PREVALENCE OF DRUG RESISTANCE AND ASSOCIATED MUTATIONS IN HIV-POSITIVE PUERTO RICANS: SEX VARIATIONS

Luis A. Cubano, PhD; Lycely del C. Sepúlveda-Torres, PhD; Greychan Sosa, BS; Nawal Boukli, PhD; Rafaela Robles, EdD; José W. Rodriguez, PhD; Lourdes Guzmán, MT; Eddy Ríos-Olivares, PhD

Introduction: A cross sectional study was conducted from 2002–2004 to record the evolution of HIV-1 infection in Puerto Rico by monitoring the expression of antiretroviral resistance-associated mutations.

Methods: Samples were analyzed by using the TRUGENE HIV-1 Genotyping Kit and the OpenGene DNA Sequencing System.

Results: Mutations in the HIV-1 virus were detected in 92.7% of men and 94.8% of women. Of these, 75.1% of men and 72.4% of women had HIV-1 with resistance to at least one medication. The average number of HIV mutations was 6.1 in men and 5.3 in women. In 2002 and 2003, strains were most frequently resistant to the antiretroviral drugs zalcitabine, lamivudine and didanosine, while in 2004, strains were most frequently resistant to zalcitabine, lamivudine, and efavirenz. The most prevalent mutations in the reverse transcriptase gene were M184V, K103N, T215Y, and M41L. The most prevalent mutations in the protease gene were L63P, M36I, L90M, A71V, and L10I.

Conclusions: Significant differences between men and women were recorded in the levels of HIV-1 expressed mutations and resistance. When comparing these results with data from 2000 and 2001, results indicate that expression of resistant mutations has remained constant. (*Ethn Dis*. 2008;18[Suppl 2]:S2-132–S2-136)

Key Words: HIV, Resistance, Mutagenesis

Department of Microbiology and Immunology (LAC, NB, JWR, LG, ERO), Center for Addiction Studies (RR), Universidad Central del Caribe, Bayamón; Department of Science and Technology, Universidad Metropolitana, San Juan (LST); Department of Natural Sciences, Universidad de Puerto Rico, Aguadilla (GS), Puerto Rico.

Author correspondence and reprint requests to: Luis Angel Cubano, PhD; Universidad Central del Caribe; PO Box 60327; Bayamón, PR 00960-6032; 787-798-3001 x 2150; 787-740-4390; luis.cubano@uccaribe.edu

INTRODUCTION

Although the incidence of AIDS cases in Puerto Rico has declined, Puerto Rico continues to have one of the highest incidences of HIV-1 infection in the United States.[1] After the advent of highly active antiretroviral therapy (HAART), the mortality of HIV-1/AIDS was substantially reduced,[2] but the genetic diversity and mutation rate of the virus[3] combined to produce numerous resistant strains, resulting in reduced treatment efficacy.[4]

As of 2003, 28,301 cases of HIV/AIDS had been reported in Puerto Rico.[1] More than 10,000 persons live with HIV/AIDS in Puerto Rico. HIV/AIDS was the major cause of death (47.7%) among drug users in Puerto Rico in 1998.[5] HIV infection in Puerto Rico is characterized by patients with a median age of 35 years, mostly Hispanic (98.8%) men, women (77.7%), and the major risk for infection is drug abuse (54.3%).[6,7] Increased risk behaviors have been associated with alcohol abuse[8] and homelessness.[9]

HIV therapy typically includes a combination of reverse transcriptase inhibitors (RTI) and protease inhibitors (PI).[10] The activity of HIV-1 reverse transcriptase is essential for viral replication and is required for the conversion of single-stranded genomic RNA into double-stranded viral DNA,[11] which is later integrated into the host genomic DNA. For this reason, HIV-1 RTIs are powerful inhibitors of HIV-1 replication and represent an important class of antiretroviral agents. HIV-1 protease cleaves viral Gag and Gag-Pol polyproteins into structure and replication proteins that are necessary for the virus to become infectious and, therefore, PI are important for HIV therapy.[11]

