MAJOR ARTICLE

HIV-1 Drug Resistance in Variants from the Female Genital Tract and Plasma

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Background. Human immunodeficiency virus type 1 (HIV-1) drug-resistance mutations may arise in a fraction of viral variants, and these variants may differ between compartments, including the genital tract and blood.

Methods. We studied 14 women with detectable HIV-1 in both the genital tract and plasma despite antiretroviral treatment. We obtained HIV-1 RNA sequences from 280 unique viral variants and then determined the resistance genotype and the predicted phenotype (*Virtual* Phenotype; Virco BVBA) of each variant.

Results. Eight patients (57%) displayed mutations conferring high-level HIV-1 drug resistance. Although we observed differences in specific mutations among viral variants, 13 of the 14 women showed highly concordant HIV-1 genotypic and predicted phenotypic resistance patterns in the 2 compartments. In 1 patient, resistance mutations appeared only in plasma; all variants in her genital tract, which displayed a low viral load, were susceptible.

Conclusions. These data suggest that, for the majority of women, determination of HIV-1 drug resistance in the plasma will approximate the drug-resistance pattern in the genital tract. Analysis of individual variants enabled us to identify minority species bearing distinctive linked mutations, which may serve as a source of novel resistance genotypes. These data are relevant to clinical management and the evolution of drug resistance.

HIV-1 drug resistance presents a challenge to successful antiretroviral therapy (ART) during both acute and chronic infection [1–12]. As HIV-1 replicates in vivo, viral mutations arise, some of which confer antiretroviral drug resistance and a selective advantage during ART. HIV-1 exists as a population of closely related but genetically distinct variants [13–15]. Previous analyses of HIV-1 variants in different anatomic sites within an individual demonstrated compartmentalization, with viral sequences from each site that were distinct yet phylogenetically related [16–18]. Because the female genital tract and blood can harbor compartmentalized HIV-1 sequences [19–23], we investigated whether drug-resistant variants were also compartmentalized, an issue that has not been studied in detail even though it carries important implications for treatment and pathogenesis.

Combination ART can suppress HIV-1 to undetectable levels in the plasma and female genital tract compartments of many patients, with suppression more effective in the genital tract than the blood in some cases [24, 25]. Nevertheless, HIV-1 has been detected in the genital tract without detection in plasma in a minority of women [26]. In addition, there are women who harbor detectable HIV-1 in both compartments despite ART [26]. Worldwide, a large fraction of HIV-1 infections are transmitted through exposure to viruses in the female genital tract during heterosexual and perinatal transmission [27–29]; such exposure underscores the relevance of drug-resistant viruses in this site to both transmission and treatment. Pioneering studies of

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drug-resistant HIV-1 in the female genital tract and plasma have been conducted [21, 30–32]. These studies evaluated population-based sequences, generally determined by use of commercial genotyping methods.

Recent advances in technology have permitted investigators studying HIV-1 drug resistance in plasma to focus on individual viral variants, enabling them to identify minority strains bearing distinctive, linked resistance mutations [33–36]. The present study investigated the contribution of individual variants from both the genital tract and plasma to the pattern of drug resistance, thereby assessing the level of concordance of drug resistance between viruses from the 2 compartments.

SUBJECTS, MATERIALS, AND METHODS

Study population. We studied participants in the New York City site of the Women's Interagency HIV Study (WIHS), a multicenter, longitudinal investigation of HIV-1 infection of women [37]. Participants were interviewed and examined semiannually; blood and cervicovaginal lavage (CVL) samples were collected. The institutional review board at each site approved the investigation, and each woman provided informed consent. We identified 14 women who reported receiving ART or having received it previously who displayed detectable virus in plasma and CVL fluid (HIV-1 RNA load >500 copies/mL).

Sample collection and analysis. CVL samples were collected and cryopreserved as described elsewhere [26], tested for semen [22], and visually inspected for blood; specimens exhibiting blood or semen were excluded. HIV-1 RNA in each compartment was quantified by use of NucliSens isolation reagents (bioMérieux).

RNA isolation, reverse-transcriptase polymerase chain reaction (RT-PCR), and sequencing. RNA was extracted and cDNA was synthesized as described elsewhere [38]. Pxlasma and CVL samples were processed separately to avoid cross-contamination, with steps taken to minimize PCR-mediated recombination [38]. cDNA served as template for amplification of a 1.6-kb fragment encompassing the protease and RT regions of HIV-1 *pol* by use of the primers listed in table 1. To obtain HIV-1 variants, we performed serial 4-fold dilutions of the cDNA to determine the end-point dilution, defined as the last dilution of cDNA yielding a positive PCR. Multiple aliquots of the end-point dilution were subjected to amplification, yielding detectable PCR products in 40%–50% of the aliquots. Both strands of the PCR products were then sequenced, with ~10 unique sequences analyzed from each site in each patient. To obtain ~10 unique variants from each compartment in every patient, it was necessary to perform an additional 2–3 parallel end-point dilutions on samples from a few women.

