin (BCG) disease received RTV until reaching 6 months of age and/or completing TB treatment, at which point they were initiated on or switched back to LPV/r. For children who suppressed and then rebounded, genotyping was done on the first sample where an elevated viral load was observed (within 3 months). For children who did not suppress, a sample was taken within 6 months. Whole blood specimens were collected at baseline (pretreatment) and during regimen failure, defined as not having achieved an HIV-1 plasma RNA <1,000 copies/ml by 52 weeks on treatment. This study was approved by the institutional review boards of Columbia University and the University of the Witwatersrand.

Genotypic analysis of Gag-PR

Viral RNA was extracted from plasma at baseline and failure using the QIAamp Viral RNA Mini kit (QIAGEN, Belgium) and reverse transcribed using a Thermoscript RT-PCR kit (Invitrogen, CA). A 1.8-kb Gag-PR fragment spanning HXB2 positions 792 to 2549 was amplified by nested polymerase chain reaction (PCR) using an Expand High Fidelity PCR kit (Roche Applied Science, Basel, Switzerland) as previously described.²³ Primers BKT03 (5' CGCAGGA CTCGGCTTGC 3') and ProOutR (5' TTGGGCCATCCA TTCCTGG 3') were used for first round PCR and primers GagNot+ (5' GCGGCGGCCGCAAGGAGAGAGATGGG TGCG 3') and ProXhoR2 (5' CTGGTACAGTCTCGAG

RGGACTRATKGG 3') for nested PCR. Population-based sequencing was performed using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA) on an ABI3130 Genetic Analyzer (Applied Biosystems, Foster City, CA). Patient sequences were aligned using Clustal W in BioEdit version 7.0.²⁴ Minor and major PR mutations were defined according to the Stanford University Drug Resistance Database (<http://hivdb.stanford.edu/DR/PIResiNote.html>). Genetic changes in Gag and PR were defined as those that developed at regimen failure compared to the baseline sequence, excluding those that reverted to the subtype C consensus sequence. The subtype C consensus sequence (year 2004) was downloaded from the Los Alamos Sequence Database (www.hiv.lanl.gov/content/sequence/NEWALIGN/align.html). The consensus C sequence was calculated by the database using the Consensus Maker tool.

Prediction of sites selected by HLA- and KIR-mediated immune pressure

HLA-associated polymorphisms in Gag were predicted from previously published mutation maps that presented the locations of common escape mutations in HIV-1 subtype B.²⁵ Briefly, the *q*-value threshold used for constructing the immune escape maps was *q* ≤ 0.05, meaning that only a 5% false-positive proportion was expected among associations displayed on the maps. Sites in Gag selected by KIR-mediated immune pressure were identified as published.²⁶

Positive selection analysis

The single ancestor likelihood counting (SLAC) test was performed using Datamonkey²⁷ to test for selection pressure and estimate the ratio of synonymous to nonsynonymous evolutionary changes (*dN/dS*). Calculations were performed for the entire dataset containing 20 Gag baseline and failure nucleotide sequences. SLAC estimates the number of *dN* and *dS* changes occurring at each codon throughout evolution based on ancestral reconstruction. A 0.05 cut-off was applied to the analysis. Positions with a *dN/dS* ratio >1 were considered to be under positive selection pressure.

Results

Clinical characteristics of study population

Of the 20 children included in this study, seven (35%) received both LPV/r and RTV while seven (35%) received RTV as the only PI and six (30%) were treated with LPV/r only. In addition to the PI, all children received two NRTIs (d4T and 3TC) (Table 1). The length of RTV-only treatment ranged from 27 to 252 days. Of the 14 children that at some point received RTV as a single PI, 10 were cotreated for mycobacterial disease while four were <6 months of age. The mean viral load and CD4 count at time of measurement of drug resistance were 103,880 copies/ml (range 1,240–750,000) and 28.4% (range 9.6–49.6), respectively. Of the 20 children, two (10%) had a CD4 count of ≤14%.

Genotypic analysis of PR and Gag mutations at PI failure

Eight children (40%) had detectable major PI resistance mutations at failure, with V82A (*n* = 8, 40%), I54V (*n* = 4,

TABLE 1. CLINICAL CHARACTERISTICS AND TREATMENT INFORMATION OF HIV-1-INFECTED INFANTS AND YOUNG CHILDREN FAILING PROTEASE INHIBITOR-BASED ANTIRETROVIRAL THERAPY

Pt. No.	Time on treatment (days)	Treatment ^a	Reason for RTV ^b	Viral load at resistance test	CD4%
1	365	RTV + LPV/r	< 6 months	7,980	46.6
2	363	RTV + LPV/r	TB	48,400	22.9
3	252	RTV + LPV/r	TB	17,800	17.0
4	251	RTV + LPV/r	TB	28,200	45.8
5	252	RTV + LPV/r	TB	15,000	16.6
6	367	RTV + LPV/r	< 6 months, TB	1,240	26.9
7	370	RTV + LPV/r	TB	100,000	9.6
8	254	RTV	TB	15,400	18.9
9	252	RTV	TB	4,980	12.6
10	261	RTV	TB	66,400	NA
11	84	RTV	TB	750,000	NA
12	85	RTV	< 6 months, TB	750,000	NA
13	27	RTV	< 6 months	6,910	NA
14	161	RTV	TB	100,000	20.4
15	254	LPV/r	—	100,000	16.4
16	384	LPV/r	—	10,100	25.4
17	369	LPV/r	—	2,010	44.2
18	363	LPV/r	—	4,220	41.2
19	253	LPV/r	—	8,720	49.6
20	734	LPV/r	—	100,000	31.0

^aRTV + LPV/r: patients received both RTV as a single PI as well as LPV/r; RTV: patients received RTV as a single PI; LPV/r: patients received LPV/r only.

^b< 6 months, age less than 6 months; TB, tuberculosis; NA, not available.

RTV, ritonavir; LPV/r, lopinavir/ritonavir.

20%), and M46I ($n=3$, 15%) identified as the only major PR mutations (Fig. 1 and Table 2). All patients who had at least one major PI mutation had received RTV therapy at some point, while patients who received LPV/r only did not have any major PI resistance mutations. Patients who developed ≥ 2 major PR mutations were more likely to have been on RTV therapy for a longer period of time (mean = 290 days, range: 251–365 days) compared to those with <2 major PR

mutations (mean = 200 days, range: 27–370 days). In addition, several minor PI mutations and polymorphisms (L10I, S12T, K20R, L33F, K43E, D60E, Q61H/E, L63P/T, K70R, and N83S) were detected in both LPV/r- and RTV-treated patients.

When analyzing the 20 patients at baseline and failing PI therapy, 10 showed no changes in PR after PI failure, and six of these had received LPV/r exclusively. The majority of these patients, however, had preexisting baseline changes in PR, relative to a consensus C sequence, that persisted following PI failure (Table 2 and Supplementary Fig. S1; Supplementary Data are available online at www.liebertpub.com/aid).

Nine of 20 patients had detectable Gag CS mutations at failure, five of whom also had PR major mutations (Fig. 2). HIV-1 drug resistance-associated CS mutations A431V (NC/p1), L449P, and P453L (both in p1/p6^{Gag}) were found in four of the nine patients. The A431V mutation was selected in two patients (numbered 1 and 10) in the presence of major PI mutations I54V and V82A. Patients 11 and 20 had no major PI mutations, but both had mutations L449P and/or P453L. The most variable cleavage site was p2/NC where four of nine patients had at least one mutation at this site at failure (positions N373A, T374A/S, and M378A). Other CS mutations occurred at NC/p1 (position E428D) and p1/p6^{Gag} (position N451S). The most conserved cleavage sites were MA/CA, CA/p2, NC/TFP, and TFP/P6^{pol} where no changes were observed.

Changes were also detected at Gag non-CS at failure that differed from the consensus C. These changes were found in the majority of patients (17/20) and occurred mainly in the matrix and capsid regions in the N-terminus of Gag (Supplementary Table S1).

