Expanded Spectrum of Antiretroviral-Selected Mutations in Human Immunodeficiency Virus Type 2

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The Journal of Infectious Diseases

MAJOR ARTICLE

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Background. HIV-1 and HIV-2 differ in their antiretroviral (ARV) susceptibilities and drug resistance mutations (DRMs).

Methods. We analyzed published HIV-2 *pol* sequences to identify HIV-2 treatment-selected mutations (TSMs). Mutation prevalences were determined by HIV-2 group and ARV status. Nonpolymorphic mutations were those in <1% of ARV-naive persons. TSMs were those associated with ARV therapy after multiple comparisons adjustment.

Results. We analyzed protease (PR) sequences from 483 PR inhibitor (PI)-naive and 232 PI-treated persons; RT sequences from 333 nucleoside RT inhibitor (NRTI)-naive and 252 NRTI-treated persons; and integrase (IN) sequences from 236 IN inhibitor (INSTI)-naive and 60 INSTI-treated persons. In PR, 12 nonpolymorphic TSMs occurred in ≥11 persons: V33I, K45R, V47A, I50V, I54M, T56V, V62A, A73G, I82F, I84V, F85L, L90M. In RT, 9 nonpolymorphic TSMs occurred in ≥10 persons: K40R, A62V, K70R, Y115F, Q151M, M184VI, S215Y. In IN, 11 nonpolymorphic TSMs occurred in ≥4 persons: Q91R, E92AQ, T97A, G140S, Y143G, Q148R, A153G, N155H, H156R, R231 5-amino acid insertions. Nine of 32 nonpolymorphic TSMs were previously unreported.

Conclusions. This meta-analysis confirmed the ARV association of previously reported HIV-2 DRMs and identified novel TSMs. Genotypic and phenotypic studies of HIV-2 TSMs will improve approaches to predicting HIV-2 ARV susceptibility and treating HIV-2–infected persons.

Keywords. HIV-2; drug resistance; nucleoside RT inhibitors; protease inhibitors; integrase strand transfer inhibitors; mutations.

Human immunodeficiency virus-2 (HIV-2) is endemic in West Africa but has achieved only limited global spread. An estimated 1–2 million people have been HIV-2–infected worldwide, including those dually infected with HIV-1 and HIV-2, although recent systematic incidence and prevalence data are lacking [1]. Compared to HIV-1, the natural history of HIV-2 infection is characterized by a much longer asymptomatic stage, significantly lower plasma viral loads, slower decline in CD4 count, and lower mortality rate due to AIDS [2–4]. However, despite its lower virulence, the majority of untreated HIV-2-infected persons will progress to AIDS and death [5].

Three antiviral drug classes—nucleoside reverse transcriptase (RT) inhibitors (NRTIs), protease (PR) inhibitors (PIs), and integrase (IN) strand transfer inhibitors (INSTIs)—are widely used to treat HIV-2 infection [1, 6, 7]. There are many studies reporting mutations emerging in viruses from antiretroviral (ARV)-experienced HIV-2 infected persons [8–24] and several reporting in vitro susceptibility data on HIV-2 isolates containing the most commonly occurring HIV-2–associated drug resistance mutations (DRMs) [8, 18, 21, 23, 25–31]. However, there has been no meta-analysis of published sequence data from ARV-naive and ARV-treated persons. Such an analysis is important to identify mutations that should be prioritized for phenotypic testing and for consideration in algorithms for HIV-2 genotypic resistance interpretation.

In this study, we reviewed published HIV-2 pol sequences to define the natural variability of HIV-2 PR, RT, and IN in the absence of therapy and the spectrum of mutations significantly associated with PI, NRTI, and INSTI therapy.

METHODS

GenBank Search

We performed a BLAST search of the National Center for Biotechnology Information (NCBI) GenBank Flat File (release date 15 August 2019) using the HIV-2 ROD *pol* sequence (accession M15390). Retrieved sequences having HIV-2 as their species according to NCBI taxonomy were grouped into submission sets sharing the same GenBank submission "Title" and "Authors" field. Human subjects' approval was not obtained to perform this meta-analysis.

Received 21 October 2019; editorial decision 15 January 2020; accepted 17 January 2020; published online January 22, 2020.

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The Journal of Infectious Diseases® 2020;221:1962–72

DOI: 10.1093/infdis/jiaa026

Submission sets meeting the following criteria were entered into the Stanford HIV Drug Resistance Database (HIVDB): (1) they could be linked to a published paper or GenBank entry containing sufficient information pertaining to the origin of the virus isolate; (2) their sequences contained at least positions 10 to 90 of PR, positions 40 to 220 of RT, or positions 50 to 270 of IN; and (3) the samples were obtained from plasma or peripheral blood mononuclear cell (PBMC) samples and had not undergone extensive viral passage or manipulation. When multiple clones from the same clinical sample were sequenced, a consensus amino acid sequence, generated from all variants that occurred in 2 or more clones, was created and included in subsequent analyses.