Although most variant sequences were derived by end-point dilution, *pol* sequences from a minority of samples, primarily CVL samples with low viral loads, could be amplified from undiluted cDNA only. Variants from these samples were obtained by performing 2–3 independent PCRs, cloning the products of each directly into TA vectors (pCR2.1-TOPO; Invitrogen), and sequencing multiple clones.

Computational analysis. Sequences were aligned and DNA distance matrix analysis was performed by use of BioEdit (version 7.0.0; available at: http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Only sequences displaying ⩾0.3% variation were included in the final analysis. After gap-stripping of columns containing gaps, phylogenetic analyses were conducted by use of MEGA (version 3.1; available at: http://www.megasoftware.net), employing the neighbor-joining method with 500 bootstrap resamplings of the data. The configuration of phylogenetic trees was confirmed in each patient by use of the parsimony method.

Genotypic drug resistance. Genotypic drug resistance was determined by submitting sequences to the April 2006 updates of the Stanford HIV drug-resistance database (available at: http://hivdb.stanford.edu), which classified resistance-associated mutations as conferring high-level (R) or intermediate-level (I) resistance. We used "WT" to denote wild-type strains, defined as those lacking resistance-associated mutations or polymorphisms.

Predicted phenotypic drug resistance. Quantitative phenotypic resistance levels were determined by analyzing the viral genotypes with the vircoTYPE HIV-1 assay (version 3.7.01; Virco BVBA). The bioinformatics engine (*Virtual* Phenotype;

Table 1. Primers used to amplify the protease–reverse transcriptase region of the HIV-1 *pol* gene.

Pair, primer name	Nucleotide position[a]	Primer sequence (5′→3′)
Outer primer pair: first PCR		
1828F	1828–1846	ATGACAGCATGTCAGGGAG
3887R	3887–3865	AGCTGCYCCATCTACATAGAAAG
Inner primer pair: second PCR		
2138F	2138–2158	AGAGCAGACCAGAGCCAACAG
3813R	3813–3794	AGGGAGGGGTATTGACAAAC

NOTE. PCR, polymerase chain reaction.

[a] Indicates nucleotide positions in HIV-1 HXB2 strain.

Virco BVBA) [39] used by this assay provides a prediction of the patient's virus phenotype (fold change in IC_{50}) by matching its genotype with other genotypes possessing known phenotype profiles in the Virco database. The derived mean fold-change values were interpreted by use of a set of biological cutoffs provided in the assay.

Statistical analysis. We applied Fisher's exact test to analyze the correlation between the presence of HIV-1 drug-resistance mutations and patient characteristics, including CD4 cell count, HIV-1 RNA load, and genital tract infections. Logistic regression was used to examine the correlation between the presence of resistance and discordant viral loads in CVL fluid versus plasma.

RESULTS

Study population. We studied 14 women with detectable HIV-1 RNA in plasma and the genital tract despite ART; their virologic, immunologic, and clinical characteristics appear in table 2. The women displayed a spectrum of HIV-1 disease progression and ART regimens. Eleven were receiving ART at the time point studied, and 3 had been treated previously (table 3). Eight showed lower genital tract infections, primarily warts, but no genital ulcers were detected.

Sequence analyses. We compared 308 unique HIV-1 RNA sequences (280 single variant and 28 population sequences) of the protease-RT region of *pol* obtained contemporaneously from CVL and plasma samples (GenBank accession numbers DQ372126-DQ372434). Computational analyses, including a BLAST search, showed no evidence of contamination, and open reading frames were intact, with no significant deletions, alterations, stop codons, or nonsense mutations. Phylogenetic analyses demonstrated that sequences from CVL and plasma samples from each patient clustered in a single branch of the evolutionary tree, and all were identified as subtype B (tree available on request).

Genotypic resistance analyses. Genotypic resistance profiles were derived from the sequences and are described in table 3. By analyzing the prevalence of each resistance mutation among variants in each sample, we found that most mutations appeared in >70% of variants from each compartment; we have called these "predominant" mutations. We also detected mutations that occurred much less frequently, in <30% of variants, and we have called these "minority" mutations (table 3). On the basis of the Stanford drug-resistance database, high-level genotypic resistance was evident in predominant variants in 8 (57%) of the 14 women.

