Characterization of complete HIV-1 proviral genomes and antiretroviral resistance mutations in HIV+ patients from Rio de Janeiro by next-generation sequencing

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INTRODUCTION

There are approximately 37 million people worldwide living with the human immunodeficiency virus (HIV). HIV-infected individuals are at higher risk for developing cancer, and have worse prognosis compared to HIV-negative patients.

The great success of antiretroviral therapy in the treatment of HIV+ patients has provided them greater longevity and a better quality of life. In the past six years, there was an increase of more than 10 million people taking antiretroviral drugs.

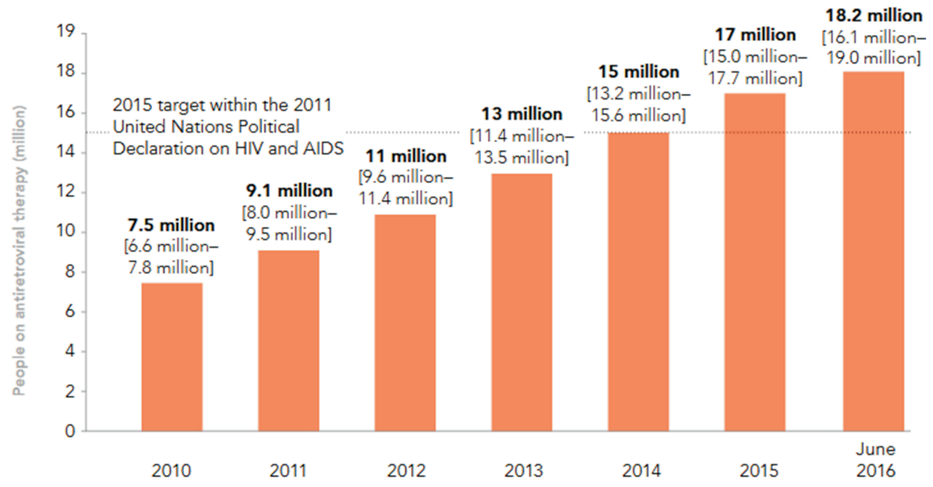

Figure 1: Number of people living with HIV on antiretroviral therapy globally, 2010-2016. Source: Global AIDS Response Progress Reporting (GARPR) 2016; UNAIDS 2016 estimates.

The high genetic HIV variability coupled with the large scale of antiretroviral drugs resulted in the appearance of drug resistance mutations. These mutations allow the repopulation of an individual by drug resistant viruses, and may compromise their future therapeutic options. Thus, it is crucial to identify resistance mutations present in the individual viral population so that therapeutic failures can be predicted before it occurs and thus avoided.

OBJECTIVES

To analyze the genetic composition of HIV integrated into PBMC, to perform virus subtyping and to determine the presence and frequency of antiretroviral drug resistance mutations in a cohort of HIV-infected patients undergoing successful HAART as first-line therapy.

METHODS

Forty HIV-1+ adult individuals from the Hospital Federal de Ipanema (HI) and the Hospital Universitário Clementino Fraga Filho (HUCFF) in Rio de Janeiro were recruited for this study.

Inclusion criteria were age equal or greater than 18 years, being under first-line HAART and with therapeutic success (undetectable viral load) for at least 12 months.

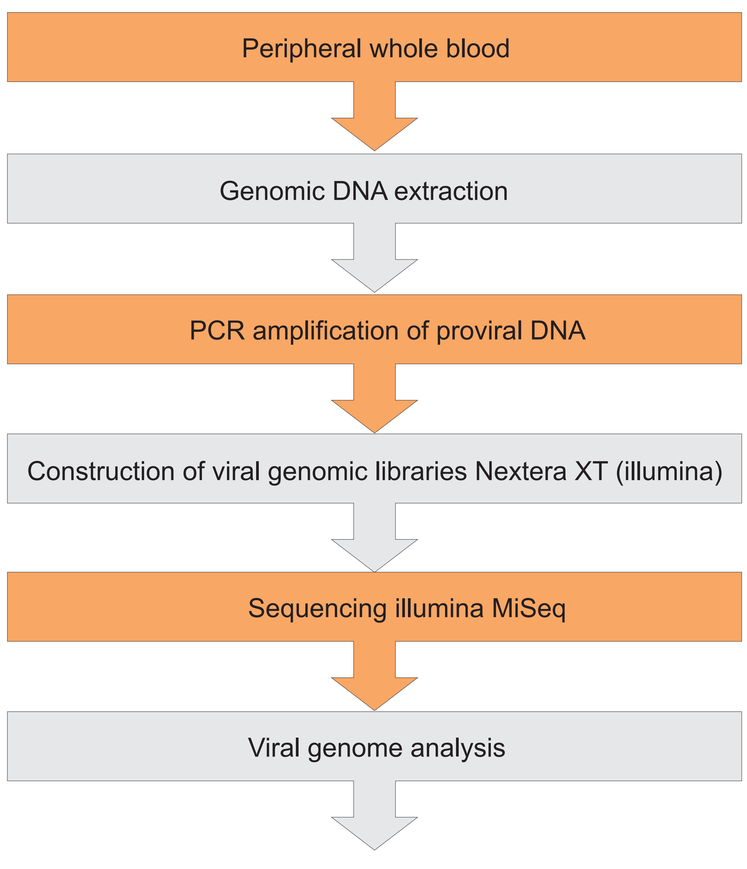

Figure 2. Flowchart of the methodology developed in the study.