A continuing challenge to maintaining the efficacy of drugs designed to impede viral reproduction is the presence of amino acid polymorphisms. Amino acid polymorphisms may occur as the result of mutations associated with drug resistance.[12] Numerous mutations with specificity for the RTIs and PIs have been identified and well characterized.[13]

As the need to assess the prevalence of resistant mutations became evident, HIV-genome sequence data have been collected and analyzed since 2000.[14] In this study, we determined the prevalence of genotypic mutations in a sample of Puerto Ricans infected with HIV-1 and analyzed sex differences in mutation expression profiles. Although clinical and demographic data, other than sex, were not compiled in the study, the data provide a starting point for more complex studies in which more variables can be measured. Since these data are relevant for mutation prevalence, especially details about exposure to antiretroviral therapy, which is directly related to the development of mutations, we are working with physicians to expand the data obtained by the study.

The purpose of the study is to establish a HIV-1 resistance-monitoring system in Puerto Rico. Furthermore, the information obtained is also important to determine if the virus found in the island differs from the virus in the continental United States. As drug resistance is a major factor in treatment success, and data from Puerto Rico are scarce, the development of the database will assist physicians in determining treatment regimens specific for the island.

METHODS

Samples

HIV-1 genotyping results from the Immunoretrovirus Research Laboratory located at the Universidad Central del Caribe in Bayamón, Puerto Rico, were used in this cross-sectional study. Samples were obtained from patients referred to the facility by their primary care physicians. The sample is a mixture of patients, ranging from those who have not been previously exposed to HAART to those who have failed on therapy. Samples were analyzed from 2002 (128 men, 62 women, 67 unknown), 2003 (221 men, 123 women, 88 unknown), and 2004 (307 men, 177 women, 83 unknown). The sex of the patients was the only demographic information collected. Patient clinical data were unknown. Total percentage of mutation or resistance included those from patients whose sex was unknown.

RNA Isolation

HIV-1 viral RNA was extracted using the QIAamp Viral RNA Mini Kit (QIAGEN, Valencia, Calif). Briefly, serum (140 μL) was added to buffer AVL containing carrier RNA in a microcentrifuge tube. Following incubation at room temperature for 10 minutes, 560 μL of ethanol was added and mixed. An aliquot of 630 μL was added to the QIAamp spin column and centrifuged at 8000 rpm for one minute (two times). The QIAamp spin column was placed into a clean 2-mL collection tube, and 500 μL of buffer AW1 was added and centrifuged at 8000 rpm for one minute, then 500 μL of buffer AW2 was added and centrifuged at 14,000 rpm for three minutes. The QIAamp spin column was placed in a clean 1.5-mL microcentrifuge tube, and 60 μL of buffer AVE was added. The tube was incubated at room temperature for one minute and centrifuged at 8000 rpm for one minute. Viral RNA was stored at −80°C.

Mutational Analysis

Whole blood from HIV-1 infected patients was collected in tubes containing ethylenediaminetetra-acetic acid (EDTA) as anticoagulant. Plasma was separated and stored at −80°C until isolation of RNA. The existence of mutations was determined by analyzing samples with the TRUGENE HIV-1 Genotyping Kit and the OpenGene DNA Sequencing System (Bayer Diagnostics, Tarrytown, NY). All of the mutations detected by the assay were tabulated. The TRUGENE system relates the mutations to resistance automatically and prepares a report indicating results in the following manner:

> **No Evidence of Resistance:** reduced susceptibility has not been associated with the mutations detected in this assay.
>
> **Possible Resistance:** mutations have been associated with an intermediate decrease in antiretroviral susceptibility in viral isolates.
>
> **Resistance:** mutations detected in this assay have been associated with a maximum reduction in susceptibility. Both possible resistance and resistance were considered as a positive result.

Statistical Analysis

SPSS 14.0 for Windows (SPSS Inc., Chicago, Ill) was used to perform the analysis, and χ^2 test was performed. Statistical significance was set at $P \leq .05$.