Sequence Alignment and Quality Control Analysis

Sequences were aligned to HIV-2 ROD and neighbor joining phylogenetic trees were created for HIV-2 PR, RT, and IN. Sequences were assigned an HIV-2 group by examining the trees containing all study sequences annotated with group assignments from the Los Alamos HIV Sequence Database [32] and the software program COMET [33]. Consensus PR, RT, and IN amino acid sequences obtained from ARV-naive individuals were created using (1) all HIV-2 sequences, (2) group A HIV-2 sequences, and (3) group B HIV-2 sequences. The overall HIV-2 consensus sequence was unweighted and therefore the same as the group A consensus sequence as more than 85% of sequences belonged to group A.

Each amino acid sequence was aligned to the group A consensus over PR positions 1 to 99, RT positions 1 to 240, and IN positions 1 to 270. RT positions beyond 240 were not analyzed because fewer data were available for these positions and because this region has rarely been associated with either HIV-1 or HIV-2 drug resistance. IN positions beyond 270 were not analyzed because this region has not been associated with HIV-1 or HIV-2 drug resistance and because, in HIV-2, this region displays length heterogeneity, complicating sequence alignment.

Possible unusual mutations were defined as mutations with a prevalence <1.0% in sequences from all persons (ARV naive and ARV treated combined). Possible signature APOBEC mutations were defined as stop codons and mutations at conserved positions likely to have resulted either from a GG→AG or GA→AA mutation. Each sequence was examined for the numbers of possible unusual mutations and of possible signature APOBEC mutations. The numbers of possible unusual mutations per sequence decreased monotonically until about 5 for PR, 8 for RT, and 10 for IN (Supplementary Figure 1A). The numbers of possible signature APOBEC mutations per sequence was rarely above 1 in PR, RT (positions 1 to 240), and IN (Supplementary Figure 1B). The few sequences with 2 or more possible signature APOBEC mutations and with more than 5 to 10 unusual mutations (depending on the gene) were excluded from analysis.

Mutation Prevalence

The prevalence of each PR, RT, and IN amino acid was calculated according to HIV-2 group (all sequences, group A sequences, group B sequences) and according to ARV status (NRTI experience for RT, PI experience for PR, and INSTI experience for IN). Mutations were defined as amino acids differing from the HIV-2 consensus sequence. Mutations also included those present as part of an electrophoretic mixture in combination with wildtype. Mutations occurring in more than 1 sequence from the same individual were counted just once. Nonpolymorphic mutations were defined as mutations with a prevalence <1.0% in persons ARV naive for the relevant drug class.

Two approaches were used to identify mutations significantly associated with therapy (ie, treatment-selected mutations [TSMs]). Fisher exact testing was performed to examine the association of each mutation with exposure to ARV treatment regardless of HIV-2 genotype. Logistic regression was performed for each mutation to adjust the association with treatment for the possible confounding effect of group. For this regression analysis, the explanatory variables were ARV treatment status (naive vs treated) and group (A vs B) and the outcome variable was the presence or absence of the mutation. Odds ratios (ORs) and *P* values of the association of each mutation with treatment were derived from the coefficient of the treatment status variable. TSMs were required to be significantly associated with therapy by both Fisher exact testing and logistic regression.

The Holm method was used to control the family-wise error rate for multiple-hypothesis testing at an adjusted *P* value ≤ .05 [34]. The adjustment for multiple-hypothesis testing was performed separately for PR, RT, and IN mutations meeting the following criteria: (1) occurring in 3 or more treated persons receiving an ARV belonging to the relevant drug class and (2) present at least 2 times more commonly in persons that were ARV experienced than those ARV naive.

Correlation Network Analysis

For each drug class, we assessed whether specific pairs or groups of TSMs were likely to co-occur among treated persons harboring 1 or more TSMs. The analysis was restricted to sequences from persons harboring a TSM to avoid the bias towards false-positive correlations between 2 TSMs that can result when large numbers of sequences lack any TSMs. We created a list of positively correlated mutation pairs defined as those (1) co-occurring in at least 3 persons, (2) having a nonparametric Spearman correlation coefficient (ρ) ≥ 0.2, and (3) having a *P* value ≤ .05. We used the R package iGraph [35] to create an undirected weighted network graph from the adjacency matrix of positively correlated amino acid substitution pairs.

RESULTS

Published Studies and Sequences

Of 111 GenBank submission sets containing HIV-2 sequences, 77 (69.4%) met inclusion criteria and were entered into HIVDB. Sequences from 34 (30.6%) submission sets were not entered into HIVDB because they contained small numbers of sequences of laboratory isolates or poorly characterized clinical isolates (18 submission sets), were not described in a published paper (10 submission sets), or contained short sequence fragments (6 submission sets). Subsequent to the analysis of GenBank submission sets, 63 PR samples from 60 PI-treated persons, 68 RT samples from 55 NRTI-treated persons, and 37 IN sequences from 33 INSTI-treated persons that were described in published papers but not in GenBank were added to our analysis [12, 20, 22, 24].