Although drug-resistance patterns varied greatly among the patients we examined, the presence or absence of high-level drug resistance in predominant variants was highly concordant between viruses in the plasma and genital tract for 13 of the 14 patients. Genotypic resistance analyses identified 4 distinct patterns of distribution of resistance mutations between viruses from the 2 compartments (table 3). The first group consisted of 6 patients (15, 29, 34, 36, 37, and 39) who displayed no drug resistance in predominant variants from either compartment. These patients all reported taking ART at the time of the analysis. Polymorphisms or mutations associated with drug resistance were detected in some, but no resistance was con-

Table 2. Virologic, immunologic, and clinical characteristics of the study women.

Patient (age in years/ethnicity)	HIV risk	HIV-1 RNA load, log copies/mL		CD4 cell count, cells/mm^3	Lower genital tract infections
		Plasma	CVL		
4 (55/L)	IDU	5.87	4.74	113	Vulvar warts
8 (51/AA)	H	4.36	3.40	254	None
15 (32/AA)	NR	3.60	4.76	367	Vulvar warts
28 (38/AA)	IDU	4.56	2.72	546	None
29 (38/AA)	H	6.17	5.32	107	Vulvar warts
30 (38/L)	H	5.20	4.40	76	None
31 (34/L)	IDU	4.71	3.56	161	Vaginal trichomoniasis
32 (49/AA)	NR	4.02	3.70	310	None
33 (36/AA)	IDU	5.23	6.18	508	None
34 (45/L)	IDU	5.23	5.52	307	BV, vaginal candidiasis
35 (34/L)	IDU	3.08	6.11	237	Vulvar warts
36 (43/AA)	H	4.08	5.70	167	Vaginal candidiasis
37 (40/AA)	NR	5.30	5.68	171	None
39 (33/AA)	NR	4.57	3.72	158	Vulvar warts

NOTE. AA, African American; BV, bacterial vaginosis; IDU, injection drug use; H, heterosexual; L, Latina; NR, risk unknown.

Table 3. Treatment, HIV-1 drug-resistance mutations, and drug-resistance profiles of variants.

		Resistance mutations in variants					
		Plasma		CVL		Drug-resistance interpretation for predominant variants[c]	
Group, patient	ART	Predominant[a]	Minority[b]	Predominant	Minority	Plasma	CVL
Group 1: no drug resistance in predominant variants							
15	AZT, 3TC, SQV	WT	PR: L63P	WT	PR: V32I,[d] G48R,[d] L63P, I93N[d] RT: K70E,[d] V179D,[d] G333E[d]	None	None
29	ddI, 3TC	PR: L33I, L63P, A71T	None	PR: L33I, L63P, A71T	PR: I50T,[d] N88S[d] RT: L74S[d]	None	None
34	d4T, 3TC, NFV	PR: L63P	None	PR: L63P	None	None	None
36	d4T, 3TC, SQV	PR: L63S	RT: D67G,[d] E44D,[e] L100S,[e] Y181C[d]	PR: L63S	PR: I50V[e] RT: K103E,[d] K219R,[d] P225L,[d] Y181H[e]	None	None
37	d4T	PR: L63Q	PR: M36I	PR: L63Q	PR: M36I, G48R, D60E,[d] L63S,[f] I84V[d] RT: E44K,[d] T69A,[d] L100F,[f] Y181C,[d] K238R,[f] Y318C[f]	None	None
39	SQV	PR: L63P, I93L	None	PR: L63P, I93L	RT: L100S,[d] F116S,[d] L210S[d]	None	None
Group 2: resistance in all variants							
4	ABC, d4T, RTV, IDV, NVP	PR: L63P, V77I RT: K103N	RT: Y181C,[d] G190R,[d] K219Q[d]	PR: L63P, V77I RT: K103N	PR: A71T[d] RT: K219Q[d]	NNRTI-R	NNRTI-R
8	None (past ART: ABC, AZT, 3TC, NVP)	PR: M36I RT: D67G, K70R, K103R, V179D, M184V, G190A, K219E	PR: V77A[d] RT: F116S,[g] T215I[g]	PR: M36I RT: D67G, K70R, K103R, V179D, M184V, G190A, K219E	PR: I84M,[d] F53S[g] RT: V106A[g]	3TC-R, NNRTI-R, ABC-I,[h] AZT-I[h]	3TC-R, NNRTI-R, ABC-I,[h] AZT-I[h]

