HIV/GENOTYPE

Prevalence of Primary and Secondary Resistant Mutations to Antiretroviral Drug in a Population of Puerto Rican Infected with HIV.

BETZY TORRES RIVERA, MS, MT*; VICTOR VALLÉS, MT†; EDDY RÍOS OLIVARES, PhD, MPH†

Introduction. **Several studies have reported increasing number of therapeutic failures with HAART in HIV-infected individuals. In order to assess the impact HIV antiretroviral resistance could have on treatment, we decided to determine the prevalence of primary and secondary antiretroviral resistant genotypes in a population of HIV -infected Puerto Ricans and compare the mutational distribution pattern with that reported in Europe and US.**

Method. **In a total of 80 plasma samples from patients with detectable viral load of over 1,000 RNA copies/ml, the Trugene Visible Genetics HIV sequencing method was used to detect antiretroviral resistance mutations.**

Results. **We found 55 subjects (69%) with high level of resistance to ZDV in the reverse transcriptase gene and 46 subjects (58%) with high level of resistance to NFV in the protease gene. Mutation frequencies to the NRTI ranged in appearance from as high as 54% (i.e., M184V) in the studied subjects to a low of less than 5 % (i.e., M184I and V75T). For the NNRTI the most common mutation was K103N in 40% of the subjects and found to confer cross resistance to NVP, DLV and EFV. Another concerning finding is the increasing trend of the frequency of primary and secondary resistant mutations from year 2000 to 20001. Nine (23%) of the total detected primary mutations, to either RTI or PI, showed an increase of at least 5% from one year to the other. Similarly, there were 6 (11%) secondary resistant mutations showing an increase of at least 5% during the two years studied.**

Conclusions. **In two year period we detected a tendency to increase in primary and secondary HIV-resistant mutation in a population of HIV-infected Puerto Ricans.**

Key words: HIV mutations, Antiretrovirals, Nucleoside reverse transcriptase inhibitors, Non-nucleoside reverse transcriptase inhibitors, Protease inhibitors, HIV-infection, Puerto Rico, HAART.

The Puerto Rico 2001 AIDS Surveillance Report showed that the Acquired Immunodeficiency Syndrome (AIDS) mortality has dropped significantly in the last decade (1). There was a decrease from 79% in 1990 to a 22% in year 2000. Similarly, in the City of New York for the same period there was a decrease of 85% to 5% respectively (2). The average decrease in the United States of America was 42% in 1997 and an additional 20% in 1998 (3). Despite this recent decrease-trend in AIDS mortality, several studies have reported increasing number of therapeutic virologic failures with highly active antiretroviral therapy (HAART) (4, 5). Some researchers suggest that this phenomenon is observed specially in patients pretreated with a drug regimen considered as sub-optimal in terms of virologic efficiency (6, 7). Other studies indicate that virologic failure of HAART is predicted by the number of resistance mutations in the reverse transcriptase (RT) and protease (P) genes of human immunodeficiency virus (HIV) and not to pharmacological factors or sub-optimal patient adherence to treatment (4, 5, 8, 9).

Irrespective of the cause of the reported impaired response of HIV to HAART, there is an emerging need to assess the prevalence of mutated sequences of RT and Protease genes of HIV in order to predict possible treatment failures when specific antiretroviral drug is used. This in turn, will provide physicians with the benefit of choosing more adequate regimen and improving the prognosis of HIV-infected persons.

Del *Programa de Maestría en Ciencias del Laboratorio Clínico, y el †Departamento de Microbiología de la Escuela de Medicina, Universidad Central del Caribe, Bayamón, Puerto Rico

This work was supported by grants from NIH-RCMI, G-12 RRA103035 and RCRII, P20-RR1106.

Address for correspondence: Dr. Eddy Rios Olivares, Department of Microbiology and Immunology, Universidad Central del Caribe, School of Medicine. Bayamón, Puerto Rico, 00960. Tel: (787) 798-4050 Fax: (787) 740-4300. Email: erios@uccaribe.edu.

HIV is a double-stranded RNA positive virus that lacks the exonuclease activity "proofreading" function, conferring frequent mutations during replication (10, 11). It has been estimated that mutations occur about once in every 10,000 nucleotides copied by the HIV reverse transcriptase enzyme (12) and because the genome of HIV-1 is approximately 10,000 nucleotides in length, one such error would occur, on average, every time a viral genome is copied. Each time the HIV infects a new cell and replicates, one mutation in its genome appears, giving rise to numerous mutations in the progeny viruses, including reduced sensitivity to antiretroviral drugs(12).