FIG. 1. Frequency of Gag and protease mutations in patient samples at protease inhibitor (PI) failure relative to their treatment. The numbers of patients with any PI drug resistance mutations (DRMs), any Gag cleavage site mutations (CSMs), and any Gag noncleavage site mutations (non-CSMs) are shown. Individual PI mutations (M46I, I54V, and V82A) and mutations occurring at Gag CS and non-CS are shown. The proportion of patients on ritonavir (RTV) + lopinavir coformulated with ritonavir (LPV/r) is indicated by black bars, RTV only is indicated by gray bars, and LPV/r only is indicated by white bars.

TABLE 2. GENOTYPIC CHARACTERIZATION OF PROTEASE AND GAG FROM HIV-1-INFECTED INFANTS AND YOUNG CHILDREN FAILING PROTEASE INHIBITOR-BASED ANTIRETROVIRAL THERAPY

Pt. No.	Treatment ^a	Protease genotype	Gag CS genotype
1	RTV + LPV/r	I54V, V82A , K20R, L23I, E35D, L63P	V378A, A431V
2	RTV + LPV/r	M46I, I54V, V82A , L10I, K20R, E35D, <u>Q61H</u> , L63P	<u>E428D</u>
3	RTV + LPV/r	V82A , K20R, <u>K45R</u>	—
4	RTV + LPV/r	M46I, I54V, V82A , K20R, <u>L33F</u> , E35D, T74S	V128T, <u>N374S</u>
5	RTV + LPV/r	—	—
6	RTV + LPV/r	K43E, N83S	<u>S373A</u>
7	RTV + LPV/r	A71T	—
8	RTV	V82A , S12T, L10I, K20R, E35D, <u>D60E</u> , <u>Q61E</u>	N451S
9	RTV	M46I, V82A , A63T	V128I
10	RTV	I54V, V82A , I36M, N37S, L63P, V77I	A431V
11	RTV	N37S, L63S, V82I	<u>V128I</u> , <u>L449P</u> , P453L
12	RTV	V82A , K20R, E35D, R57K, D60E, L63P	—
13	RTV	<u>E35D</u> , L63V, T74S	E428K, L449P, P453L
14	RTV	L10M, I36M, N37K, K41I, L63T, <u>K70R</u>	—
15	LPV/r	K20R, E35D, L63V	—
16	LPV/r	I36M, K41R, T74S, V77I	I437L
17	LPV/r	L63V	—
18	LPV/r	V82I	Y132F
19	LPV/r	I36L, N37E, K41R, L63V	<u>T374A</u> , P453L
20	LPV/r	L63P, V77I	<u>L449P</u>

^aRTV + LPV/r: patients received both RTV as a single PI as well as LPV/r at some point; RTV: patients received RTV as a single PI only; LPV/r: patients received LPV/r only.

Protease mutations in bold represent major PI mutations. Mutations *underlined* represent changes that developed following PI-based therapy.

For 10 patients (5, 7, 11, 13, 15–20) there were no changes in protease at failure, although nine had baseline protease polymorphisms.

Predicted HLA and KIR immune escape mutations in Gag

Previously published immune escape maps were used to determine if some of the identified changes in Gag that developed at failure were due to immune pressure. Seven of 20 patients had changes at the failure time point relative to

baseline, at sites shown to be selected by known HLA A, B, and C alleles reported to occur in South African black and white populations (Supplementary Table S2). In total, eight sites were predicted to be associated with HLA or KIR-mediated immune pressure (Fig. 3). One site at codon 374 was also a Gag CS (p2/NC) mutation and another at codon 79 was at a Gag non-CS previously shown to be associated with PI

