

Genotypic and Phenotypic Analyses of Drug Susceptibility in HIV-1 Isolates from Drug-Naïve Patients in Nigeria

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Background

- One of the major concerns in the AIDS pandemic is the extensive genetic variability of HIV-1. This affects the development of candidate vaccines and may impact antiretroviral therapy. Most genotypic and phenotypic analyses of resistance in HIV-1 have been done using only subtype B viruses. Comparatively little is known about antiretroviral drug resistance for non-B subtypes. Results from drug susceptibility testing, as well as pol-subtype designations for treatment-naïve Nigerian patients will provide much needed information on the impact of HAART regimens on the HIV pandemic.

Methods

- Envelope subtypes were determined for each of the 18 samples using a modified gp41-based heteroduplex mobility assay earlier described by us. Patient-derived sequences from a 1.2 KB fragment in the pol gene (spanning the entire protease (PR) region and most of the reverse transcriptase (RT) region) were isolated and cloned into retroviral expression vectors. The sensitivity of the resultant PR and RT proteins to all available antiretroviral drugs was assayed using a version of Virologic's PhenoSense HIV assay modified for assaying non-B subtypes. Nucleotide sequences for these patient pool were also obtained. Amino acid codons at positions associated with decreased susceptibility to drug therapy were characterized, and nucleotide data from the 1.2 KB region of pol used in phylogenetic analysis.

Results

- Analysis of HIV-1 PR and RT positions associated with mutations conferring resistance to antiretroviral drugs revealed no known primary resistant-associated mutations. For example, compared with the NL4_3 consensus sequence, we found between 9 and 17 amino acid substitutions within the protease region, all of which would be classified as secondary mutations or polymorphisms. The phenotypic profiles of the viruses correlate well with the observed genotypes. All but one isolate were susceptible to all of the 15 drugs analyzed. This isolate exhibited low-level reductions in susceptibility to nelfinavir (3. 8-fold) and retonavir (3. 4-fold) relative to the drug-sensitive control. Such reduced susceptibility to these two drugs has also been observed in subtype B viruses in the absence of primary mutations. Relative to our NL4_3-based reference, this isolate exhibited the following PR genotype: L10I, I13V, K14R, I15I/V, K20I, E35Q, M36I, N37D, R41K, R57K, L63L/P, I64I/M, C67E, H69K, T74T/S, V82I, AND L89M. From the gp41-based heteroduplex mobility assay, 17 of the 18 patients were infected with subtype G, and one patient was infected with subtype A. From phylogenetic analyses of 1212 nucleotides of pol, 13 of the 17 isolates were strongly grouped with other subtype G reference strains throughout PR and RT regions. One of these was more distinct from the rest, although it is more closely related to subtype G strains than to any other subtypes. Of the remaining four samples, one most closely resembled CRF_02_AG, while the 3 others (including the one sample with a subtype A env designation) were sister taxa to the CRF_02_AG clade. These three isolates are recombinants with varying degrees of G and A subtype fragments within PR and RT.