30	ABC, AZT, 3TC, NVP	PR: M36I, L63P RT: T69N, Y181C	PR: N88S,[d] I93V[d] RT: M41V,[d] E44G,[d] A62T[d]	PR: M36I, L63P RT: T69N, Y181C	RT: K65E,[d] K103D[d]	NNRTI-R	NNRTI-R
31	d4T, 3TC	PR: A71T RT: M184V	PR: I93L	PR: A71T RT: M184V	PR: I93L RT: Y181F,[d] L234R,[d] Y318H[d]	3TC-R	3TC-R
32	ddI, ABC, NFV	PR: L63P, A71T V77I, L90M RT: M184V, G190A	RT: Q151R,[d] T215F,[d] T215S,[d] T215I	PR: L63P, A71T, V77I, L90M RT: M184V, G190A	PR: D60H[i] RT: F77Y[i]	3TC-R, NNRTI-R, NFV-I[j]	3TC-R, NNRTI-R, NFV-I[j]
Group 3: mixed resistant and sensitive variants							
33	ddC, d4T, SQV	PR: L10I, L63P, L90M RT: K70R	PR: K20R, M36I, G48V	RT: K70R	PR: L10I, K20R, M36I, G48V, L63P, L90M RT: K219R[d]	SQV-I,[k] AZT-I[k]	AZT-I[k]
35	None (past ART: AZT, 3TC)	RT: M184V	WT[l] PR: L10F,[m] L63P[m] RT: D64G,[m] L234P[m]	RT: M184V[n]	WT[l] RT: K65E[d]	3TC-R	3TC-R
Group 4: discordant resistance							
28	None (past ART: AZT, 3TC, NVP)	RT: K103N, M184V	PR: M36I RT: V75I, V118I[d]	WT	PR: M36I,[d] D60N[d]	NNRTI-R, 3TC-R	None

NOTE. 3TC, lamivudine; ABC, abacavir; ART, antiretroviral therapy; AZT, zidovudine; CVL, cervicovaginal lavage; d4T, stavudine; ddC, zalcitabine; ddI, didanosine; I, intermediate-level resistance; IDV, indinavir; NFV, nelfinavir; NNRTI, nonnucleoside reverse-transcriptase inhibitor; NVP, nevirapine; PR, protease; R, high-level resistance; RT, reverse transcriptase; RTV, ritonavir; SQV, saquinavir; WT, wild type.

[a] Predominant resistance mutations are those appearing in ⩾70% of variants.

[b] Minority resistance mutations are those appearing in <30% of variants.

[c] This table focuses on genotypic resistance patterns. Predicted (on the basis of the *Virtual* Phenotype database) phenotypic drug resistance (v-PHT) was also determined for every variant, with minor differences detected between the v-PHT and genotypic resistance profiles in 3 patients (see footnotes h, j, and k).

[d] Mutations were each detected in only 1 variant from this compartment in this patient; each mutation appeared on a different variant.

[e] E44D and L100S were both seen on the same variant; I50V and Y181H were seen on another variant.

[f] L63S and Y318C were both seen on the same variant; L100F and K238R were seen on another variant.

[g] F116S and T215I were both seen on the same variant in plasma; F53S and V106A were both seen on the same variant in CVL fluid.

[h] v-PHT resistance: 3TC-R, NNRTI-R, and ABC and AZT sensitive.

[i] D60H and F77Y were both seen on the same variant.

[j] v-PHT resistance: 3TC-R, NNRTI-R, and NFV-R.

[k] v-PHT resistance: none.

[l] WT variants were seen in 2 of 8 plasma and 3 of 7 CVL sequences.

[m] L10F and L63P were both seen on the same variant; D67G and L234P were seen on another variant.

[n] M184V mutations were present in 57% of CVL variants from this patient.

ferred. An evolutionary tree constructed of sequences from patient 15 exhibited unique susceptible variants that were intermingled between the 2 sites (figure 1*A*). Intermingled variants were phylogenetically related and were distributed in both sites.

Group 2 consisted of 5 patients (4, 8, 30, 31, and 32) exhibiting drug-resistance mutations present in all variants from each compartment. A phylogenetic tree composed of sequences from patient 30 depicted variants with high-level drug resistance that are intermingled between the 2 compartments (figure 1*B*).

Patients in Group 3 (33 and 35) harbored a mixed population of susceptible and drug-resistant variants in both compartments (tables 3 and 4). As illustrated by patient 35, 3 of 7 variants from the CVL sample and 2 of 8 from the plasma sample were sensitive to all classes of antiretroviral drugs (figure 1*C*). The remaining variants in both compartments displayed high-level resistance. Phylogenetic analysis displayed partial compartmentalization, with variants from CVL fluid and plasma clearly related, yet forming largely distinct clusters in the evolutionary tree. Patient 33 exhibited high-level drug resistance in 2 of 8 plasma-derived and 2 of 7 CVL-derived variants. The remaining variants in both compartments displayed intermediate-level genotypic resistance (table 3).

Group 4 consisted of the sole subject, patient 28, who displayed discordant drug-resistance patterns in the plasma and CVL samples (table 3). Each HIV-1 variant in plasma displayed drug-resistance mutations, yet each CVL variant was susceptible to all antiretroviral drugs. The viral loads in the 2 compartments also varied markedly, with a plasma viral load of 36,307 copies/mL and a CVL viral load of only 527 copies/mL. Phylogenetic analysis displayed sequences that were strongly compartmentalized, suggesting independent viral evolution in the 2 compartments (figure 1*D*).

