Design of a Genotyping Assay to Identify and Monitor HIV-2 Drug Resistance

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Introduction

Human immunodeficiency virus type 2 (HIV-2) was characterized in the US in 1987 as a causative agent of acquired immunodeficiency syndrome (AIDS).[1,2] It is distinct from the more prevalent HIV-1 and a closer relative of simian immunodeficiency virus (SIV). HIV-2 viruses can be classified into groups based on genome sequence similarity. Only two groups, A and B, are epidemiologically significant. Antiretroviral drugs that act against specific viral proteins have been developed to treat HIV-1. HIV-2 can be treated with some antiretrovirals, specifically, certain protease inhibitors (PI) and nucleoside and nucleotide reverse transcriptase inhibitors (NRTIs). These drugs act on the protease (PR) and reverse transcriptase (RT), respectively. HIV-2 is intrinsically resistant to others, specifically, some PIs and all non-nucleoside reverse transcriptase inhibitors (NNRTI). HIV-1 and HIV-2 can rapidly acquire mutations which confer drug resistance. Genotyping assays for HIV-1 are used routinely to identify drug resistance mutations and guide treatment. There are no HIV-2 genotyping assays approved for clinical use in the US.

Objectives

Our overall objective is to develop and validate an HIV-2 drug resistance genotyping assay for clinical use.

Specific objectives of this project:

- To develop a set of *pol* region amplification and sequencing primers
- To collect HIV-2 drug resistance mutation information and organize by genomic region with evidence for resistance

Methods

HIV-2 information was gathered from literature and public databases. Amplification and sequencing primers were aligned for compatibility with reference sequences from HIV-2 group A and B using sequence analysis software and tools available from the Los Alamos National Laboratory (LANL) HIV databases (http://www.hiv.lanl.gov).

Table 1. Reference Sequences for HIV-2 and Closely Related Viruses

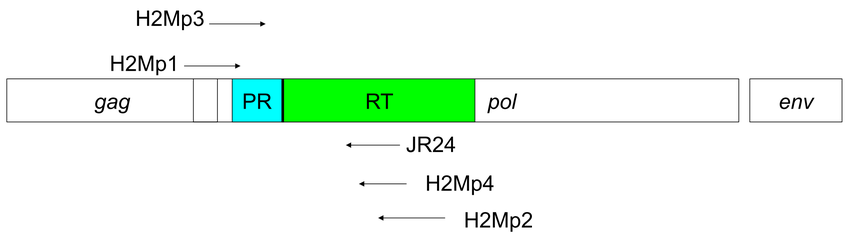
Virus:	Name:	Genbank Accession Number:
SIV	MAC 239	M33262
HIV-1 Group M	HXB2	K03455
HIV-2 Group A	ROD	M15390
HIV-2 Group B	EHO	U27200

Full gene maps for MAC239 and HXB2 were available from LANL HIV Databases. (http://www.hiv.lanl.gov/content/sequence/HIV/MAP/landmark.html)

Using these as models, we created *pol* region gene maps for HIV-2 group A (ROD) and group B (EHO) reference sequences.

Results

Figure 1. HIV-2 Genome and Relative Primer Locations



After collecting primer information from the literature, new primers were developed from H2Mp1[3], H2Mp2[3], H2Mp3[3], H2Mp4[3] and JR24[4] that successfully amplified and sequenced the *pol* region of HIV-2 group A viruses. Details related to primer development, amplification and sequencing will be described elsewhere.

Figure 2. Portion of Annotated *pol* Region HIV-2 Gene Map

Position in ROD	ROD DNA Sequence	Amino Acid ORF1	Codon	HIV-1 DR Mutation	Position in EHO	EHO DNA Sequence	Amino Acid ORF1	Codon
2291	a	-	-		2816	a	-	-
2292	a	K	70	K70E	2817	g	R	70
2293	g	-	-		2818	a	-	-
2294	g	-	-		2819	g	-	-
2295	t	V	71	A71V/I/L/T	2820	t	V	71
2296	a	-	-		2821	a	-	-
2297	c	-	-		2822	a	-	-
2298	g	R	72		2823	g	R	72
2299	g	-	-		2824	a	-	-
2300	g	-	-		2825	g	-	-
2301	c	A	73		2826	c	A	73
2302	c	-	-		2827	a	-	-
2303	a	-	-		2828	a	-	-
2304	c	T	74	T74S	2829	c	T	74
2305	c	-	-		2830	a	-	-
2306	a	-	-		2831	g	-	-
2307	t	I	75		2832	t	V	75
2308	a	-	-		2833	a	-	-
2309	a	-	-		2834	a	-	-
2310	t	M	76	L76V	2835	t	M	76
2311	g	-	-		2836	g	-	-
2312	a	-	-		2837	a	-	-
2313	c	T	77		2838	c	T	77
2314	a	-	-		2839	a	-	-
2315	g	-	-		2840	g	-	-
2316	g	G	78		2841	g	G	78
2317	c	-	-		2842	g	-	-
2318	g	-	-		2843	g	-	-
2319	a	D	79		2844	a	D	79
2320	c	-	-		2845	c	-	-
2321	a	-	-		2846	a	-	-
2322	c	T	80		2847	c	T	80
2323	c	-	-		2848	c	-	-
2324	c	-	-		2849	c	-	-
2325	c	P	81		2850	c	P	81
2326	a	-	-		2851	a	-	-

This figure shows a section of the annotated *pol* region gene map created using HIV-2 group A and B reference sequences to be used for sequence analysis and comparison with closely related viruses. The gene map has been annotated at positions known to confer drug resistance in HIV-1 including those positions which confer native resistance to NNRTIs. The full gene map includes codons 1-99 of the protease and codons 1-255 of the reverse transcriptase (1062 nucleotides).

Figure 3. Selected Mutations Associated or Possibly Associated with Drug Resistance

PR		RT	
HIV-1	HIV-2	HIV-1	HIV-2
L90M	L90M (Gottlieb 2009, Ruelle 2008, Charpentier 2011, Treviño, 2011, Adjé-Touré, 2003, Colson 2004)	Q151M	Q151M (Smith 2009, Gottlieb 2009, Ruelle 2008, Chapentier 2011, Boyer 2012, Treviño 2011, Jallow 2007, Brandin 2003)
			Q151M/L (Rodes 20000)

This figure represents the drug resistance associated mutation information for HIV-1 vs HIV-2. Where HIV-1 and HIV-2 mutations differed, HIV-2 mutations were included with first author and year of the publication describing them. HIV-2 resistance associated mutations were classified at a total of 42 positions in the protease and reverse transcriptase, not including the 21 positions in these regions known to confer native drug resistance, where polymorphisms occurred.

Figure 4. Selected Drug Resistance Information for HIV-2

Author, Year	Region of Genome	Resistance Mutation	Drug	Drug Class	Evidence of Resistance	Wild Type Reference
Ruelle, 2008	PR	V71I	NFV	PI	Sample from patient on multidrug therapy	HIV2ROD
	RT	Q151M	3TC	NRTI	Sample from patient on multidrug therapy	HIV2ROD

All published drug resistance information for HIV-2 was organized into a single spreadsheet. This included relevant information pertaining to mutation, region of the genome, drug to which resistance was conferred, class of drug, evidence for resistance and wild type reference used in the paper.

Conclusions

- We have made significant progress toward the development and clinical validation of an HIV-2 drug resistance genotyping assay.
- Our future goals are to optimize the assay, validate it for clinical use and create a rules-based drug resistance interpretation tool.

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