Quality-control filtering resulted in the removal of 14 PR sequences from 14 persons, 62 RT sequences from 48 persons, and 18 IN sequences from 13 persons, resulting in a dataset containing 983 PR sequences from 739 persons, 852 RT sequences from 611 persons, and 385 IN sequences from 321 persons. Overall, 1411 (63.6%) samples were obtained from plasma specimens and 809 (36.4%) from PBMC specimens. Direct polymerase chain reaction Sanger sequencing was performed for 1922 (86.6%) of samples; the remaining samples comprised 1 or more molecular clones.

Figure 1A depicts PR variability in 483 PI-naive persons, Figure 1B depicts RT variability in 333 NRTI-naive persons, and Figure 1C depicts IN variability in 236 INSTI-naive persons.

PR Variability and PI-Associated Mutations

Of the 983 PR samples from 739 individuals, 888 (90.3%) were group A, 75 (7.6%) were group B, and 20 (2.0%) belonged to other groups or were AB recombinants. Overall, 523 (53.2%) samples were from 483 PI-naive persons, 408 (41.5%) were from 232 PI-treated persons, and 52 (5.3%) were from persons with uncertain PI treatment history. Of the 232 PI-treated persons, approximately 75% were noted to have received indinavir (IDV) and/or lopinavir/ritonavir (LPV/r). The proportion of samples from treated persons did not differ significantly between groups A (42.7%) and B (36.0%) ($P = .3$; Fisher exact test).

The HIV-2 group A reference sequence ROD (GenBank accession M15390) and group B reference sequence EHO (accession U27200) had 2 and 14 PR amino acid differences, respectively, from the HIV-2 consensus PR sequence. Among the PR sequences from PI-naive persons, 62 mutations at 38 positions had a prevalence ≥1.0% (Figure 1A).

Compared with the consensus HIV-1 PR amino acid sequence, different HIV-2 consensus amino acids were present at 5 major HIV-1 PI-resistance positions: (1) at positions 32, 46, and 47, the HIV-1–associated PI-resistance amino acids (V32I, M46I, and I47V) were the consensus amino acids; (2) at position 82, the HIV-1–associated polymorphism V82I was the HIV-2 consensus; and (3) at position 76, L76M, a variant not reported in HIV-1 strains, was the HIV-2 consensus.

Sixteen mutations occurring in 10 or more PI-treated persons were significantly associated with PI therapy using both Fisher exact tests and logistic regression (Table 1). Twelve of these mutations were nonpolymorphic, including V33I, K45R, V47A, I50V, I54M, T56V, V62A, A73G, I82F, I84V, F85L, and L90M. Four were polymorphic, including V10I, I64V, V71I, and L99F. Additional nonpolymorphic mutations at known HIV-1 PI-resistance positions included F53Y, I54L, A73T, M76L, N83D, I84L, and I89T each in 3 persons and G48V and I82L each in 2 persons.

Eighteen pairs of PI-selected mutations had a correlation coefficient ≥0.2 and a P value ≤.05 including 10 pairs with a correlation coefficient ≥0.3 and a P value <.001 (Table 2). Among the nonpolymorphic PI-selected mutations that are not known HIV-1 resistance mutations, T56V significantly correlated with I54M and I64V; V33I with V47A, I50V, and I64V; K45R with I47A; and F85L with A73G. V47A and I54M were strongly negatively correlated ($\rho = -0.34$; $P < .001$). Supplementary Figure 2A contains a weighted network graph from the adjacency matrix of significantly correlated PR amino acid substitution pairs.

RT Sequences and Mutation Prevalence

Of the 852 RT samples from 611 persons encompassing the 5′ RT polymerase coding region including positions 1 to 240, 780 (91.5%) were group A, 59 (6.9%) were group B, and 13 (1.5%) belonged to other groups or were AB recombinants. Overall, 336 (39.4%) samples were from 333 NRTI-naive persons, 466 (54.7%) were from 252 NRTI-treated persons, and 50 (5.9%) were from persons with uncertain NRTI-treatment history. Of the 252 NRTI-treated persons, approximately 50% had a history of receiving zidovudine/lamivudine (AZT/3TC) with or without other NRTIs; 15% had a history of receiving stavudine (d4T), didanosine (ddI), tenofovir disoproxil fumarate (TDF), or abacavir (ABC); and 35% had an unspecified NRTI treatment history. The proportion of samples from NRTI-treated individuals was slightly higher for group A than group B (56.4% vs 40.7%; $P = .02$; Fisher exact test).

The HIV-2 group A reference sequence ROD and group B reference sequence EHO had 9 and 33 amino acid differences, respectively, over the first 240 RT positions. Among the RT sequences from NRTI-naive persons, 134 mutations at 83 positions between positions 1 and 240 had a prevalence of ≥1.0% (Figure 1B).

Compared with the consensus HIV-1 RT amino acid sequence, different HIV-2 consensus amino acids were present at 3 positions at which HIV-1 thymidine analog mutations (TAMs) commonly occur: N210 (rather than L210), S215 (rather than T215), and E219 (rather than K219). Among the HIV-1 associated DRM positions at which non-TAMs have been reported, I rather than V was the consensus amino acid at position 75.

