Characterization of near full-length HIV-1 proviral genomes and antiretroviral resistance mutations by next-generation sequencing in HIV+ patients from Rio Grande do Sul

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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.

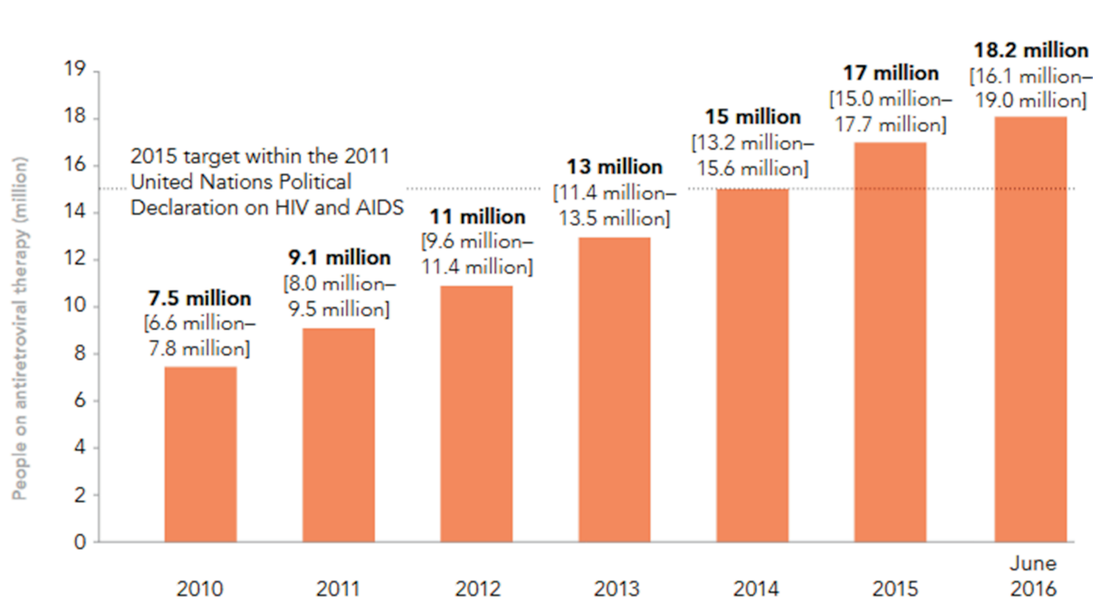

Figure 1: Number of people living with HIV on antiretroviral therapy globally, 2010-2016. Source: Global AIDS Response Progress Reporting (GARPR) 2016; UNAIDS 2016 estimates

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.

OBJECTIVE

To analyze the genetic composition of HIV integrated into the genome of cells, 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 Universitário Doutor Miguel Riet Corrêa Junior (FURG) in Rio Grande 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.

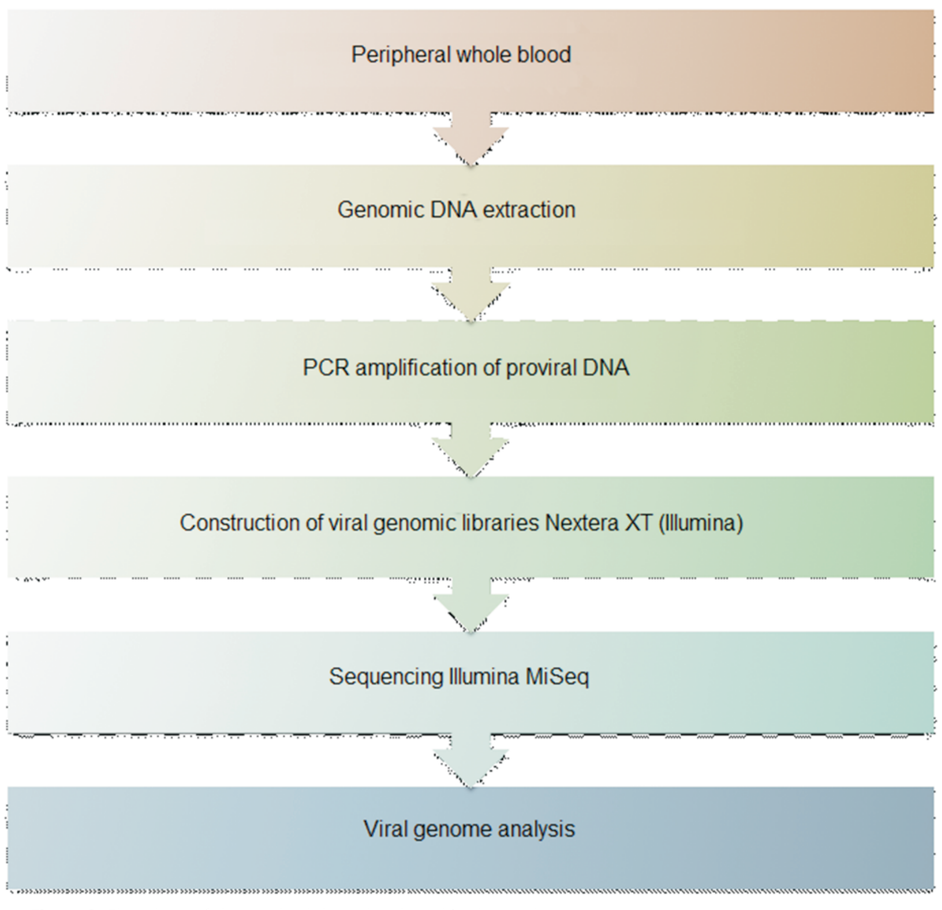

Figure 2. Flowchart of the methodology developed in the study.

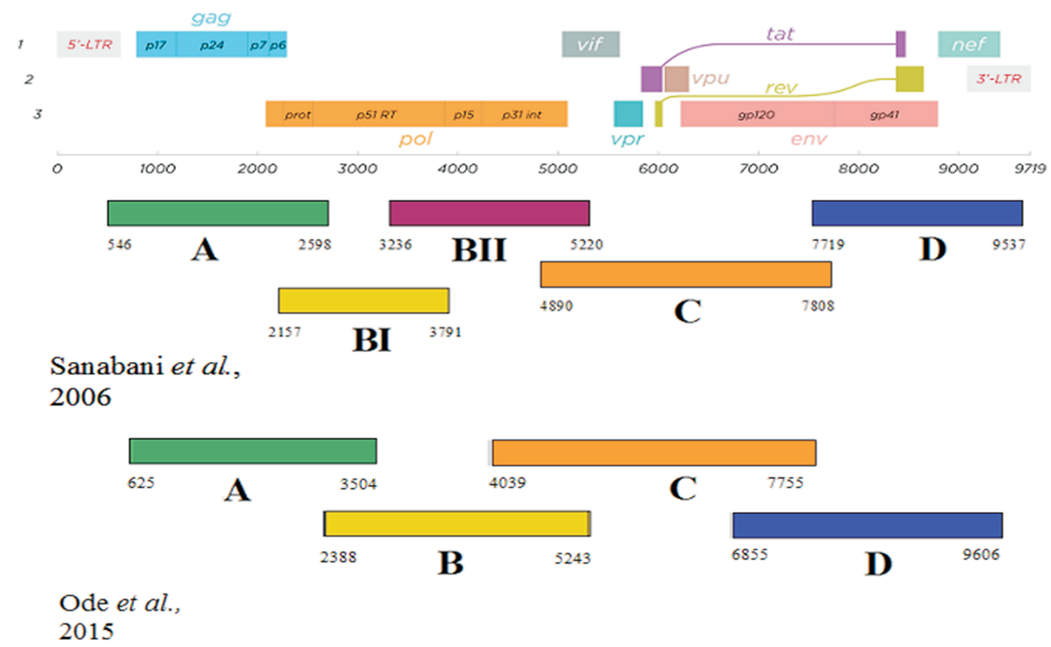

Figura 3. Strategy of amplification of the HIV near full-length genome.

Reads were mapped with the HXB2 HIV reference and the 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

Of the 40 samples collected, 14 were sequenced, while the remaining continues to go through the PCR amplification and sequencing steps. Of the 14 samples sequenced, analyses were performed for nine and of the nine samples analysed, five were near full-length genome.

According to the phylogenetic analyses most of the samples belonged to the C subtype (n = 6) and three were recombinant forms, CRF31_BC, URF-BC and URF-BF1

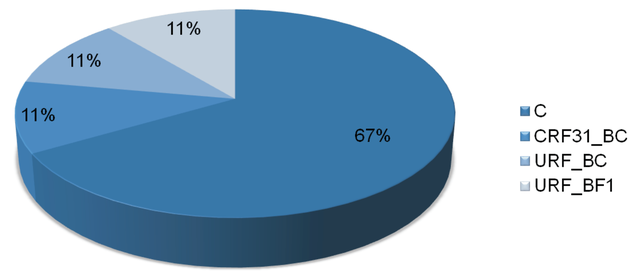

Figure 4: HIV-1 subtype diversity. URF: unique recombinant form; CRF: circulating recombinant form.

Six samples showed drug resistance mutations and all of them were in the reverse transcriptase gene. Mutations of samples 1S, 3S, 6S, 12S and 14S confers resistance to drugs of the nucleoside reverse transcriptase inhibitor class and mutations of samples 1S, 6S, 7S, 12S and 14S confers resistance to drugs of the non-nucleoside reverse transcriptase inhibitors class.

Table 1: Characteristics of the drug resistance mutations found in samples.

Sample	Resistance mutation	Resistant drug	Resistance level	Current therapeutic regimen
1S	E138G	3TC	Susceptible	TDF+3TC+EFV
		TDF	Susceptible	
		EFV	Potential low-level	
3S	K65R	AZT	Susceptible	Biovir(3TC+AZT)+EFV
		3TC	Intermediate	
		EFV	Susceptible	
6S	K103N	AZT	Susceptible	TDF+3TC+AZT+RTV
		3TC	Susceptible	
		TDF	Susceptible	
7S	K65R	3TC	Intermediate	TDF+3TC+DRV+RTV
	K70R	TDF	High-level	
	E138G			
12S	K103N	3TC	Susceptible	TDF+3TC+EFV
		TDF	Susceptible	
		EFV	High-level	
14S	K219E	AZT	Potential low-level	Biovir(3TC+AZT)+AZT+RTV
		3TC	Susceptible	

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: Setor de Edição e Informação Técnico-Científica / INCA

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