RESULTS

Of 1256 patients analyzed, 92.7% of the men and 94.8% of the women had infections with mutated HIV virus, and 75.1% of men and 72.4% of women had infections that were resistant to at least one medication. The average number of mutations in viruses in men was 6.1, while the average number of mutations in viruses in women was slightly less (5.3).

As shown in Table 1, comparison of HIV-1 resistance to antiretroviral drugs from 2002 to 2004 demonstrated that in 2002, the highest rates of resistance were to zalcitabine (58.0%), didanosine (58.0%), and lamivudine (51.4%). For 2002, there was a significant difference between men and women in rates of viral resistance to didanosine ($P=.05$), tenofovir ($P=.04$), and zalcitabine ($P=.05$). In 2003, the highest rates of HIV-1 resistance were to zalcitabine (59.0%), didanosine (51.6%), and lamivudine (50.2%). For 2003, there was a significant difference between men and women in rates of viral resistance to ritonavir ($P=.02$), nelfinavir ($P=.04$), and indinavir ($P=.04$). In 2004, the highest rate of resistance to antiretroviral drugs were to zalcitabine (54.1%), lamivudine (50.7%), and efavirenz (41.2%). A significant difference between men and women in rates of viral resistance to ritonavir ($P=.04$), nelfinavir ($P=.04$), saquinavir ($P=.04$), indinavir ($P=.04$), and forscarnet ($P=.05$) was also observed in 2004.

Reverse Transcriptase Resistance-Associated Mutations

Table 2 compares the levels of reverse transcriptase resistance-associated specific mutations. Analysis of reverse transcriptase mutations for 2002 demonstrated that the highest degree of expression was for M184V (45.1%), K103N (33.9%), M41L (19.8%), and T215Y (19.1%). There were no significant differences between men and women in the expression of reverse transcriptase-specific resistant mutations during this year. Reverse transcriptase mutations for 2003 with the highest degree of expression were M184V (44.9%), K103N (29.9%), T215Y (25.2%), and M41L (23.1%). Significant differences between men and women for the expression of D67N ($P=.01$), K70R ($P=.01$), and M184I ($P=.006$) were recorded. In 2004,

Table 1. Rates of HIV-1 resistance to antiretroviral drugs, Puerto Rico, 2002–2004

	2002 (% of resistant isolates)			2003 (% of resistant isolates)			2004 (% of resistant isolates)		
Antiretroviral Drug	Men	Women	Total	Men	Women	Total	Men	Women	Total
Zidovudine	40.6	33.9	39.3	44.3	40.7	45.1	39.1	35.6	36.2
Didanosine	63.3	48.4	58.0 *	49.3	50.4	51.6	32.9	28.8	30.2
Zalcitabine	63.3	48.4	58.0 *	59.3	54.5	59.0	54.7	50.8	54.0
Lamivudine	57.0	43.5	51.4	50.7	47.2	50.2	51.8	49.2	50.6
Stavudine	35.2	27.4	33.9	41.2	37.4	41.9	40.1	35.0	36.9
Abacavir	39.1	33.9	37.7	44.3	37.4	44.4	43.3	34.5	39.2
Tenofovir	10.2	1.6	8.9 *	22.2	13.8	19.9	30.0	25.4	27.0
Forscarnet	1.6	0	0.8	0.9	0.8	0.9	28.9	30.5	35.1
Nevirapine	40.6	38.7	44.0	37.6	40.7	39.4	41.4	37.7	40.7 *
Delavirdine	39.1	38.7	43.2	38.0	39.8	39.4	39.1	36.7	38.6
Efavirenz	39.1	38.7	43.2	37.6	41.5	40.3	42.3	37.3	41.1
Saquinavir	28.1	24.2	30.7	32.6	23.6	31.9	27.7	19.2	25.4 *
Indinavir	31.3	24.2	32.7	38.0	26.8	35.9 *	28.0	19.8	25.7 *
Ritonavir	31.3	24.2	32.7	38.0	26.0	35.6 *	28.0	19.8	25.7 *
Nelfinavir	36.7	27.4	37.0	44.8	33.3	41.9 *	34.9	28.8	33.0
Amprenavir/fosamprenavir	20.3	16.1	23.7	30.7	22.8	27.8	24.7	17.5	22.0
Lopinavir + ritonavir	17.22	16.1	20.6	25.8	17.9	24.3	20.5	15.3	19.0
Atazanavir	0	0	0	2.7	0.0	2.3	28.3	22.0	26.3