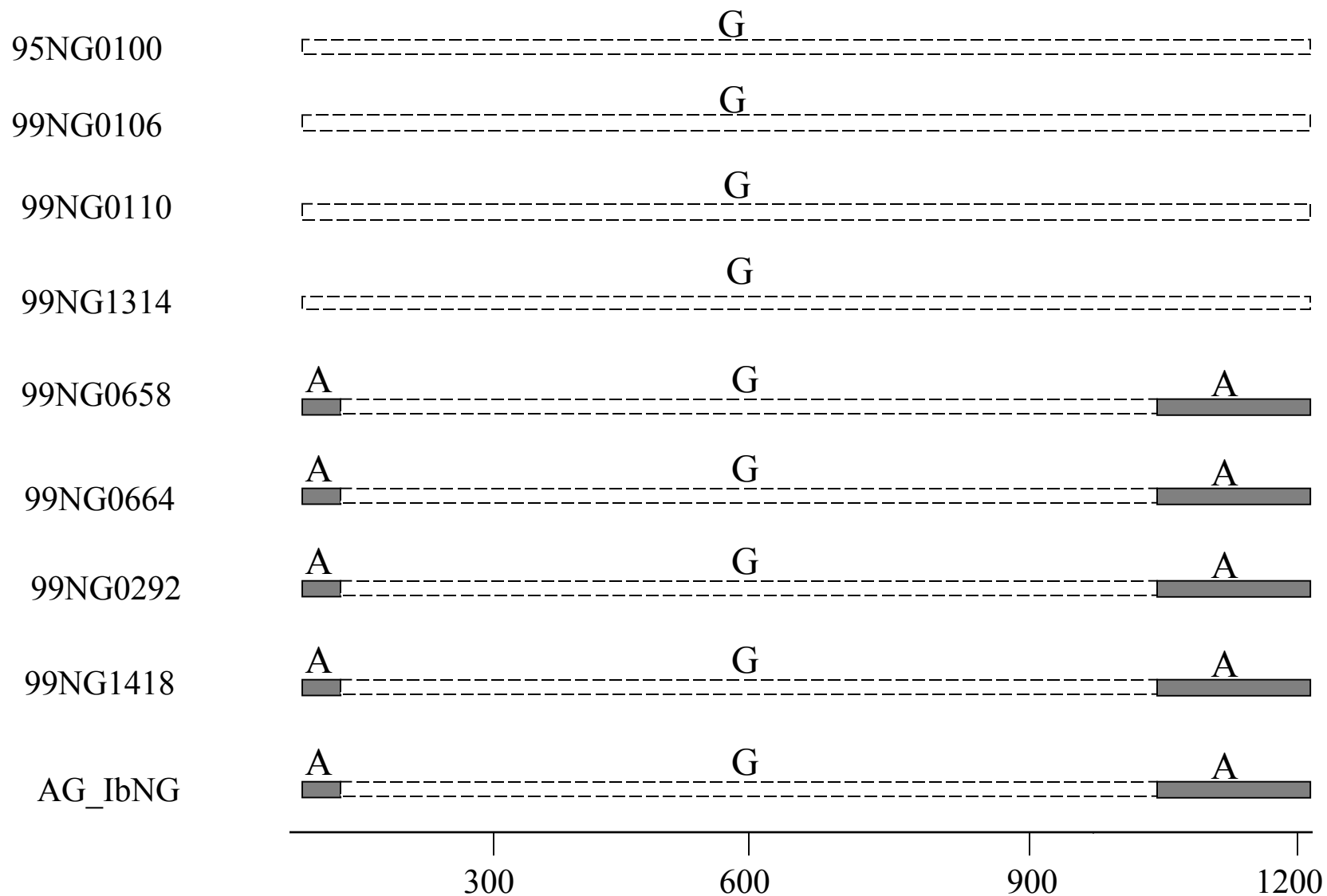


Fig. 2. Schematic diagram of eight Nigerian (prefix 99NG) protease-RT sequences depicting subtype designations resulting from bootscanning (Window:500, Step:10, GapStrip:On, Reps:100, Max Likelihood, T/t:2.0, Neighbor) . One Nigerian protease-RT reference strains from GenBank was included for comparisons AG_IbNG (the prototype CRF02_AG). Dashed lines indicate regions of subtype G, gray boxes = subtype A. Protease and partial RT gene region sequences span approximately 1-1200 nucleotides. Eighteen sequences were analyzed totally, the ten not shown are pure subtype G's as those above.

Table 1. Drug Susceptibility of Viruses from drug-naïve Nigerian Patients

Specimen Identifier	State/City/ or Ruyal	Protease-RT Clade	VL ID	gp41	NRTI							NNRTI			PI				
					ABC	ADV	ddi	3TC	d4T	ddc	ZDV	DLV	EFV	NVP	AMP	IDV	NFV	RTV	SQV
99NG0100	Enugu	G	3-022877	G	0.7	0.8	0.8	0.7	0.8	0.7	0.6	0.4	0.5	0.3	0.4	0.4	0.6	0.7	0.6
99NG0106	Kaduna	G	3-022878	G	0.6	0.7	0.6	0.7	0.6	0.6	0.4	0.9	0.3	0.2	0.7	0.4	1	1.2	0.4
99NG0110	Kaduna	G	3-022879	G	0.6	0.9	0.8	0.9	0.8	0.8	0.8	0.7	0.6	0.6	0.5	0.4	0.7	0.7	0.6
99NG0134	Niger	G	3-022880	G	1.1	0.8	0.9	0.9	1	0.9	0.8	0.5	0.8	0.4	0.5	0.3	0.7	0.8	0.7
99NG0202	Ondo	G	3-022881	G	0.8	0.7	1	1.1	1	1	0.6	0.5	0.4	0.4	2	1.3	3.8	3.4	1.3
99NG0280	Kaduna	G	3-022882	G	1.2	0.9	1	1	1	0.9	1	0.4	0.4	0.5	0.7	0.5	0.9	0.9	0.6
99NG0282	Kaduna	G	3-022883	G	0.7	0.8	0.9	1	0.8	0.9	0.7	0.4	0.5	0.4	0.4	0.4	0.7	0.9	0.7
99NG0292	Osun	AG	3-018910	G	0.6	0.8	0.9	0.7	1	0.9	1.1	0.6	0.5	0.3	0.3	0.4	0.5	0.4	0.6
99NG1418	Oyo	AG	3-018899	A	0.8	0.8	1.1	1	0.7	0.7	0.7	0.2	0.4	0.1	0.2	0.4	0.4	0.3	0.2
99NG0634	Kano	G	01-100083	G	0.9	1.0	1.1	1.0	0.9	0.9	0.6	0.7	0.7	1.6	0.9	1.3	2.4	2.5	1.1
99NG0646	Kano	G	01-100084	G	0.6	0.8	1.0	0.8	0.8	1.0	0.3	0.4	0.2	1.4	0.8	1.1	1.8	1.4	1.0
99NG0658	Kano	AG	01-100085	G	0.9	0.9	1.0	0.8	0.9	1.0	1.0	1.1	1.3	0.4	0.4	0.5	0.4	0.4	0.5
99NG0660	Kano	G	01-100086	G	0.9	1.0	1.2	1.1	1.0	1.3	1.1	0.8	1.2	0.9	0.6	0.9	1.4	1.5	0.9
99NG0664	Kano	AG	01-100087	G	0.7	1.0	1.1	0.9	0.9	1.2	0.4	0.4	0.2	0.6	0.6	0.6	0.8	0.6	0.5
99NG0742	Adamawa	G	01-100088	G	0.8	0.8	0.8	0.9	0.7	1.0	0.5	0.5	0.6	0.5	0.4	0.6	1.1	1.2	0.5
99NG0748	Adamawa	G	01-100089	G	0.7	0.8	0.9	0.7	0.8	1.0	0.5	0.6	0.6	0.7	0.4	0.5	0.8	1.0	0.6
99NG1310	Yobe	G	01-100090	G	1.1	1.0	1.3	1.1	0.9	1.1	0.7	0.7	0.8	1.4	0.6	0.9	1.3	1.3	0.8
99NG1314	Borno	G	01-100091	G	0.9	1.0	1.2	0.9	1.0	1.2	0.6	0.6	0.4	1.0	0.5	0.7	0.8	1.3	0.7

Conclusions

- Based on our analyses, subtype G and CRF02_AG were susceptible to all classes of antiretroviral drugs and should respond to HAART regimens. Analysis of a larger sample set is required to determine whether non-B clades may exhibit a wide range of baseline susceptibilities than do B clade. The significance of the secondary mutations reported here can only be determined in a well monitored human trials in Nigeria where wide use of ART is ongoing.