INTEGRASE DRUG RESISTANCE MUTATIONS IN PROVIRAL DNA OF HIV-1 INDIVIDUALS IN PUERTO RICO

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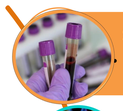
BACKGROUND

The Commonwealth of Puerto Rico is among the top ten states and territories having the highest prevalence of HIV infection, rate of HIV diagnosis and highest accumulated cases of Acquired Immune Deficiency Syndrome (AIDS) (6,7). During the period of 1981 to 2019, approximately *49,791* people had been diagnosed with human immunodeficiency virus type 1 (HIV-1) in PR (6). Recent studies have shown high prevalence of drug resistance mutations (DRM) to Protease Inhibitors (PIs) and Reverse Transcriptase Inhibitors (RTIs) in PR (13, 17, 18). Currently, integrase strand transfer inhibitors (INSTIs) are being used as part of first-line ART and as rescue therapy for heavily treated HIV-1 infected patients. Their combination with PIs and RTIs have synergistic effect for the reduction of virus replication (2, 13). Though INSTIs show high genetic barrier to drug resistance, it does occur, and transmission of DR has been reported in ART-naïve patients (1, 3). As the use of INSTIs increases, including the surveillance of DRM in the integrase gene becomes increasingly more important as part of the clinical management of HIV patients. As the number of patients treated with INSTIs increases, the surveillance of DRM comes to be an important part of clinical management of HIV patients. INSTIs have been used in PR for several years, however the *prevalence of integrase drug resistance mutations has not been determined for DNA integrase sequences of HIV patients.*

OBJECTIVE

To assess the prevalence of HIV-1 drug resistance mutations to integrase drug inhibitors (INSTIs) present in the proviral DNA of HIV-1 individuals in Puerto Rico from the period comprising from 2016 to 2019.

METHODS



Sample selection

- Samples received for HIV drug resistance genotyping tests from 2016 to 2019. A total of 185 sequences were analyzed.

DNA and RNA extraction

- PBMCs: proviral DNA extracted with QIAamp DNA Blood mini kit
- Plasma samples: extraction of viral RNA with QIAamp RNA kit

PCR amplification

Integrase gene was amplified using a WHO-accredited HIV-1 RNA genotyping protocol and a modified nested PCR for DNA samples. PCR was done with PE Applied BioSystem GeneAmp PCR System 9700/9800

Sequencing and Data Processing

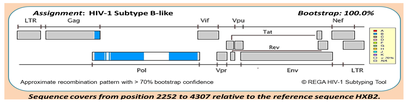
- Big Dye Terminator Cycle Sequencing kit (Applied Biosystems)
- AB 3730XL DNA Sequence Analyzer
- Sequence editing and alignment using ReCall and BioEdit software and CPR

Drug Resistance Interpretation and Analysis

A. DNA integrase resistance interpretation with Stanford Drug Resistance Database

B. Comparison of DNA and RNA integrase DRM interpretation

RESULTS



Sequence covers from position 2252 to 4307 relative to the reference sequence HXB2.

The studied HIV-1 DNA integrase sequences cover positions 2252 to 4307 relative to reference HXB2 (REGA software) and subtyping was checked using COMET. All 185 DNA integrase sequences were classified as HIV-1 subtype B.

Of the **185 proviral DNA integrase** sequences that were analyzed, **six sequences (3.3%)** were identified to contain **INSTI drug resistance mutations** (DRM) according to analysis using the Stanford Drug Resistance Database. These six samples with integrase resistance mutations corresponded to all *male* individuals, with an average age of *42.8 yrs* (range 22-62) and an average CD4+ count of 488.16 (range 175-819).

In the proviral DNA integrase sequences, we detected **several major DRM** in DNA integrase sequences **(E138K, G140S,Q148H,R263K)** but **no known DR accessory mutations.** We also detected other DR mutations or polymorphisms present in the DNA integrase sequence that are listed in **Table 1**.

Comparing the presence of integrase DR mutations in proviral sequences with those RNA integrase sequences from population in our database comprising the same time period (2016-2019), we found that 4.3% of DNA integrases showed DRMs compared to 7.6% of RNA integrase sequences (n=171).