* Significant difference between men and women.

reverse transcriptase mutations with the highest degree of expression were M184V (47.3%), K103N (29.8%), M41L (20.5%), and T215Y (19.8%). There were significant differences between men and women in the expression of G190A (P=.009) and K65R (P=.003).

Protease Resistance-Associated Mutations

As shown in Table 3, in 2002, the protease resistance-associated specific mutations with the highest degree of expression were L63P (73.6%), M36I (18.6%), L90M (25.2%), L10I (20.5%), and A71V (14.7%). There was a significant difference between men and women for L63P (P=.03). Protease mutations for 2003 with the highest degree of expression were L63P (71.3%), M36I (28.0%), L90M (24.1%), L10I (22.7%), and A71V (18.1%). Significant differences were recorded for G73S (P=.02), L10I (P=.007), L90M (P=.03), L24I (P=.027), and M46I (P=.001). For 2004, the protease mutations with the highest degree of expression were L63P (71.3%), M36I (25.4%), L90M (18.3%), A71V (18.2%), and L10I (15.0%). In 2004, a significant difference was found for L90M (P=.01), 154M (P=.05), and V82T (P=.05).

DISCUSSION

This study is an effort to establish an HIV-1 resistance monitoring system in Puerto Rico and a continuation of a report published in 2002 that examined the prevalence of HIV-1 resistance mutations in 2000–2001.[14] The 2002 report showed higher levels of expres-

Table 2. Frequency of the 10 most prevalent resistance mutations in HIV-1 reverse transcriptase, Puerto Rico, 2002–2004

	2002 (% of mutations)			2003 (% of mutations)			2004 (% of mutations)		
Mutation	Men	Women	Total	Men	Women	Total	Men	Women	Total
M184V	50.8	41.9	45.1	46.6	42.3	44.9	46.9	46.9	47.3
K103N	31.3	27.4	33.9	29.4	29.3	29.9	29.0	27.1	29.8
M41L	18.8	19.4	19.8	22.6	24.4	23.1	22.5	20.3	20.5
T215Y	17.2	17.7	19.1	23.5	26.0	25.2	20.5	20.9	19.8
D67N	18.0	9.7	16.3	24.0	13.0	21.3*	21.8	16.4	19.2
K70R	16.4	6.5	13.2	19.5	8.9	17.1*	17.6	14.1	16.2
L210W	10.2	14.5	13.2	12.2	16.3	15.0	15.0	10.7	9.0
V118I	10.2	6.5	11.7	10.0	13.8	12.0	11.4	14.1	12.2
K219Q	10.9	9.7	9.7	13.1	7.3	12.0	11.4	10.7	10.9
L74V	4.7	6.5	5.8	9.0	8.1	8.1	10.7	7.3	9.0

* Significant difference between men and women.

Table 3. Frequency of the 10 most prevalent resistance mutations in HIV-1 protease, Puerto Rico, 2002–2004