Some mutations can be silent, missence or non-sense mutations (13). In HIV, mutations are classified as primary and secondary mutations. Primary mutations are those that alter the binding of a drug to its target, resulting in increased amounts of drug necessary to inhibit the target enzyme and secondary mutations, those that increase the level of resistance by improving the virus carrying the primary mutations (11).

In 1987-1988, zidovudine (AZT) was the first drug approved for treatment of patients with late-stage HIV disease (14). Anti-retroviral therapy, with this drugs and others, for HIV-1 played an important role in reducing patient viremia, but treatment failure or inefficiency alter drug metabolism contributing to impart resistance (14). Initially, effective antiviral drug implies an effective reduction of HIV RNA in the blood and gradually raises levels of CD4 lymphocytes. After several months or years, the level of HIV RNA reflects changes in the activity of antiviral drugs (the drugs are less effective)(15).

Thus, resistance to zidovudine (AZT) begins to emerge in patients after months to years of therapy (16). The most common genotypes associated with high level of zidovudine resistance and clinical failure are combinations of mutations at positions 41, 215, 67, 70 y 219 (17, 18) of the RT gene. In addition in drug naive HIV- infected patients of USA, there is phenotypic and genotypic evidence of antiretroviral drug resistance (19).

Two testing methods are available to identify HIV drug resistance: genotypic assay, which detects genetic mutations and phenotypic assay, which measures the concentration of drug required to inhibit viral replication in cell cultures (11). There are different opinions as to the advantage and disadvantages for the use of these two methodologies. However, since this topic is beyond the scope of the present study, no further discussion will be considered on this subject. HIV Genotyping by the Visible Genetics Inc. (Suwance, GA) is the only antiretroviral drug suscetibility assay that has been FDA approved.

In this study we determined the prevalence of different primary and secondary resistant genotypic mutations in a population of Puerto Ricans infected with HIV. In addition we measured the frequency in which primary and secondary mutations appeared combined. These mutational distribution patterns were compared with those reported in Europe and US.

Material and Methods

This study analyzes data from the Immunoretrovirus Research Laboratory at the Universidad del Caribe (Bayam©n, P.R.). Plasma sample from 80 patients were analyzed using TruGene HIV genotypic assay (Visible Genetics Inc., Suwance, GA). The demographic and clinical data of these patients were unknown.

HIV genotyping requires plasma from whole blood, collected in tubes containing ethylenediaminetetra-acetic acid (EDTA) as anticoagulant. Plasma is separated and kept frozen at -80°C. HIV viral RNA was extracted using ultra centrifugation and a column purification method (QIAamp Viral RNA). RNA was reverse transcribed to cDNA and amplified by RT-PCR, creating a 1.3 kb amplicon covering the protease and the first codons of the reverse transciptase (RT) gene. Then, the amplicon is sequenced in 4 sections protease gene (P), reverse transcriptase gene (RT)-beginning, middle and end) using a proprietary sequencing chemistry generating a sequencing ladder in forward and reverse directions labeled with two fluorescent dyes (Cy5 and Cy5.5). TRUGENE™ protocol, to secure acrylarmide cartridge (6% ready to use) to SureFill™ Injector was used. The resulting sequences were compared to Gene Librarian that contains known resistance mutations.

Statistical analysis. Since this is a population selected by convenience, in order to determine frequency or prevalence of the mutations that confer HIV resistance to the antiretrovirals, our data compares: (a) Relationship of the percentage (%) of primary to secondary mutations, in a Puerto Rican population (b) type and frequency (%) of combinational mutations (combination of primary, secondary or both), (c) comparative analysis of the pattern and frequency (%) of mutations reported in U.S, Europe and Puerto Rico.

Results

Total resistance mutations in reverse transcriptase (RT) and protease (PT) genes detected during a two-year period (2000-2001). Table 1 shows the resistant mutations most frequently found in RT gene of viruses isolated from 80 studied subjects. These were, in RT gene, for nucleoside reverse transcriptase inhibitors (NRTI's): M184V (54%), T215Y (46%), M41L (46%) and for non-nucleoside reverse

transcriptase inhibitors (NNRTI's): K103N (40%). The least frequent for NRTI's were: M184I (<5%), V75T (<5%) and for the NNRTI's, V179D (<5%), A98G (<5%). Similarly, in table 2 appears the mutations most frequently observed in the P gene. These were, L63P (70%), L90M (50%), V71I (42%), and A71V (30%), and the least frequent L10F, L24I, L33F all with <5%.