Table 1a shows interpretation of levels of resistance to INSTIs present in these six proviral integrase sequences. **Two proviral HIV integrase for have high resistance to EVG and RAL with intermediate levels for BIC and DTG. A third individual has intermediate resistance to DTG and EVG.**

TABLE # 1. DNA Integrase INSTIs Drug Resistance Mutations and Other Mutations

Sample ID	Major INSTI DRM	Other Integrase Mutations																	
271102DNA	E138K	S24S/N	I60M	K71Q	I72V	V110V/I	I113V	S119R	W132W	G134G/R	E138E/K								
273698DNA	G140G/S, Q148Q/H	S17N	G59G/A	I72V	E96E/D	L101I	T124A	T125A	K156N	K160K/R	V201I	T218T/S	I220I/V						
274260DNA	R263R/K	E11D	S24S/G	L45L/I/V	M50I	L101I	I113V	T125A	V151I	K215N	D256E	D288D/N							
278584DNA	G140G/S, Q148Q/H	L101I	S119T	T124N	G163E	F181L	V201I	D256D/E											
284499DNA	E138E/K	S24N	V31V/I	E35Q	L45V	I72V	L101I	K111R	S119P	T122I	T124A	G163E	A205S	K211R	T218I	S230N	D288N		
286656DNA	R263R/K	S17N	L28I	I72V	L101I	S119T	T124N	T125A	I135V	A205S	S230N	D256E	R262R/K	A265V	S283G				

Table 1a. Resistance Levels to INSTIs in DNA Integrase

Sample ID	BIC	DTG	EVG	RAL
271102DNA	pot Low	pot Low	Low	Low
273698DNA	Inter	Inter	High	High
274260DNA	Low	Inter	Inter	Low
278584DNA	Inter	Inter	High	High
284499DNA	pot Low	pot Low	Low	Low
286656DNA	Low	Inter	Inter	Low

These six individuals also had DR genotyping available. Mutations present in Protease and Reverse Transcriptase sequences are shown on **Tables 2** and **Table 3**. Sequences from two of these individuals showed resistance to protease inhibitors while the other 4 remained susceptible but presented other mutations in gene, the some of which are considered accessory mutations that enhance resistance once the major DR mutation is selected. One patient has DR to NRTIs but remained susceptible to PIs. A second shows resistance to both NRTIs and NNRTs and also has resistance to PIs. In those patients with lower to intermediate levels of DR to INSTIs in DNA integrase, it was accompanied by susceptibility to PIs and either susceptibility or low resistance to some of the NRTI/NNRTI.

TABLE # 2. Protease Drug Resistance Mutations and Other Mutations

Sample ID	Major PI DRM	Other Protease Mutations													
271102DNA	---	I13IV	I15V	E35D	N37D	D60E	L63P								
273698DNA	M46I, I85V, L90M	I13IV	K20KT	P39PS	R41RK	M46MI	L63LP	I64IV	A71AV	T74TS	I85IV	L90LM	I93IL		
274260DNA	---	E35D	K45KR	R57K	L63P										
278584DNA	M46I, I50V, V82A	L10LF	L33LF	E34EQ	M46MI	I50IV	L63LP	V82VA							
284499DNA	---	T12P	L19IV	162V	L63P	C67D	I72S	V77I							
286656DNA	---	I13IV	K14KR	G16GE	L63S	I64V	E65D	V77I							

TABLE # 3. Reverse Transcriptase Drug Resistance Mutations and Other Mutations

Sample ID	Major NRTI / NNRTI DRM	Other Reverse Transcriptase Mutations																	
271102DNA	M184I	V60I	V90VI	A98S	D123E	I142V	D1773	M184MI	E203EK	R211K	A272P	K277R	P294Q						
273698DNA	---	---					---					---							
274260DNA	---	E6D	K122KE	I202V	R211K	V245M	T286P	1293V											
278584DNA	M41L, D67G, T69D, M184V, L100I, K103N	V35T	M41L	V60I	D67G	S68G	T69D	V90V1	L100LI	K103KN	K122E	A158S	S162A						
	L210W, T215Y, K219E	M184MV	T200A	Q207E	L210LW	R211K	T215Y	K219KE	V245VM	D250DE									
284499DNA	---	K20R	V35I	I135IL	I142IN	I159V	K173Q	R211K											
286656DNA	---	V35VI	K122E	I135IT	I142V	Q207E	L210LS	R211K	F214FL	T215TA	V245K	D250E							

Of the two patients with high resistance to INSTIs, one case was accompanied by resistance to most PI, high resistance to NRTI, however remained susceptible to NNRTIs. In the second case, high resistance to INSTIs was accompanied by intermediate to high resistance to PIs, NRTI and NNRTIs.

CONCLUSIONS

- We found low frequency (3.3%) of INSTIs DRM in the HIV-1 DNA integrase latent reservoir in sequences spanning from 2016 to 2019.
- The identified resistance mutations are associated with providing high to intermediate resistance to INSTIs, and include DRM associated with intermediate resistance to dolutegravir.
- We found intermediate resistance to INSTIs in patients with little or no resistance to PI and NRTI/NNRTI that may be due to random viral mutation or maybe due to transmission of the INSTI resistant virus.
- In this study we found there was lower frequency of DR in the proviral DNA integrase compared to INSTIs drug resistance mutations present in plasma virus.
- The clinical significance and impact of those DRM present in the proviral reservoir remains unclear and underscore the importance of continued genotypic monitoring including the integrase gene as more individuals are treated with INSTIs as part of their first-line ART regimens.

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