To rule out PCR selection and resampling bias favoring discordant resistance patterns, we took several steps. We confirmed our findings by analyzing a second aliquot of viral RNA from each compartment, repeating the procedure in parallel to obtain and sequence individual variants. Resistance patterns agreed with those originally obtained. Furthermore, the phylogenetic tree displayed considerable heterogeneity among the variants in each compartment, and DNA distance matrix analysis supported this finding. Despite the low viral load in the CVL fluid, we observed up to 2.26% variation among CVL strains (range, 0.3%–2.26%) and 1.51% among those from plasma (range, 0.37%–1.51%). The level of heterogeneity within each compartment provides additional evidence for adequate variant sampling.

Although the presence and type of predominant resistance mutations were concordant in each compartment for the great majority of patients, we did identify minority variants carrying resistance mutations detected in only 1 site (table 3); frequently such mutations appeared in only 1 variant in the patient. For example, Y181C, which is associated with nonnucleoside reverse-transcriptase inhibitor (NNRTI) resistance, was identified in minority variants derived from a sole compartment in patients 4 and 37. The presence of HIV-1 drug-resistance mutations among these patients did not correlate with CD4 cell count, HIV-1 loads, lower genital tract infection, or discordance between CVL and plasma viral loads.

HIV-1 variant versus population-based sequence analyses. Comparison of population-based and individual sequences revealed that drug-resistant minority variants were missed by the bulk sequencing method (table 4). Individual variants also displayed linkage of mutations on the same viral genome. Patients 33 and 35 illustrate these observations (table 4). Patient 33's population-based plasma sequence displayed multiple protease mutations and the K70R mutation, which is associated with zidovudine resistance. Analysis of individual variants demonstrated that all 8 displayed at least 1 protease mutation and that 5 of 8 displayed K70R, but none displayed the linked set of mutations observed in the population-based sequence. In the genital tract, differences between the population-based and individual sequences were also apparent. Minority variants in CVL fluid representing 28.6% of the viral population showed high-level protease resistance but were missed by population-based sequencing.

Population-based and individual sequences from patient 35 illustrated more-subtle differences (table 4 and figure 1*C*). Variant analysis demonstrated that both compartments harbored a mixture of wild-type and resistant strains. In the CVL fluid, 4 of 7 variants displayed M184V and 3 displayed wild-type sequences, with population-based sequences indicating a mixture of wild-type and mutant strains. In the plasma, 6 of 8 variants displayed M184V and 2 carried wild-type sequences, but, in this case, population-based sequencing detected the resistant variants only.

Predicted phenotype analyses. We also analyzed all sequences by use of the vircoTYPE assay for predicted phenotypic resistance. As with genotypic resistance, predicted phenotypic resistance was concordant between CVL fluid and plasma for all but 1 patient, patient 28. Minor differences between genotypic and predicted phenotypic resistance profiles were detected in patients 8, 32, and 33 (table 3, footnotes).

DISCUSSION

To understand the mechanisms of HIV-1 drug resistance and pathogenesis, it is important to analyze resistance patterns in multiple viral variants and in different compartments. By examining 280 unique variants from 14 women, we identified 4 distinct patterns of distribution of drug resistance between the genital tract and plasma. Although minority variants with distinct resistance mutations were detected in each compartment,