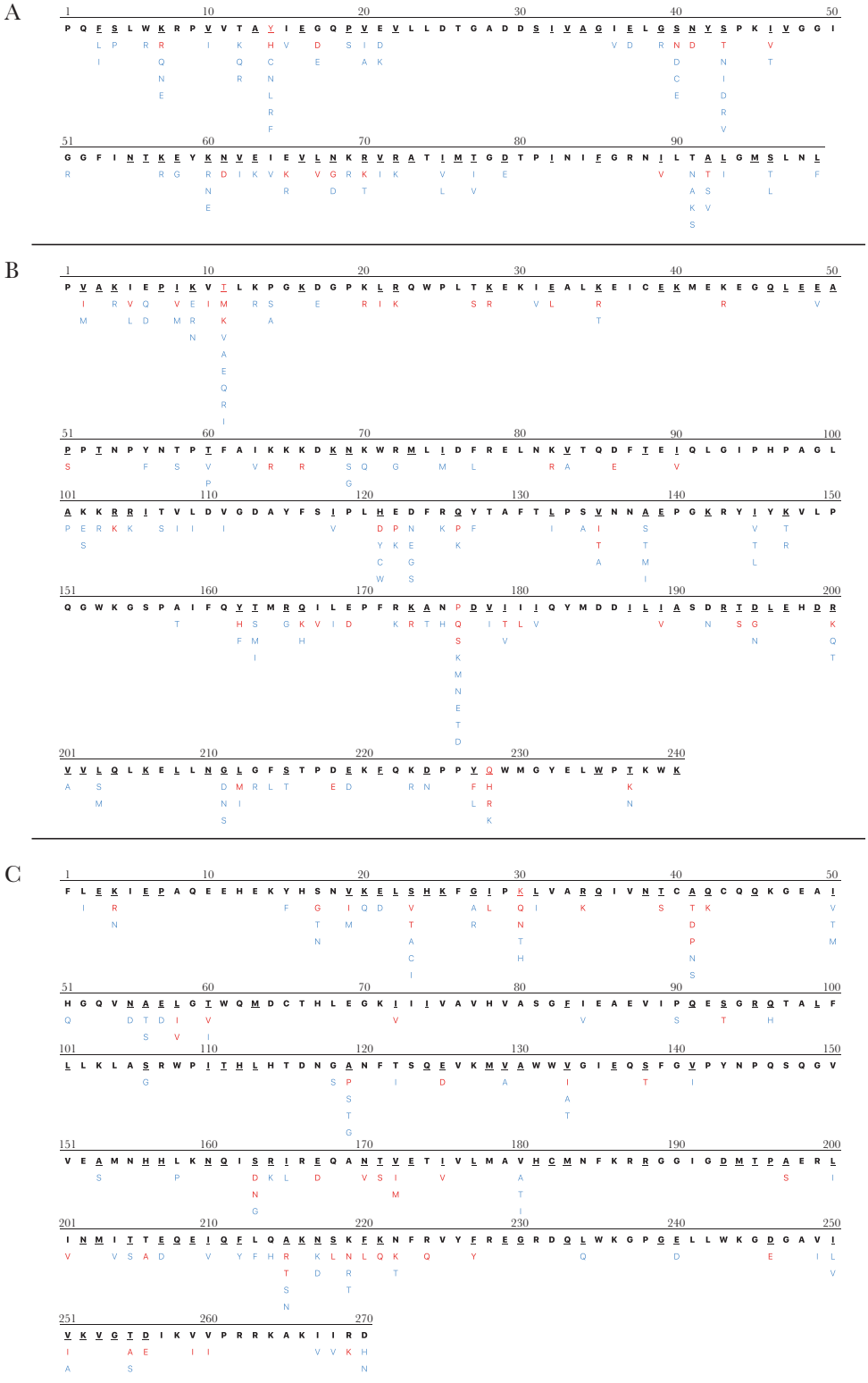

Figure 1. Natural variability of HIV-2 PR in 483 PI-naive persons (*A*), RT in 333 NRTI-naive persons (*B*), and IN in 236 INSTI-naive persons (*C*). Underlined amino acids are consensus HIV-2 mutations that differ from the group M HIV-1 consensus. Mutations in bold black have a frequency above 50% in antiretroviral-naive persons. Mutations in red have a frequency between 10% and 50%. Mutations in blue have a frequency between 1% and 10%. Abbreviations: HIV, human immunodeficiency virus; IN, integrase; INSTI, integrase strand transfer inhibitor; NRTI, nucleoside reverse transcriptase inhibitor; PI, protease inhibitor; PR, protease; RT, reverse transcriptase.