	Gag Cleavage Sites							Protease Major Mutations		
	MA/CA	CA/p2	p2/NC	NC/p1	p1/P6 ^{44G}	NC/TFP	TFP/P6 ^{44I}			
Cons C	¹²⁸ VSQNY/PIVQN ¹³⁷	³⁵⁹ KARVL/AEAMS ³⁶⁸	³⁷³ NTNIM/MQRSN ³⁸²	⁴²⁸ ERQAN/FLGKI ⁴³⁷	⁴⁴⁴ RPGNF/LQNR ⁴⁵³	⁴²⁸ ERQAN/FLREN ⁴³⁸	⁴³⁶ ENLAF/QQGEA ⁴⁴⁵	M 46	I 54	V 82
Pt. 1	/.....	/.....	/A.....	...V../.....	/.....	/.....	/.....	.	V	A
Pt. 2	/.....	/.....	/.....	D...../.....	/.....	/.....	/.....	I	V	A
Pt. 4	/.....	/.....	.S../.....	/.....	/.....	/.....	/.....	I	V	A
Pt. 6	/.....	/.....	A...../.....	/.....	/.....	/.....	/.....	.	.	.
Pt. 8	/.....	/.....	/.....	/.....	/..S..	/.....	/.....	.	.	A
Pt. 10	/.....	/.....	/.....	...V../.....	/.....	/.....	/.....	.	V	A
Pt. 11	/.....	/.....	/.....	/.....	/P...L	/.....	/.....	.	.	.
Pt. 19	/.....	/.....	.A...../.....	/.....	/.....	/.....	/.....	.	.	.
Pt. 20	/.....	/.....	/.....	/.....	/P.....	/.....	/.....	.	.	.

FIG. 2. Position of Gag CS amino acid changes in patients at PI failure. Nine patients with Gag CS changes at PI failure are shown. Of these, five also had major PI mutations (positions 46, 54, and 82). Gag CS mutations are reported relative to the baseline sample. The sequences and amino acid positions at consensus C cleavage sites are shown.

FIG. 3. Changes in Gag following PI-based therapy failure. Amino acid positions in Gag ($n=53$) that change following PI-based therapy were classified according to those at CS ($n=8$) and those at non-CS ($n=45$). Non-CS changes were further classified as those known to be associated with PI exposure from the published literature ($n=3$), those predicted to be HLA/KIR-associated polymorphisms ($n=8$), and those under positive selection pressure ($n=5$). *Position 93 was predicted to be an HLA and KIR-associated polymorphism. A total of 21/53 sites were therefore considered to be under selective pressure. The remaining 32 positions that could not be accounted are likely due to natural variation.

exposure.²⁵ The E93D KIR-associated polymorphism in Gag was identified in one patient (patient 8, Fig. 3) and has been associated with HLA-mediated immune pressure.^{25,26}

Positive selection in Gag

A total of 53 amino acid residues were identified in Gag that had changed between baseline and failure among these 20 patients (Fig. 3). To determine which of these changes were under positive selection pressure following PI failure, a *dN/dS* analysis on the matched baseline and failing sample was performed (Supplementary Table S3). A total of five sites in Gag were found to be under significant selection pressure (Fig. 3). Position 62, which has previously been reported to be associated with PI exposure,²⁶ had the highest *dN/dS* ratio of 16.318 ($p=0.013$) and was found in four patients. The remaining four positively selected sites (positions 15, 54, 69, and 339) have not previously been associated with PI exposure nor predicted to be HLA/KIR. Two additional sites in Gag have been reported to be associated with PI failure,^{17,28} including the HLA/KIR-selected position 79, but were not found to be positively selected in this analysis. In total, 21 of the 53 changes identified in the entire Gag gene following PI failure showed some evidence of selection pressure (Fig. 3; Supplementary Table S3).

Discussion

This is the first study to describe the genotypic changes in both the Gag and PR genes in HIV-1 subtype C pediatric

patients failing PI-based therapy. The study showed that virological failure during PI exposure may have led to the selection of PR resistance mutations as well as Gag CS and non-CS mutations. In this study only patients on an RTV regimen were observed to have developed major PI mutations as well as Gag CS mutations while LPV/r selected for Gag CS mutations only. Mutations at Gag CS were found in some cases to occur in the absence of major PR mutations implicating them as potentially resistance conferring. Furthermore, sites in Gag non-CS were predicted to be under selection pressures following PI failure. Collectively these data warrant further investigation of the contribution of the Gag gene to PI drug resistance.