Table 1. Total Frequency (%) of Mutations in Reverse Transcriptase Gene in HIV Positive Subjects During Two-Year Period (2000 and 2001)

Nucleoside RT inhibitors	Frequency%(n)	Non-nucleoside RT inhibitors	Frequency
*M184V	54% (43)	K103N	40% (32)
T215Y	46% (37)	Y181C	9% (7)
*M41L	46% (37)	G190A	5% (4)
L210W	31% (25)		
*K70R	25% (20)	L100I	14% (11)
*K219Q	21% (15)		
M41L/T215Y/D67N/L210W	14% (11)		
*H208Y	10% (8)		
L74V	9% (7)	Y188L	9% (7)
T69D	9% (7)	A98S	5% (4)
T69N	8% (9)		
M41L/T215Y/L210W	8% (9)		
K219E	6% (5)		
T215F	5% (4)		
M184L/V75T	<5% (2)	A98G/V179D	<5% (2)

*Associated with multiple drug resistance combinations

Table 2. Total Frequency (%) of Mutations in Protease Gene in HIV Positive Subjects During Year (2000 - 2001) (n=80)

Protease Inhibitor	Frequency % (n)
*L63P	70% (56)
*L90M	50% (40)
V77I	42% (34)
A71V	30% (24)
L10I	28% (22)
M36I	21% (17)
A71T	18% (14)
*M46I	18% (14)
*I54V	18% (14)
G73S	16% (13)
*V82A	13% (10)
*I84V	10% (8)
*M46L	10% (8)
*D30N	9% (7)
*L10V	9% (7)
L63P/A71V/V77I	9% (7)
L90M L63P/A71V	9% (7)
L33F	6% (5)
L10I L63P/A71V/L90M	6% (5)
*G48V	5% (5)
I54V/A71V/L90M	5% (5)
L10F, L24I, L33F	<5%

*Associated with multiple drug resistance combinations

Comparison of frequency of primary and secondary mutations detected in HIV positive subjects in the Years 2000 and 2001. Table 3 compares the frequency of primary mutations found in year 2000 with those detected in year 2001. It can be seen that several of the most frequent mutations (i.e., T215Y, K03N, M46I, H208Y, M46L, T69D, N88D, T69N and Y181C) showed a mark increase in appearance from one year to the other. However, others (i.e., M184V, L90M, K70R, D30N, L74V) did not present frequency variation during the two-year period studied. Few mutations (i.e., M41L and V82A) decreased in their frequency from one year to the other. The most frequent secondary mutations were: L63P, M184V, L90M, V77I, D67N and the least frequent were L10V, Y181C, Y188L and more than 40 with <10%. Moreover, these was an increase of at least 4% from year 2000 to 2001 in the following secondary mutations: L63P, M184V, L90M, V77I, A71T and M46I, and at least 4% decrease in mutation D67N. (Data not shown).

Table 3. Comparison of the Primary Mutations Detected in Years 2000 and 2001 in HIV Positive Subjects

Primary Mutations	% of mutations in year 2000n=54	% of mutation in year 2001 n= 26	% Total of mutations 2000 2001 n=80
NRTI's			
M184V	51.85 (28)	57.69 (15)	54 %
T215Y	44.44 (24)	50 (13)	46 %
M41L	48.15 (26)	42.31 (11)	46 %
K70R	24.07 (13)	26.92 (7)	25 %
H208Y	7.41 (4)	15.38 (4)	10 %
L74V	9.6 (5)	7.69 (2)	9 %
T69D	5.56 (4)	15.38 (4)	9 %
T69N	5.56 (4)	11.54 (3)	8 %
NNRTI's			
K103N	31.48(17)	57.69(15)	40 %
Y181C	3.75(3)	15.38(4)	9%
PI's			
L90M	48.15(26)	53.85(14	50 %
M46I	14.81(8	23.08(6	18 %
V82A	16.67(9)	3.84(1)	13 %
I84V	11.11(6)	7.69(2)	10 %
M46L	7.41(4)		10 %
D30N	5.56(3)	5.56(4)	9 %
N88D	3.70(2)	15.38(4)	8 %

Prevalence of commonly detected mutations and Associated Antiretroviral Drugs. The frequency of the most common detected mutations in HIV positive subjects and the specific drugs associated were: M184V appeared conferring resistance to Didanoside (RTI's), Lamiduvine(RTI's) and Zalcitabine in 54% of the total sample studied; L90M to Saquinavir(PI's) and Nelfinavir (PI's) in 50%. T215Y and M41L to Zidovudine(RTI's) in 46%, and K103N to Delavirdine (NNRTI's), Efavirenz, (NNRTI's), and Nevirapine (NNRTI) in 40%. At least 15 mutation appeared with a frequency =25%.(Data not shown).

Frequency of primary and secondary mutations appearing combined and the associated drugs 2000 to 2001. The frequency in which primary mutatios appear combined and the corresponding associated drugs were: M41L & T215Y (28%), L90M & M184V (26%), L90M & M41L, L90M & T215Y with (24%). These corresponded to Abacabir/Zidovudine, Didanosine/Zalcitabine, Nelfinavir/Saquinavir, respectively. The most common secondary mutations appearing combined were: L63P& L90M (39%), L63P & M184V (36%), which corresponded to Saquinavir/Nelfinavir, Didanoside/Zalcitabine/ Lamiduvine respectively. More than 20 combinations appeared with a frequency of =14%.(Data not shown).

Prevalence of primary mutations appearing combined with secondary mutations and associated antiretroviral drugs (2000 to 2001). The prevalence of primary and secondary mutations appearing combined were: L90M& L63P to Saquinavir/Nelfinavir (36%), M41L & D67N to Zidovudine/Abacabir (30%), M41L & L210W to Zidovudine/Abacabir(30%) and the least frequent was M461& L90M to Saquinavir/Nelfinavir with 15% . More than 10 combinations appeared with a frequency of =13%.(Data not shown).

Frequency of appearance of individual resistant mutation to specific RTI drug in HIV positive subjects during the two year period reported (2000 to 2001). Table 4 presents the most frequently primary and secondary resistant mutations reported to specific RTI. These were: to Zidovudine M41L 21% (primary), T215Y 21% (primary), D67N 18% (secondary), and L210W 14% (secondary); to Zalcitabine L74V 10% (primary), and M184V 58% (secondary). To the non-nucleoside reverse transcriptase inhibitors (NNRTs) the most frequent resistant mutations observed were: to Efavirenz K103N 45% (primary), and L100I 15% (secondary); to Delavirdine K103N 72% (primary).

Frequency of appearance of individual resistant mutation to specific PI drug in HIV positive subjects during the two year period reported (2000 to 2001). Table 5 presents the most frequently primary and secondary

Table 4. Frequency of Appearance of Individual Primary and Secondary Mutation to Specific RTI antiretroviral Drugs in HIV Positive Subjects from Year 2000 to 2001

Drugs (n)	Primary mutations (%)	Secondary mutations (%)
NRTI	M41L (21), T215Y (21),	D67N (18), L210W (14),
Zidovudine (ZDV) (180)	K70R (11),K70K (1), T215F (2),M41V (0.5)	K219Q (8), K219E (2), K219K (0.5), L210L (0.5)
Zalcitabine (ddC) (72)	L74V (10), T69D (11), T69N (8), L74L (1)	M184V (58), M184M (40), M184I (3), V75T (3), T215C (1)
Didanosine (ddI) (57)	L74V (12), L74L (2)	M184V (72), M184M (9), V75T (4), M184I (2)
Abacabir (ABC) (55)	M184V (76), L74V (13), M184M (5), M184I (4), L74L (2)	0
FTC (48)	M184V (90), M184M (6), M184I (4)	0
Lamiduvine (3TC) (46)	M184V (91), M184M (7), M184I (2)	0
MDR (4)	0	A62V (50), V75I (25), V75V (25)
Stavudine (d4T) (3)	I50V (33), V75T (70)	0
NNRTI		
Nevirapine (NVP) (83)	K103N (74), Y181C (8), Y181Y (4), G190A (5), G190G (2), G190S (1), K103D (1), G190E (1), V106A (1), Y181I (1)	L100I (12), Y188L (8), A98G (4), A98S (4), V108I (2), Y188F (2), A98A (1), V118I (1), A93S (1)
Efavirenz (EFV) (71)	K103N (45), K103D (1)	L100I (15), Y188L (6), V108I (3), V179D (6), Y181C (4), Y181Y (3), Y188F (3), Y188Y (1), K103N. (1), V1108I (1)
Delavirdine (DLV) (44)	K103N (72), Y181C (16), Y181Y (7), K103D (2)	P236L (2)

resistant mutations reported to specific PI. These were: to Indinavir M461 6% (primary), L63P 23% (secondary), L90M 16% (secondary), and A71V 10% (secondary); to Nelfinavir L90M 19% (primary), L63P 27% (secondary), and V77I 16% (secondary). To Ritonavir L90M 26% (secondary), and A71V 14% (secondary); to Saquinavir L90M 37% (primary), and L10I 20% (secondary): to Amprenavir I84V 29% (primary) and M46I 50% (secondary).

Table 5. Frequency of Appearance of Individual Primary and Secondary Mutation to Specific PI antiretroviral Drug In HIV Positive Subject from Year 2000 to 2001

Drugs (n)	Primary mutations (%) (n)	Secondary mutations (%) (n)
PI		L63P (23), L90M (16),
Indinavir (IDV)	M461 (6), V82A (4),	A7I (10), L10I (9),
(242)	M46L (2), V82F (2),	I54V (6), I84V (3),
		A71T (5), L10V (3)
		G73A (1), A71I (1),
		L90L (1), I541 (0.4),
		K20R (0.4), I54L (0.4),
		L10R (0.4), L63P (0.4),
		V32I (0.4), V77I (0.4),
		L10L (0.4), I84I (0.4)
Nelfinavir (NFV)	L90M (19), D30N (3),	L63P (27), V77I (16),
(211)	N88D (2), M184V (0.5),	A71V (11), M36I (7),
	L90L (1)	M46I (7), I84V (7), V77V (1),
		I84I (0.5)
Ritonavir (RTV)	V82A (6), M46I (9),	L90M (26), A71V (14),
(159)	N88D (2), L90L (1),	M36I (11), I54V (9), I84V (6)
	M184V (0.4)	A71T (9), L33F (3), A7II (11),
		K20R (1), L90L (1), I54L (1),
		I54I (1), I84I (0.5)
Saquinavir (SQV)	L90M (37), L90L (2),	L10I (20), G73S (12),
(108)	G48V (2), G48G (1)	I54V (13), I84V (6),
		G73G (2), I82V (2),
		I54I (1), I84I (1)
Amprenavir (APV)	I84v (29), I50V (4),	M46I (50), L10F (14)
(28)	I84I (4)	

Frequency of HIV-infected subjects with primary and secondary resistant mutations to antiretroviral drugs. Table 6 shows that more than 40% of the subjects presented primary resistant mutations that confer resistance to Nelfinavir, Saquinavir, Zidovudine, Nevirapine, Abacavir, FTC, 3TC, Efavirenz, Delavirdine and secondary resistant mutations that confer low level resistance to Nelfinavir, Indinavir, Ritonavir, Saquinavir, Zalcitabine, Didanisine, Zidovudine. Interestingly, more than 50% of the studied subjects harbored HIV with secondary level of resistance to Zidovudine, Didanosine, Zalcitabine, Ritonavir, Indinavir and Nelfinavir. Secondary mutations to antiretrovirals such as: Abacabir, Adefovir, FTC, Lamiduvine, Stavudine, Delavirdine in the group of RTI were not detected in the HIV positive population.

Table 6. Frequency of HIV-infected Subjects with Primary and Secondary Mutations to Anti-Retrovirals Drugs (n=80)

Drugs	#subjects with primary mutations	%	# subjects with secondary mutations	%
NRTI's				
Zalcitabine (ddC)	20	25 %	46	58 %
Didanosine (ddI)	7	9 %	45	56 %
Zidovudine(ZDV)	55	69 %	45	50 %
Abacavir (ABC)	47	59 %	0	0
Adefovir	1	1 %	0	0
FTC	43	54 %	0	0
Lamiduvine(3TC)	42	53 %	0	0
Stavudine (d4T)	3	4 %	0	0
NNRTI's				
Efavirenz (EFV)	34	43 %	24	30 %
Nevirapine(NVP)	40	50 %	24	30 %
Delavirdine(DLV)	36	45 %	0	0
MDR	0	0	3	4 %
PI				
Nelfinavir (NFV)	46	58 %	76	95 %
Indinavir (IDV)	23	29 %	71	89 %
Ritonavir (RTV)	23	29 %	52	65 %
Saquinavir (SQV)	43	54 %	32	40 %
Amprenavir(APV)	9	11%	16	20 %

Total frequency of resistance observed to individual drug in HIV positive subjects during the period of the study (2000 to 2001). Figure 1 illustrates the total resistant mutations detected to individual antiretroviral. The resistance to the NRTI, Zidovudine was the most frequently scored, followed by Zalcitabine, Didanosine, Abacabir, FTC, Stavudine. Among the NRTIs, the drug showing the highest frequency resistance mutations was Efavirenz, followed by Delavirdine. Among the PI, Indinavir was the most frequently detected with resistant mutations, followed by Nelfinavir, Ritonavir, Saquinavir and Amprenavir.

Frequency of primary drug resistance reported from France, Spain, USA and Puerto Rico. Figure 2 compares the prevalence of mutations to antiretroviral drugs (RTIs and PIs) reported in France, Spain and USA with the results presented in this study for Puerto Rico. There is a general trend in France, Spain and USA to a decrease in the frequency of resistant mutations to RTIs from 1995 to 1999. However, contrary to this decreasing trend, in PR the tendency in the last two years (2000 and 2001) has been

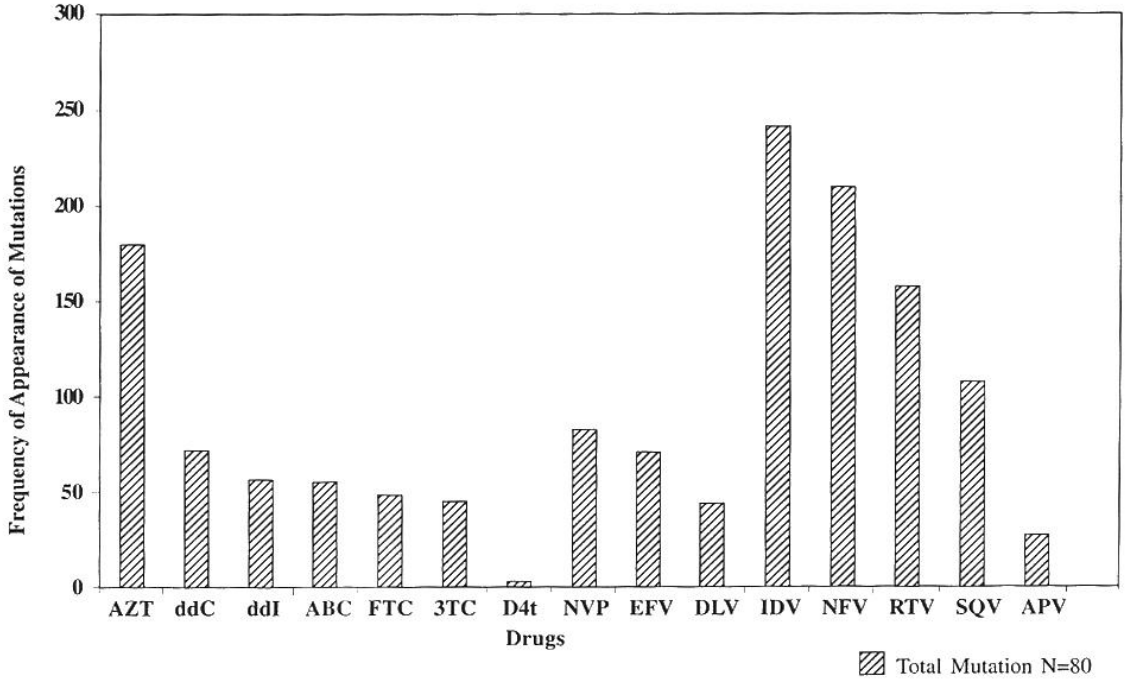

Figure 1. Total Frequency on Resistance to Specific Antiretroviral Drugs in HIV Positive Subjects From Year 2000 to 2001 (n=80)

an increase in the number of resistant mutation to the RTIs, although these differences are not statistically significant (P>0.05). Similarly, whereas in France, Spain and USA, the percent of resistant mutations in the last three years to the PI is less than 10% (P<0.05), in PR the frequency is above 15% with a tendency to increase. Although, the increase registered for PR from 2000 to 2001 is also not statistically significant ($x^2 = .741$, P=0.389.)

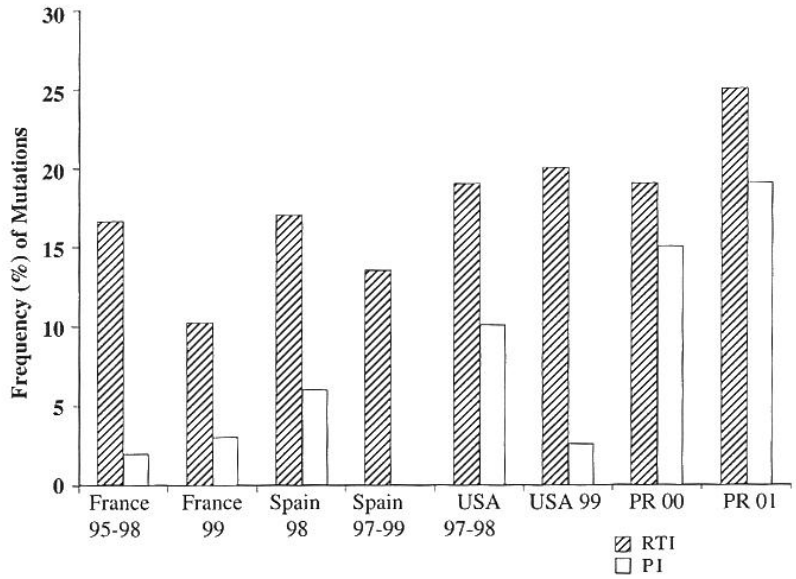

Figure 2. Frequency of Primary Drug Resistance Reported for France*, Spain*, USA* and Puerto Rico
* As reported in the literature Ref. (21)

Discussion and Conclusions

In this study we analyzed the frequency of primary and secondary antiretroviral resistant mutations in the retro-transcriptase (RT) and protease (P) genes of HIV from a population of 80 infected Puerto Rican during years 2000 and 2001. We have shown that there is a broad spectrum in the frequency of appearance of resistant mutations to the NRTIs, NNRTIs and PIs. Mutation frequencies to the NRTIs ranged in appearance from as high as 54% (i.e., M184V) in the study subjects to a low of less than 5 % (i.e., M184I and V75T). Similar variation patterns were observed for the NNRTIs and the PIs. NNRTI resistant mutations occur in two sites and emerge rapidly, of these the most common mutation is K103N that was detected in 40% of the subjects and found to confer cross-resistance to Nevirapine, Delavirdine and Efavirenz (10, 11). On the other hand, in agreement with previous reports, PI mutations appear in greater number of sites than for RTIs. (10). Mutations that confer resistance to Adefovir were the least frequent (1%). This NRTI has been used successfully to treat patients with resistant mutations to Zidovudine, Didanosine, and Lamiduvine (19).

Another concerning finding is the increasing trend of the frequency of primary and secondary resistant mutations from year 2000 to 20001. Nine (23%) of the total detected primary mutations, to either RTIs or PIs, showed an increase of at least 5% from one year to the other. Similarly, there were 6 (11%) secondary resistant mutations showing an increase of at least 5% during the two years studied. Although this is not a sufficiently long period of observation time to establish it significance, it does flashes a warning. On the other hand and contrary to this increase of mutation frequencies from one year to the other, studies on prevalence of primary drug resistance in France, Spain and USA show a general tendency to decrease with time.

In our study, the primary mutations most frequently associated with specific antiretroviral were: M184V to Didanoside, Lamivudine and Zalcitabine, L90M to Saquinavir and Nelfinavir, T215Y and M41L to Zidovudine and K103N to Delavirdine, Efavirenz and Nevirapine. The result showing association of T215Y and M41L to Zidovudine agrees with results published elsewhere (11, 16, 17). Similar to our results, there have been report of high frequency (50%) of a resistant mutation (M184V) to Lamivudine that appears quickly after treatment and confers low level of cross resistance to Zalcitabine, Abacavir and Didanosine (10, 11, 15).The secondary mutations found in high frequency (>50%) were L63P, M184V and L90M. L63P, a mutation detected in 70% of the studied subjects, is commonly found in viruses that have never been exposed to PIs and in patients whom regimens have failed (19). Mutations and their associated antiretroviral most frequently (>25%) found together in the virus preparation from the same subject (combined) conferring resistance were: Primary, M41L & T215Y to Abacabir and Zidovudine, L90M & M184V to Didanoside and Zalcitabine and L90M & M184V to Nelfinavir, Saquinavir, and Lamivudine; Secondary, L63P & L90M to Saquinavir and Nelfinavir, L63P & M184V to Didanoside, Zalcitabine and Lamiduvine, Primary combined with secondary, L90M & L63P to Nelfinavir and Saquinavir. Other combined mutation confering resistance to PIs were: M41L & D67N, M41L & L210W. To our knowledge, there have been no reports in the literature tabulating the frequency of appearance of two combined mutations in the HIV preparation from the same individual. This type of data could have important application in transmission studies.

Taking this findings together, it can be suggested that a selected group of mutations appears to belong to "hot spots" (mutation hypersensitive loci) in the RT and PR genes sites. This would explain the high frequency in which they were detected coding for primary and/or secondary resistance to specific antiretrovirals. In Puerto Rico, it would appear that there is no significant difference in the frequency of resistant mutations to RTIs from that reported in the last years (1998-2001) for France, Spain and USA. However, this different was significant for PIs. Contrary to France and Spain, in Puerto Rico there was a tendency to increase in both primary and secondary mutations to RTIs from year 2000 to 2001. This increasing trend could be explained by the lack of a uniform health policy for the treatment of HIV individuals that could minimize the impact of emerging resistant mutations in the population. It has been held that virological failure to HAART is not predicted by pharmacological factors or sub-optimal patient adherence to treatment (4, 5, 8, 9). However, in Puerto Rico, the major HIV risk factor behavior (injecting drug use), has a definite impact on drug compliance, use of sub-optical concentrations and inappropriate combinations. Therefore, continuous monitoring on emerging resistant mutations should be mandatory.

Resumen

Varios estudios han reportado un aumento en la resistencia a drogas terapeuticas (antiretrovirales) utilizadas para el tratamiento de pacientes HIV positivos. Teniendo en cuenta el potencial efecto de la resistencia del virus al tratamiento, hemos decidido determinar la prevalencia de las secuencias genotípicas en una población de puertoriqueños infectados y comparar los patrones de mutación con las reportadas en Europa y EU.

Un total de 80 muestras de plasma de pacientes con una carga viral sobre 1,000 copias RNA/ ml, fueron analizadas mediante el método de Trugene Visible Genetics, para detectar genotipicamente la resistencia a los diferentes antiretrovirales conocidos.