Table 1. Prevalence of Nonpolymorphic and Polymorphic HIV-2 PR Mutations in Protease Inhibitor (PI)-Naive and PI-Treated Persons Significantly Associated With PI Therapy

Mutation	PI Naive, No. (%) (n = 483)[a]	PI Treated, No. (%) (n = 232)[b]	Fold Change	Fisher Exact Test *P*[c]
Nonpolymorphic				
V33I	4 (0.83)	24 (10.34)	12.4	<.000001
K45R	0 (0)	26 (11.21)	>>>	<.000001
V47A	0 (0)	39 (16.81)	>>>	<.000001
I50V	2 (0.41)	16 (6.90)	16.7	.000001
I54M	2 (0.41)	43 (18.53)	44.8	<.000001
T56V	1 (0.21)	29 (12.50)	60.4	<.000001
V62A	1 (0.21)	19 (8.19)	39.6	<.000001
A73G	2 (0.42)	11 (4.74)	11.4	.006
I82F	2 (0.42)	42 (18.10)	43.5	<.000001
I84V	1 (0.21)	20 (8.62)	41.5	<.000001
F85L	2 (0.42)	28 (12.07)	29.0	<.000001
L90M	1 (0.21)	39 (18.66)	87.9	<.000001
Polymorphic				
V10I	12 (2.52)	39 (17.03)	6.8	<.000001
I64V	8 (1.66)	41 (17.67)	10.7	<.000001
V71I	40 (8.32)	100 (43.10)	5.2	<.000001
L99F	44 (9.36)	47 (23.04)	2.5	.0002

[a]n = total number of persons from whom sequences were obtained. Sequences encompassing positions 10, 33, 45, 71–85, 90, and 99 were available from 476, 481, 482, 481, 471, and 470 (rather than total 483) PI-naive persons, respectively.

[b]Sequences encompassing positions 10, 90, and 99 were available from 229, 209, and 204 (rather than total 232) PI-treated persons, respectively.

[c]Adjustment for multiple hypothesis testing for all mutations occurring ≥3 times in PI-experienced persons and ≥2 times more commonly in PI-experienced compared with PI-naive persons was performed using the Holm method.

Twelve mutations occurring in 10 or more NRTI-treated persons were significantly associated with therapy using both Fisher exact tests and logistic regression (Table 3). Nine of these mutations were nonpolymorphic including K40R, A62V, K65R, K70R, Y115F, Q151M, M184V/I, and S215Y. Three were polymorphic including N69S, V111I, and F214L. Six additional nonpolymorphic mutations at NRTI-resistance positions (M41I, D67N, N69T, K70N, I75V, and T215F) occurred in 6

Table 2. Statistically Significant Correlations Between Pairs of PI-Selected Mutations[a]

Mut X	Mut Y	No. with Mut X + Mut Y[b]	No. with Mut X alone[b]	No. with Mut Y alone[b]	No. without Mut X or Mut Y[b]	ρ	P
10I	82F	29	14	20	105	0.49	<.001
50V	64V	13	5	29	116	0.37	<.001
56V	64V	18	13	23	109	0.37	<.001
73G	85L	8	3	23	127	0.37	<.001
64V	84V	14	28	7	112	0.36	<.001
54M	82F	24	22	21	98	0.35	<.001
33I	47A	14	11	27	113	0.30	<.001
33I	50V	8	17	9	130	0.30	<.001
45R	47A	14	13	25	107	0.29	<.001
84V	90M	13	8	28	91	0.30	<.001
73G	82F	8	3	38	115	0.27	.001
45R	99F	15	8	33	81	0.28	.001
54M	56V	15	31	14	101	0.24	.002
54M	71I	37	8	66	50	0.24	.002
33I	64V	12	13	30	111	0.22	.004
54M	73G	7	38	4	111	0.21	.006
56V	82F	14	16	32	102	0.20	.012
56V	84V	8	21	13	116	0.20	.012

Abbreviations: Mut, mutation; PI, protease inhibitor; ρ, Spearman correlation coefficient.

[a]Correlations among PI-selected mutations in sequences from persons containing 1 or more PI-selected mutations.

[b]The number of persons for which each pair of mutations could be evaluated ranged between 137 and 168 because some sequences did not encompass all positions.

Table 3. Prevalence of Nonpolymorphic and Polymorphic HIV-2 Reverse Transcriptase Mutations Between Positions 1 and 240 in NRTI-Naive and NRTI-Treated Persons Significantly Associated With NRTI Therapy

Mutation	NRTI Naive, No. (%) (n = 332)[a]	NRTI Treated, No. (%) (n = 252)[b]	Fold change	Fisher Exact Test *P*[c]
K40R	3 (0.93)	14 (6.33)	6.8	.03
A62V	2 (0.61)	19 (7.72)	12.7	.0003
K65R	1 (0.30)	56 (22.49)	74.0	<.000001
K70R	0 (0)	18 (7.20)	>>>	.00001
Y115F	0 (0)	10 (3.98)	>>>	.01
Q151M	3 (0.90)	51 (20.24)	22.4	<.000001
M184I	1 (0.30)	17 (6.75)	22.4	.0003
M184V	3 (0.90)	147 (58.33)	64.6	<.000001
S215Y	0 (0)	14 (5.62)	>>>	.0003
Polymorphic				
N69S	9 (2.74)	24 (9.60)	3.5	.03
V111I	31 (9.34)	80 (31.87)	3.4	<.000001
F214L	7 (2.12)	30 (12.05)	5.7	.00008

Abbreviation: NRTI, nucleoside reverse transcriptase inhibitor.

[a]n = total number of persons from whom sequences were obtained. Sequences encompassing positions 40, 62, 65–70, and 214–215 were available from 321, 328, 329, and 330 (rather than total 332) NRTI-naive persons, respectively.

[b]Sequences encompassing positions 40, 62, 65, 69–70, 111–115, and 214–215 were available from 221, 246, 249, 250, 251, and 249 (rather than total 252) NRTI-treated persons, respectively.

[c]Adjustment for multiple hypothesis testing for all mutations occurring ≥3 times in NRTI-experienced persons and ≥2 times more commonly in NRTI-experienced compared with NRTI-naive persons was performed using the Holm method.

to 9 NRTI-treated persons but were not significantly associated with therapy after correction for multiple comparisons. The HIV-1–associated TAM M41L and the HIV-1 associated non-TAMs, L74V/I, F77L, and F116Y did not occur.

Fifteen pairs of mutations had a correlation coefficient ≥0.2 and a *P* value ≤.05 including 13 pairs with a correlation coefficient ≥0.3 and a *P* value <.001 (Table 4). Among the NRTI-selected mutations that are not known HIV-1 resistance mutations, the nonpolymorphic mutation K40R was significantly correlated with S215Y and the polymorphic mutation V111I was significantly correlated with A62V, K65R, N69S, Q151M, and F214L. Supplementary Figure 2B contains a weighted network graph from the adjacency matrix of significantly correlated RT amino acid substitution pairs.

IN Sequences and Mutation Prevalence

Of the 385 IN samples from 321 persons, 320 (83.1%) of the samples were group A, 51 (13.2%) were group B, and 14 (3.6%) belonged to other groups or were AB recombinants. Overall, 252 (65.5%) were from 236 INSTI-naive persons, 88 (22.9%) were from 60 INSTI-treated persons, and 45 (11.7%) were from persons with uncertain INSTI treatment history. Of the 60

Table 4. Statistically Significant Correlations Between Pairs of NRTI-Selected Mutations[a]

Mut X	Mut Y	No. with Mut X + Mut Y[b]	No. with Mut X alone[b]	No. with Mut Y alone[b]	No. without Mut X or Mut Y[b]	ρ	*P*
40R	215Y	9	5	1	152	0.74	<.001
65R	69S	24	39	1	139	0.53	<.001
65R	111I	47	16	37	108	0.46	<.001
62V	65R	18	1	41	135	0.46	<.001
62V	69S	11	11	15	159	0.39	<.001
151M	214L	21	33	11	131	0.38	<.001
69S	111I	22	4	64	119	0.33	<.001
65R	151M	28	31	28	113	0.28	<.001
62V	111I	17	4	67	116	0.27	<.001
70R	151M	12	6	42	136	0.28	<.001
65R	214L	18	41	13	127	0.27	<.001
65R	70R	12	46	6	136	0.26	<.001
69S	214L	10	13	23	152	0.26	<.001
111I	214L	21	59	12	112	0.22	.002
111I	151M	33	48	26	100	0.22	.002

Abbreviations: Mut, mutation; NRTI, nucleoside reverse transcriptase inhibitor; ρ, Spearman correlation coefficient.

[a]Correlations among NRTI-selected mutations in sequences from persons containing 1 or more NRTI-selected mutations.

[b]The number of persons for which each pair of mutations could be evaluated was not the same and ranged between 167 and 209 because some sequences did not encompass all positions.

INSTI-treated persons, 52 had received raltegravir and 6 had received raltegravir followed by dolutegravir. The proportion of samples from treated individuals was higher for group B than A (45.1% vs 19.1%; $P = .0001$; Fisher exact test).

The HIV-2 group A reference sequence ROD and group B reference sequence EHO had 11 and 27 IN amino acid differences, respectively, over the first 270 amino acids of the HIV-2 consensus IN sequence. Among the IN sequences from INSTI-naive persons, 145 mutations at 93 positions had a prevalence ≥1.0% (Figure 1C).

Fourteen mutations occurring in 4 or more INSTI-treated persons were significantly associated with therapy using both Fisher exact tests and logistic regression (Table 5). Eleven of these mutations were nonpolymorphic, including Q91R, E92A/Q, T97A, G140S, Y143G, Q148R, A153G, N155H, H156R, and an R231 5-amino acid insertion. Three mutations were polymorphic, including I84V, A119T, and V141I. Additional nonpolymorphic HIV-1 INSTI-resistance mutations included Y143C/R and Q148K, each in 3 INSTI-treated persons, and H51Y, E92G, G118R, G140A, Y143H, S147G, Q148H, and R263K each in 1 or 2 INSTI-treated persons.

Fourteen pairs of mutations had a correlation coefficient ≥0.2 and a P value ≤.05 including 7 pairs with a correlation coefficient ≥0.5 and a P value <.001 (Table 6). Among the nonpolymorphic INSTI-selected mutations that are not known HIV-1 resistance mutations, Q91R was significantly correlated with A119T; A153G with I84V, E92A, and N155H; and H156R with E92Q. Supplementary Figure 2C contains a weighted network graph from the adjacency matrix of significantly correlated IN amino acid substitution pairs.

DISCUSSION

Structural, biochemical, and in vitro susceptibility studies have reported differences in the activity of several ARVs against HIV-2 compared with HIV-1 [1, 6]. First, HIV-2 is intrinsically resistant to the nonnucleoside RT inhibitor (NNRTI) class of drugs by virtue of multiple natural occurring amino acid differences between HIV-1 and HIV-2 in the hydrophobic pocket to which NNRTIs bind [36]. Second, the mechanism of AZT resistance differs between HIV-1 and HIV-2. While HIV-1 resistance to AZT is usually caused by pyrophosphorolysis-mediated primer unblocking, the TAMs responsible for this process occur less commonly in HIV-2 isolates under AZT selection pressure [37]. The absence of primer unblocking in HIV-2 is partially explained by differences between HIV-1 and HIV-2 at amino acids in the critical β3-β4 hairpin loop extending between residues 67 and 75 and at other sites involved in pyrophosphorolysis [37, 38]. Third, differences in the HIV-1 and HIV-2 PR substrate cleft amino acids appear responsible for the reduced activity of multiple PIs against HIV-2 [39, 40]. Indeed, changing several of the amino acids that represent the HIV-2 consensus to the HIV-1 consensus restores PI susceptibility [39].

Most studies, however, suggest that the mutations that develop in HIV-2 during therapy with NRTIs, PIs, and INSTIs occur at HIV-1 DRM positions. Several notable previously reported exceptions include V111I in RT [21, 41, 42], L99F in PR [9, 18, 43], and an insertion at position 231 in IN [23]. Other differences in the spectrum of mutations observed in NRTI, PI, and INSTI-treated persons arise because the consensus amino

Table 5. Prevalence of Nonpolymorphic and Polymorphic HIV-2 Integrase Mutations in INSTI-Naive and INSTI-Treated Persons Significantly Associated With INSTI Therapy

Mutation	INSTI Naive, No. (%) (n = 236)[a]	INSTI Treated, No. (%) (n = 60)[a]	Fold	Fisher Exact Test P[b]
Nonpolymorphic				
Q91R	1 (0.42)	8 (13.33)	31.5	.0005
E92A	0 (0)	5 (8.33)	>>>	.01
E92Q	0 (0)	12 (20.00)	>>>	<.000001
T97A	0 (0)	19 (31.67)	>>>	<.000001
G140S	0 (0)	7 (11.67)	>>>	.0004
Y143G	0 (0)	4 (6.67)	>>>	.05
Q148R	0 (0)	7 (11.67)	>>>	.0004
A153G	1 (0.42)	10 (16.67)	39.3	.00003
N155H	1 (0.42)	17 (28.33)	66.9	<.000001
H156R	2 (0.85)	10 (16.67)	19.7	.0001
R231 insertion	0 (0)	7 (12.28)	>>>	.0003
Polymorphic				
I84V	15 (6.36)	19 (31.67)	5.0	.00004
A119T	4 (1.69)	8 (13.33)	7.9	.02
V141I	3 (1.27)	8 (13.33)	10.5	.008

Abbreviation: INSTI, integrase strand transfer inhibitor.

[a]n = total number of persons from whom sequences were obtained. However, sequences encompassing position 231 were available from 57 (not 60) INSTI-treated persons.

[b]Adjustment for multiple hypothesis testing for all mutations occurring in ≥3 times in INSTI-experienced persons and ≥2 times more commonly in INSTI-experienced compared with INSTI-naive persons was performed using the Holm method.

Table 6. Statistically Significant Correlations Between Pairs of INSTI-Selected Mutations[a]

Mut X	Mut Y	No. with Mut X + Mut Y[b]	No. with Mut X alone[b]	No. with Mut Y alone[b]	No. without Mut X or Mut Y[b]	ρ	*P*
153G	155H	10	0	7	35	0.70	<.001
92A	153G	5	0	5	40	0.67	<.001
91R	119T	5	4	3	40	0.51	<.001
84V	153G	9	11	1	30	0.51	<.001
92Q	97A	10	3	9	31	0.49	<.001
140S	148R	4	3	3	39	0.50	<.001
92A	155H	5	0	12	35	0.47	<.001
140S	141I	4	3	4	37	0.45	.001
84V	92A	5	15	0	31	0.41	.003
84V	155H	12	9	6	26	0.40	.003
92Q	156R	6	8	4	36	0.37	.006
97A	156R	7	12	3	30	0.34	.014
92Q	155H	8	5	11	30	0.31	.022
97A	119T	6	15	2	30	0.30	.026
141I	148R	3	5	4	37	0.29	.041

Abbreviation: INSTI, integrase strand transfer inhibitor; ρ, Spearman correlation coefficient.

[a]Correlations among INSTI-selected mutations in sequences from persons containing 1 or more INSTI-selected mutations.

[b]The number of persons for which each pair of mutations could be evaluated was not the same and ranged between 48 and 54 because some sequences did not encompass all positions.

acid at many PR, RT, and IN positions differ between HIV-1 and HIV-2. Additionally, different mutations are likely to have different functional effects because of subtle differences in the structures of HIV-1 and HIV-2 enzymes [44, 45].