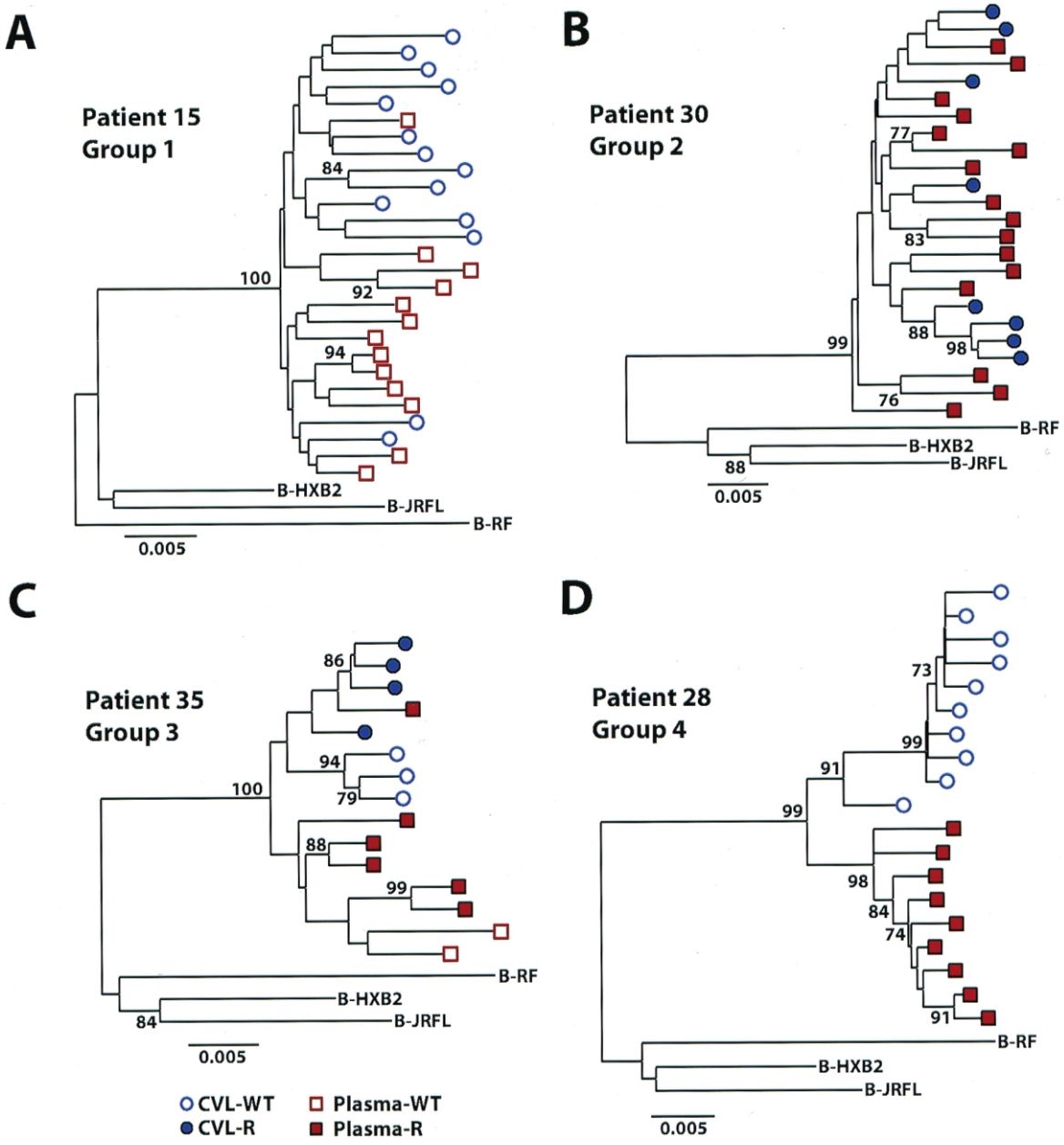

Figure 1. Phylogenetic trees of *pol* sequences from plasma and genital tracts of 4 representative patients. Each tree depicts the plasma and genital tract (cervicovaginal lavage [CVL]) sequences derived from a single patient and subtype B reference strains HXB2, JRFL, and RF. The nos. at branch points indicate bootstrap values. Blue circles represent CVL sequences, and red squares represent plasma sequences. Open circles and squares represent sequences harboring no drug resistance, and filled circles and squares represent sequences with mutations conferring high-level (R) drug resistance. *A,* Patient 15—no HIV-1 drug-resistance mutations in either compartment; intermingling of CVL and plasma sequences. *B,* Patient 30—high-level resistance in both compartments; intermingling of CVL and plasma sequences. *C,* Patient 35—sequences in both plasma and CVL fluid harbored a mixture of wild-type (WT) and resistant variants; 2 of 8 variants from plasma and 3 of 7 from CVL were WT. Sequences were partially compartmentalized between the 2 sites. *D,* Patient 28—all variants in plasma displayed mutations conferring high-level resistance, although all variants in the genital tract were sensitive in genotype and virtual phenotypic HIV-1 drug-resistance analysis. Sequences were strongly compartmentalized between the 2 sites.

Table 4. HIV-1 drug-resistance mutations in population-based sequences vs. individual variants in plasma and cervicovaginal lavage (CVL) fluid—patients 33 and 35.

Patient, site, sequence type	Variants, no. (%[a])	Resistance mutations								
		Protease						Reverse transcriptase		
33										
Plasma										
Population-based sequence	…	L10I	K20R	M36I	G48V	–	L90M	K70R	–	–
Individual variant sequences	2 (25)[b]	L10I	K20R	M36I	G48V	L63P	L90M	–	–	–
	2 (25)	L10I	–	–	–	L63P	L90M	K70R	–	–
	1 (12.5)	L10I	K20R	–	–	L63P	L90M	K70R	–	–
	1 (12.5)	L10I	–	–	–	–	L90M	K70R	–	–
	1 (12.5)	L10I	–	–	–	–	L90M	–	–	–
	1 (12.5)	–	–	–	–	–	L90M	K70R	–	–
CVL fluid										
Population-based sequence	…	–	–	–	–	–	–	K70R	–	–
Individual variant sequences	4 (57.1)	–	–	–	–	–	–	K70R	–	–
	1 (14.3)	L10I	K20R	M36I	G48V	L63P	L90M	K70R	–	–
	1 (14.3)	L10I	K20R	M36I	G48V	L63P	L90M	–	–	–
	1 (14.3)	–	–	–	–	–	–	K70R	–	K219R
35										
Plasma										
Population-based sequence	…	–	–	–	–	–	–	–	M184V	–
Individual variant sequences	6 (75)	–	–	–	–	–	–	–	M184V	–
	2 (25)	–	–	–	–	–	–	–	–	–
CVL fluid										
Population-based sequence	…	–	–	–	–	–	–	–	WT/M184V[c]	–
Individual variant sequences	4 (57)	–	–	–	–	–	–	–	M184V	–
	3 (43)	–	–	–	–	–	–	–	–	–

[a] Percentage of variants displaying each pattern of drug-resistance mutations.
[b] According to the Stanford HIV drug-resistance database, patient 33 exhibited high-level drug resistance in 2 of 8 plasma variants and in 2 of 7 CVL-derived variants. The remaining variants in both compartments displayed intermediate-level genotypic resistance.
[c] The population sequence derived from the CVL fluid revealed a mixture of wild-type and mutant variants.

both the presence and type of predominant resistance mutations were concordant between the 2 sites in 13 of 14 patients. Predicted phenotypic resistance patterns were also highly concordant between the 2 compartments.

These findings have clinical relevance. The female genital tract is a crucial site of exposure to HIV-1 during heterosexual and mother-to-child transmission (MTCT). With increased availability of ART, drug resistance has emerged in women treated to prevent MTCT [40]. Because HIV-1 can vary between the genital tract and plasma [19–23], it was suspected that some women might simultaneously harbor predominantly resistant viral strains in the genital tract and susceptible strains in the plasma. In that case, drug-resistant virus in the genital tract of pregnant women would be missed if antiretroviral susceptibility testing were performed on blood alone, as is currently done. This detailed analysis of HIV-1 variants from each compartment suggests that the results of HIV-1 drug-resistance testing for plasma approximates the pattern of resistance in the genital tract in the majority of infected women.

Only 1 patient in this series, patient 28, exhibited uniformly resistant variants in one compartment and susceptible strains in the other; in this patient, the HIV-1 loads and resistance patterns differed markedly between the 2 sites. The 9 variants obtained from plasma, which showed an HIV-1 load of 4.56 $\log_{10}$ copies/mL, displayed resistance to lamivudine and NNRTIs, yet the 12 found in CVL fluid, which had a viral load of only 2.74 $\log_{10}$ copies/mL, were uniformly susceptible to antiretroviral agents. On initiation of ART, patient 28's plasma HIV-1 load decreased from 5.11 to 3.60 $\log_{10}$ copies/mL, but plasma viremia rebounded to ~4.5 $\log_{10}$ copies/mL 1 year later. Although plasma viral loads remained at ~4.5 $\log_{10}$copies/mL during the 18 months before the time point studied here, HIV-1 in contemporaneous CVL fluid was suppressed to undetectable levels. Furthermore, HIV-1 drug resistance in plasma first emerged at the visit described here and persisted during 13 months of additional follow-up; HIV-1 in CVL fluid was undetectable during that period.

These data suggest that the HIV-1 population detected in patient 28's CVL fluid may resemble the low-level plasma viremia that can emerge after a period of suppression. Although

previous studies have revealed that drug resistance can develop in plasma when the level of HIV-1 is low, fully sensitive virus has also been rigorously documented [41, 42]. As observed with low-level viremia, it is plausible that the susceptible HIV-1 variants detected in patient 28's CVL fluid reflect transient reemergence of wild-type virus that entered the latent reservoir before the initiation of ART [42]. The phylogenetic analysis supports this view (figure 1*D*); although sequences from patient 28's plasma and CVL fluid were derived from a common ancestor, they have evolved separately. A previous study, which analyzed HIV-1 drug-resistance mutations in the 2 compartments over time, described the appearance of HIV-1 resistance in the plasma before it was detected in the female genital tract [21]. This finding may be a consequence of more-effective HIV-1 suppression in the genital tract than in the plasma, which has been observed in some cases [25].

The present analysis does not exclude the possibility of a population of predominantly drug-resistant HIV-1 variants replicating solely in the genital tract [43]. Our study did identify resistant minority species in some patients that were detected solely in CVL fluid. Such variants may multiply under the selective pressure of ART, resulting in transmission. Because one of the goals of ART is to reduce transmission, comparison of resistance profiles in the 2 sites merits further investigation, particularly in the setting of MTCT and with the inclusion of non-B subtypes. The relationship of HIV-1 load to drug resistance in the female genital tract also merits investigation. An epidemiologic study of WIHS participants showed that 33% of women who were viremic during ART harbored fully sensitive virus in plasma [44]. Similarly, a large cross-sectional investigation reported that 28% of patients with viral loads >30,000 copies/mL failed to exhibit plasma HIV-1 resistance to any drug [1]. Comparable studies examining HIV-1 resistance in the female genital tract, however, have not yet been conducted.

Genomic variation is a key characteristic of HIV-1, with viral sequences varying between and even within anatomic compartments in the same patient [13–15, 22, 23]. Previous studies pioneered the use of single-genome sequencing to investigate viral variants in plasma, documenting that drug-resistant minority variants appear commonly [33–36]. We extended this approach to include the female genital tract and made several observations that were not appreciated by use of population-based sequencing. We demonstrated multiple minority variants as well as mixtures of sensitive and drug-resistant viruses in each compartment (tables 3 and 4). Furthermore, examination of individual variants revealed the linkage of resistance mutations on the same RNA molecule. Patient 33, for example, harbored a complex mixture of viruses exhibiting high- and intermediate-level resistance. The resistance profile of patient 33's population-based plasma sequence, however, fails to reflect the pattern of linked mutations of any of the 8 variants (table 4). These results relate to clinical considerations and HIV-1 disease pathogenesis. Rare or minority variants may serve as the source of new drug-resistant strains that predominate during the course of infection. Analyses of such variants promise to increase our understanding of the evolution of drug resistance.

The significance of minority drug-resistance mutations is likely to depend on clinical circumstances and linkage to other mutations, as illustrated by the nevirapine (NVP) resistance–associated mutation Y181C. Patient 4, who was treated with NVP, displayed K103N in every variant in both sites, resulting in high-level genotypic and phenotypic NVP resistance (phenotypic fold change of 40.3 [Virco cutoff, 8]). One plasma strain, however, bore both K103N and Y181C, leading to a predicted phenotypic fold change of 65.7. Under the selective pressure of NNRTI-based therapy, this minority variant would be likely to multiply and spread to other compartments, perhaps eventually predominating in this patient. Patient 37 also displayed 1 variant bearing Y181C, but she displayed no predominant resistance mutations, nor had she taken any NNRTIs. It seems unlikely that variants bearing Y181C would eventually predominate under these circumstances.

Previous studies have reported discordant patterns of HIV-1 drug resistance between the female genital tract and plasma [21, 30–32]. We believe the differences are largely due to variable methods of sequencing, genital tract sampling, and the levels of disparity termed discordant. Most discordance reported previously involved secondary drug-resistance mutations, polymorphisms, or mixtures of resistant and wild-type sequences in each compartment. Our conclusions are mainly based on mutations conferring high-level resistance, because these mutations are most immediately relevant to treatment and transmission. The other major distinction between this report and previous ones is that the analysis of individual variants reported here permits the detection of predominant and minority drug-resistance genotypes, providing a more-detailed picture of the resistance profile. Commercial genotyping methods used in previous reports generally detect HIV-1 species comprising >20% of the viral population.

Differences in sampling methods and genital tract viral loads are also likely to have contributed to varying results. Only one of our patients, patient 28, displayed a CVL viral load <1000 copies/mL. In other studies, however, HIV-1 loads were often low, especially in the genital tract [31, 32]. A very low viral load may reflect reemergence of wild-type virus that entered a latent reservoir before the initiation of ART, resulting in contemporaneous populations of sensitive and drug-resistant variants [42], as is likely in patient 28. Furthermore, by examining CVL fluid (the result of a wash of the endocervix, vagina, and vaginal fornix), we studied viral variants from a large portion of the lower genital tract. Others have used swabs or cervical

wicks [30–32], which permit a comparison of drug-resistance mutations found in viruses from each specific area.

The general concordance between predominant drug-resistance patterns in CVL fluid and plasma reported here underscores ART's powerful selective pressure in both compartments. Concordance may result from parallel evolution and selection of drug-resistant variants or from seeding of one compartment by resistant viruses from another. Recombination between HIV-1 variants from plasma and the genital tract may also be involved. We have previously documented multiple instances of intrapatient recombination between variants from plasma and the genital tract of the same individual [22, 23]. Moreover, HIV-1 multidrug resistance can result from recombination in vitro [45–47] and recently was documented to stem from recombination between plasma and CVL strains in vivo as well (K. S. Kemal, C. Kitchen, H. Burger, T. Klimkait, F. Hamy, B. Foley, D. Mayers, K. Anastos, K. Petrovic, M. Suchard, V. Minin, P. Huggins, and B. Weiser, unpublished data). Additional studies of the evolution of drug resistance in the genital tract and plasma are necessary to develop more-effective strategies for HIV-1 prevention and treatment.

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