Encontramos que 55 (69%) de los sujetos poseían resistencia a ZDV en el gene de transcriptasa reversa y 46 (58%) sujetos poseían resistancia a NFV en el gene de proteasa. Las frecuencias de mutaciones a NRTI en los sujetos estudiados aparecieron de un 54%(ej. M184V) a menos de un 5% (ej. M184I, V75T). Para NNRTI la mutación mas común fue K103N en 40% de los sujetos, confiriendo resistencia a NVP, DLV, y EFV. Otro de los hallazgos encontrados fue una tendencia de aumento en las resistencias primarias y secundarias para el periodo 2000-2001. Un total de nueve (23%) de las mutaciones primarias detectadas tanto para RTI como PI manifiestaron un aumento de por lo menos un 5% de un año al otro y un 6 (11%) de las mutaciones secundarias mostraron un aumento de por lo menos de un 5% durante el mismo periodo.

Durante el periodo estudiado, hemos detectado una tendencia de aumento en las mutaciones primarias y secundarias en una población de puertorriqueños infectada con HIV.

References

1. Puerto Rico Department of Health Surveillance Report Acquired Immunodeficiency Syndrome. Casos de SIDA confirmados en Puerto Rico hasta 2/28/2001. Surveillance Report 2001.
2. Surveillance Report 2000 of New York.
3. Check W. Discovering why HIV drugs fail. College of American Pathology 1999;13:1-12.
4. Rousseau M, Vergne L, Montes B, et al. Patterns of resistance mutations to antiretroviral drugs in extensively treated HIV-1 infected patients with failure of highly active antiretroviral therapy. J AIDS 2001;26:36-43.
5. Young B, Johnson S, Bahktiari M, et al. Resistance mutations in protease and reverse transcriptase genes of human immunodeficiency virus type 1 isolates from patients with combination antiretroviral therapy failure. J Infect Dis 1998; 178:1497-1501.
6. Bratt G, Karlsson A. Leandersson AC, et al. Treatment history and baseline viral load, but not viral tropism or CCR-5 genotype, influence prolonged antiviral efficacy of highly active antiretroviral treatment. AIDS 1998;12:2193-202.
7. Ledergerber B, Egger M, Opravil M, et al. Clinical progression and virological failure on highly active antiretroviral therapy I HIV-1 patients: a prospective cohort study. Lancet 1999;353:863-8.
8. Cabana M, Clotet B, Martinez MA. Emerge and genetic evolution of HIV-1 variants with mutations conferring resistance to multiple reverse transcriptase and protease inhibitors. J Med Virol 1999;59:480-90.
9. Lorenzi P, Opravil M, Hirschel B, et al. Impact of drug resistance mutations on virologic responses to salvage therapy. AIDS 1999;13:F17-21.
10. Kartsonis N A, D'Aquila R T. Clinical monitoring of HIV-1 infection in the era of antiretroviral resistance testing. Inf Dis Clin North Am 2000;14:4.
11. Wilson J W, Bean P. A physician's primer to antiretroviral drug resistance testing. The AIDS reader. 2000.
12. Condra J H, Emini E A. Preventing HIV-1 drug resistance. Science & Medicine. 1997. January / February 2-11.
13. Rokoski R. Biochemistry. Saunders Text and Review. 1996;271.
14. Mayers, Douglas L, Resistance and cross resistance to nucleoside reverse transcriptase inhibitors. Advance in Research and Therapy.1996;6:2.
15. Active HIV replication continuously generates viral variants that are resistance to antiretroviral drugs. International Association of Physicans in AIDS Care. Education clinical guidelines 2000.
16. Richman, Douglas D. Resistance to drugs for HIV infection. 1998. IAPAC.
17. Kellam P, Larder, Brendan A. Retroviral recombination can lead to linkage of reverse transcriptase mutations that confer increased zidovudine resistance. J Virol 1995;669-674.
18. Larder, Brendan A, Arinder Kohli, Stuart Bloor, et al. Human immunodeficiency virus type 1 drug susceptibility during zidovudine (AZT) monotherapy compared with AZT plus 2',3'-dideoxyinosine or AZT plus 2',3'-dideoxycytidine combination therapy. J Virol Sept 1996,5922-5929.
19. Weinstock H, Zaidi I, Respess R, et al. Genotypic and phenotypic evidence of antiretroviral drug resistance among drug-naïve persons recently diagnosed with HIV-1,USA,1997-1999. Antiviral Therapy 2000;5 (Supplement 3):135.
20. Kozal et.al Nat Med. 1996;2:753-759.
21. Bureau of HIV/AIDS, STD and TB Update Series Center for Infectious Disease Prevention and Control; May 2001.