This study provides the first comprehensive summary of the natural variation of HIV-2 PR, RT (positions 1 to 240), and IN (positions 1 to 270). It also provides the first meta-analysis of the prevalence of PR, RT, and IN mutations in ARV-naive and treated persons using published sequence data. Despite the much lower number of HIV-2 sequences compared with the number of HIV-1 sequences, it is possible to show that even after adjustment for multiple comparisons, 16 mutations in PR, 12 mutations in RT, and 10 mutations in IN are selected by ARV therapy. These numbers will likely increase as more data become available as 26 additional PR, RT, and IN mutations, including 14 significantly associated with ARV therapy, were observed at nonpolymorphic positions associated with drug resistance prior to adjusting for multiple comparisons.

Of the 16 PI-associated mutations, 6 are major HIV-1–associated PI-resistance mutations: V47A, I50V, I54M, I82F, I84V, and L90M. Each of these have also been shown to be associated with reduced HIV-2 susceptibility to LPV, with higher levels of reduced susceptibility observed in multiple compared with single cycle phenotypic assays [18, 46, 47]. The remaining 10 PI-associated mutations have not been studied in vitro. Seven of these (V10I, V33I, K45R, I56V, I64V, V71I, and A73G) were significantly correlated with V47A, I50V, I54M, I82F, or I84V. Five (V10I, V33I, V71I, A73G, and F85L) are at accessory HIV-1–associated resistance positions. Three (K45R, V62A, and I64V) are polymorphic in HIV-1 but not HIV-2. Two are at novel positions, including T56V (which is a nonpolymorphic position in HIV-1 and HIV-2) and the aforementioned L99F (which is polymorphic in HIV-2 sequences).

Of the 15 NRTI-associated mutations, 8 are HIV-1–associated NRTI-resistance mutations, including A62V, K65R, K70R, Y115F, Q151M, M184VI, and S215Y. Four of these (K65R, Y115F, Q151M, and M184V) have been shown to reduce HIV-2 susceptibility to 1 or more NRTIs including 3TC, ABC, AZT, and TDF, whereas the polymorphic mutation V111I has been shown to increase the fitness of viruses containing K65R or Q151M [21, 26, 27]. The novel nonpolymorphic mutation K40R was present in most viruses containing S215Y. The rare occurrence of several nonpolymorphic NRTI-resistance mutations, including K65R, Q151M, and M184V, in NRTI-naive persons has been attributed to likely transmitted drug resistance as these positions are conserved in all primate lentivirus species [42, 48, 49].

Of the 14 INSTI-associated mutations, 5 are common HIV-1–associated INSTI-resistance mutations, including E92Q, T97A, G140S, Q148R, and N155H. Three (E92A, Y143G, A153G) are uncommon mutations at HIV-1–associated resistance positions and 6 (I84V, Q91R, A119T, V141I, H156R, and R231 insertions) are at positions that have not been associated with HIV-1 drug resistance. The position 231 insertion is of particular interest because it has been shown to markedly reduce susceptibility to raltegravir (RAL), elvitegravir (EVG), and dolutegravir (DTG), even in the absence of other INSTI-selected mutations [23]. It has been reported in 2 group A and 5 group B viruses from persons receiving raltegravir [23], and in 1 nonhuman primate infected with SIV_{mac} treated with the investigational INSTI cabotegravir [50]. The number of INSTI-selected mutations would likely have been higher had sequences been

available from more than 60 INSTI-treated patients. Indeed, 11 nonpolymorphic HIV-1–associated resistance mutations were reported in 1 to 3 sequences, including the major INSTI-resistance mutations Y143CR and Q148HK and the signature DTG-resistance mutations G118R and R263K.

In conclusion, this first meta-analysis of published PR, RT, and IN sequences from ARV-naive and ARV-experienced persons confirmed the association with therapy of many previously reported HIV-2 drug-resistance mutations and identified novel HIV-2–associated nonpolymorphic TSMs. Several of the novel mutations, particularly those that occurred commonly and were nonpolymorphic, should be studied for their effects on in vitro susceptibility. Further genotypic and phenotypic studies of HIV-2–associated TSMs will improve approaches to predicting HIV-2 ARV susceptibility and providing ARV treatment to HIV-2–infected persons.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. The authors thank the HIV-2 study authors whose HIV-2 pol sequences were contributed to GenBank, without whom this study would not be possible; and the UW-Senegal HIV-2 Study Group (http://www.uwsenegalresearch.com).

Financial support. This work was supported the National Institutes of Health National Institute of Allergy and Infectious Diseases (grant numbers R24AI136618 to R. W. S., and 2R01-AI060466 and R01-AI120765 to G. S. G.).

Potential conflicts of interest. G. S. G. has received research grants and research support from the Bill and Melinda Gates Foundation, Gilead Sciences, Alere Technologies, Merck & Co. Janssen Pharmaceuticals, Cerus Corporation, ViiV Healthcare, Roche Molecular Systems, Bristol-Myers Squibb, and Abbott Molecular Diagnostics. R. W. S. has received research funding from Janssen Pharmaceuticals. All other authors report no potential conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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