	2002			2003			2004		
Mutation	**Men**	**Women**	**Total**	**Men**	**Women**	**Total**	**Men**	**Women**	**Total**
L63P	78.9	64.5	73.9*	70.6	76.4	71.3	73.0	68.4	71.3
M36I	19.5	22.6	18.7	27.6	31.7	28.0	23.5	27.1	25.4
L90M	24.2	17.7	25.3	25.3	15.4	24.1*	20.5	13.0	18.3*
L10I	20.3	19.4	20.6	26.2	13.8	22.7*	15.6	13.6	15.0
A71V	15.6	11.3	14.8	20.4	14.6	18.1	17.9	19.2	18.2
A71T	12.5	8.1	13.6	14.5	8.1	11.3	14.3	10.2	12.5
M46I	10.9	9.7	14.0	17.2	4.9	12.7*	12.7	10.2	11.6
I54V	11.7	4.8	10.9	13.6	10.6	12.0	11.4	9.6	10.9
D30N	6.3	1.6	5.8	10.4	12.2	9.7	10.1	11.3	10.2
N88D	6.3	3.2	5.8	8.6	9.8	8.1	8.0	11.9	10.2

* Significant difference between men and women.

sion of some mutations when compared to the current study. The reduction in the percentage of mutations when compared to the 2000–2001 study is likely due to better treatment compliance, increased use of HAART, or to sample size, as the 2000–2001 study was conducted with only 80 subjects.

From a sample size of 1256 patients, 92.7% of the men and 94.8% of the women analyzed had infections with mutated HIV virus, and 75.1% of men and 72.4% of women had infections that were resistant to at least one medication. The different in percentages is due to the fact that not all mutations in the HIV genome result in resistance.

Increases in the level of resistance to forscarnet, which increased from .8% in 2002 to 35.2% by 2004; tenofovir, which increased from 8.9% to 27.0% in two years; and to atazanavir, which increased from 2.3% to 26.3% in one year are due mainly to two factors. One is the sample size, and the second and more relevant factor is software upgrades in the TRUGENE system. New versions of the software that result from the discovery of new links between mutations and resistance to existing or new medications impose a technical limitation to this study; data cannot be analyzed again as the patient data are not stored in the computer because of privacy issues. For example, 2004 is the first year for which complete data are available for atazanavir. As more data are obtained, it is expected that the average of five full years of data will help reduce fluctuations in average due to sample size.

We report both the 10 most common mutations and the current interpretation (antiretroviral resistance). It is possible that in the future, as new algorithms are developed, the interpretation might change, but the link between resistance and the major resistance associated mutations should remain constant.

When comparing Puerto Rico with the United States, the rates of HIV-1 resistance are very similar: 76% of isolates are resistant to at least one antiretroviral drug in the United States[15] , and 75.1% of isolates from men and 72.4% of isolates from women were resistant to at least one drug in our study. This finding could, in part, be due to the shared infection pattern between the United States and Puerto Rico, maintained by the high level of travel between the two countries.[16]

Poor HAART adherence coupled with increased risk behaviors can lead to drug resistance and infection of partners with virulent mutated strains. Although the treatment of HIV infection was revolutionized by the introduction of HAART and the subsequent reduction in mortality, the high rates of resistance to various antiretroviral drugs increase the odds of a person's being infected with a drug-resistant strain. By 2002, the rates of new infections with drug-resistant viruses were reported to be as high as 12%.[17] The rapid development of resistance to some antiretroviral drugs points out the need for the constant development of new drugs in all four categories: nucleoside/nucleotide RTIs, nonnucleoside RTIs, protease inhibitors, and integrase inhibitors. Studies indicate that patients acquire on average more than one antiretroviral drug resistance mutation per year.[18] This high degree of mutational variation makes clinical decisions challenging.[19] Therefore, it is necessary to continue the surveillance of HIV resistance mutations to assist physicians in the selection of adequate medical regimens for HIV-infected patients.

Statistically significant differences were observed in mutation incidence between sexes. These differences can be due to treatment compliance, absorbance and retention of the drugs as a result of metabolic differences, or psychosocial factors with different prevalence rates.[20] These aspects require further study and are outside the scope of this investigation. Furthermore, lack of any other demographic data makes it impossible to perform multivariate analysis that could shed more light on the differences found.

Implications for Improving Health Disparities

That fact that there are differences between sexes in HIV mutations could point out possible differences in efficacy of antiretroviral drugs. Understanding these possible differences in efficacy will lead to better treatments, reducing health disparities between men and women.

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