Similar to what has been reported by others, all PI resistance-associated mutations were selected in patients who had received RTV compared to those treated with LPV/r exclusively.^{21,30} The major PI mutations detected at failure were M46I, I54V, and V82A, all of which are well described as causing high-level resistance to multiple PIs.³¹ Of interest, nearly half of the patients in this study acquired changes at Gag CS at failure. Gag and PR function as a single unit with enzyme and substrate coevolving to optimize viral replication.³² Mutations at the Gag CS A431V, L449P, and P453L have been shown to contribute to PI resistance.^{10,11} A431V has been found to occur in combination with V82A and can be selected as compensatory or as a direct PI mutation in the absence of PR mutations.¹¹ The CS mutation L449P has been directly associated with exposure to ritonavir, indinavir, fosamprenavir, nelfinavir, saquinavir, and atazanavir, while P453L has been found to enhance resistance in the presence of PR mutations I50V and I84V *in vitro*.^{9,10,33} In this study these well-described Gag-CS mutations were found in four patients, two in the absence of PR mutations, and it is likely that they contributed to PI failure in these patients. In addition, five patients had Gag-CS mutations that are less well defined, mainly at p2/NC, which is a more variable CS.¹¹ In some cases these also occurred in the absence of PR mutations. Whether these Gag CS changes contributed to LPV/r and RTV failure in these patients is unknown and would require further phenotypic testing.

A large number of changes at Gag non-CS were also detected at failure in this cohort. Eight sites were predicted to be under HLA/KIR-mediated immune pressure suggesting that they may be cytotoxic CD8⁺ T lymphocyte escape mutations.²⁵ One of these, codon 79, has previously been associated with PI exposure, thus suggesting dual pressure from antiretroviral drugs and the immune system.²⁸ HLA alleles associated with these predicted sites have all been identified in the South African population.³⁴ However, it is important to note that the immune escape maps used to determine HLA-associated changes were based on HIV-1 subtype B Gag sequences. Therefore these data may underestimate the sites associated with HLA immune pressure in subtype C patients. Only one KIR-associated polymorphism at position 93 in Gag was identified in one patient suggesting this was not a major contributor to the Gag changes. However, a more in-depth analysis involving high-resolution HLA and KIR typing is required to determine whether these alleles were present in these patients and selected these mutations. Nevertheless, this prediction analysis does suggest that some of the Gag non-CS changes seen in this cohort are the result of immune selection pressures rather than drug pressures.

Positive selection analysis on the Gag region identified five sites as being under selection pressure, one of which was previously shown to be associated with PI exposure (position 62).²⁹ The remaining four sites under positive selection pressure occurred in four patients, two of whom developed Gag CS changes at failure and one who had a preexisting CS change from baseline. Therefore, these changes may be indicative of adaptive change possibly offering a fitness advantage and warrant further study. Of the 53 changes identified in this study, 21 could be accounted for by various selection pressures. The remaining 32 changes may represent natural variation, which is a hallmark of HIV-1. However, they occurred mainly in the conserved matrix and capsid regions of Gag and as such may have a function that is yet to be defined.

A limitation of this study is the use of population-based sequencing, which would not detect minority variants in PR and Gag present at baseline that may impact the response to treatment. Furthermore, due to the small sample size, caution should be taken in generalizing these findings to patients on RTV and/or LPV/r treatment and additional studies on pediatric patients receiving PI-based therapy are warranted. Nevertheless, almost half of the patients in this study failed RTV and LPV/r treatment without any detectable changes in PR or Gag CS. Poor adherence and suboptimal drug levels in patients presenting with wild-type PR and Gag cannot be excluded, but these data were not available. A recent study identified changes in gp41 of the *envelope* gene as an alternate route of PI resistance suggesting that sites outside of PR and Gag may also be involved.³⁵ Others have suggested that due to the short half life of PIs, there is a limited mutation selection window such that wild-type virus emerges faster than mutant virus resulting in the restricted evolution of drug resistance.³⁶

Thus further studies are needed to determine the reasons why patients fail PIs in the absence of detectable resistance mutations. Nevertheless, analysis of pre- and posttreatment samples allowed us to identify and correlate Gag mutations selected during PI failure in the presence or absence of PR mutations in a few patients. These data suggest that it may be useful to include the Gag gene when screening for PI resistance. Further studies on the effects of Gag mutations on PI resistance, and the development of drug resistance interpretation algorithms for both Gag and PR, may prove beneficial.

Sequence Data

Sequences used in this study correspond to GenBank accession numbers KP056164–KP056203.

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Author Disclosure Statement

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