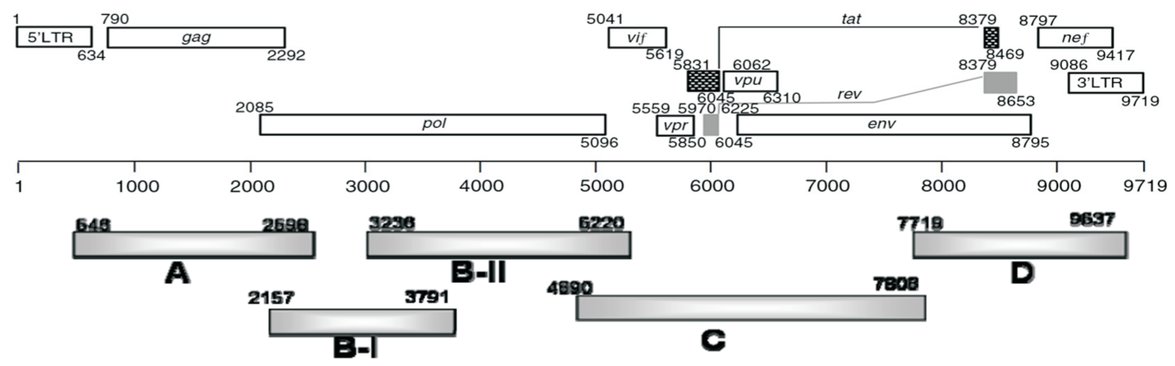

Figura 3. Strategy of amplification of the HIV complete genome (Sanabani et al., 2005).

Reads were mapped with the HXB2 HIV reference and their consensus sequence was extracted using the Geneious R9 program. After consensus extraction, the resistance mutations were evaluated manually. Finally, all the consensuses were aligned with the sequences of the different group M subtypes and this alignment was subjected to phylogenetic inference in MEGA5 for subtype assignment.

RESULTS

Eight samples from HUCFF and 32 from HI were included in the study. Of these 40 samples, 21 have been already sequenced, while the remaining continues to go through the PCR amplification and sequencing steps. Of the 21 samples sequenced, analyses were performed for 14.

According to the phylogenetic analyses most of the samples belonged to the B subtype (n = 10) and four were unique recombinant forms, one URF-BC and three URF-BF.

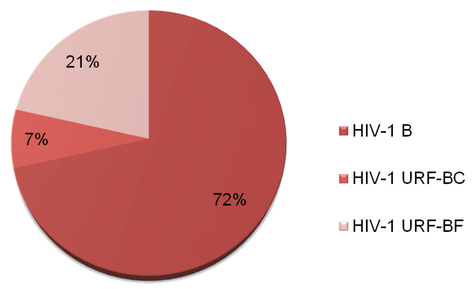

Figure 4: HIV-1 subtype diversity. URF: unique recombinant form.

Five samples showed drug resistance mutations. Four of them (11, 21, 22 and 32) were in the reverse transcriptase gene and one (16) in the protease gene. Mutation of samples 11 and 21 confers resistance to drugs of the nucleoside/nucleotide reverse transcriptase inhibitor class; mutation of samples 22 and 32 confers resistance to drugs of the non-nucleoside/nucleotide reverse transcriptase inhibitors class; and the mutation of sample 16 confers resistance to drugs of protease inhibitors class.

Table 1: Characteristics of the drug resistance mutations found in samples 11, 16, 21, 22 and 32

Sample	Resistance mutation	Resistant drug	Resistance level	Current therapeutic regimen
11	M41L	AZT	Low-level	TDF+3TC+EFV
		D4T	Low-level	
		DDI	Potential low-level	
16	G73S	ATV	Potential low-level	AZT+3TC+FPV+RTV
		FPV	Potential low-level	
		IDV	Low-level	
		NFV	Low-level	
		SQV	Low-level	
21	L210W	ABC	Low-level	EFV+TDF+FTC
	T215S	AZT	Intermediate	
		D4T	Intermediate	
		DDI	Low-level	
		TDF	Low-level	
22	E138K	EFV	Potential low-level	AZT+3TC+EFV+TDF
		ETR	Potential low-level	
		NVP	Potential low-level	
		RPV	Intermediate	
32	V179D	EFV	Potential low-level	TDF+3TC+EFV
		ETR	Potential low-level	
		NVP	Potential low-level	
		RPV	Potential low-level	

Despite the therapeutic success, antiretroviral resistance mutations were found in the patients investigated, which evidences the need for careful management when there are changes in their therapeutic regimen.

Projeto Gráfico: Serviço de Edição e Informação Técnico-Científica / INCA

SUPPORT: