prevalence in HCM in 2003[6] and consistent with the threshold survey in Hanoi in 2006.[7] Further monitoring is necessary to establish a useful database of ARV-resistant HIV-1 in Vietnam. There is also a need for a consensus algorithm, based on what is known about drug-resistance mutations in subtype B strains, which can be used to predict the clinical outcomes of people who are infected with non-subtype B HIV-1 strains.[39]

Sequence Data

The GenBank accession numbers of the sequences reported in this study are as follows: from FJ006949 to FJ007345 for *pol-PR/RT*, FJ007346 to FJ007369 for *pol-PR*, and FJ007370 for *pol-RT*.

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Disclosure Statement

No competing financial interests exist.

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Changes in the HIV Type 1 Envelope Gene from Non-Subtype B HIV Type 1-Infected Children in Kenya

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Abstract

A switch of coreceptor usage from CCR5 to CXCR4 occurs in about half of HIV-1-infected individuals in the natural course of infection. To investigate whether antiretroviral therapy (ART) enhances the coreceptor switch of HIV-1, we genotypically analyzed the env-V3 amino acid sequences from 81 HIV-1-infected children in Kenya whose plasma samples were obtained between 2000 and 2007. Of 41 children on ART, 35 had HIV-1 using CCR5 as a coreceptor at baseline. In 7 (20%) of them HIV-1 switched the coreceptor usage during the follow-up period. The mean duration of ART to the time of coreceptor switch was 2.6 years (range: 0.5–5.2). Of the remaining 40 children without ART, 32 had HIV-1 using CCR5 as a coreceptor at baseline and in 3 (9.4%) HIV-1 switched the coreceptor usage. The mean age of the children with HIV-1 coreceptor switch with and without ART was 7.3 and 9.7 years, respectively. The difference in the rate and age of coreceptor switch between treated and untreated children was not significant ($p = 0.38$ and 0.31, respectively). Of the HIV-1-infected children, 10 started ART by the age of 5 years (rapid progressors) and 23 did not need ART by the age of 10 years (slow progressors). The rate of coreceptor switch was strongly higher in rapid progressors (40%) than slow progressors (8.7%) ($p = 0.053$). These results suggest that switching of coreceptor usage from CCR5 to CXCR4 among HIV-1-infected children is not influenced by ART, but by factors responsible for rapid disease progression.

Introduction

Human immunodeficiency virus type 1 (HIV-1) coreceptor usage plays a critical role in the virus tropism. HIV-1 infection requires interactions between the viral envelope (env) glycoprotein (gp120) and cellular receptors, CD4 as a major receptor and CCR5 or CXCR4 as a coreceptor.[1] Based on the coreceptor usage, HIV-1 variants are classified as CCR5-tropic (R5 variants), CXCR4-tropic (X4 variants), and dual tropic (R5/X4 variants).[2] R5 variants are responsible for the establishment of HIV-1 infection and predominate in the early stage of HIV-1 infection.[3–5] X4 variants emerge later as disease develops.[6–9] A switch in HIV-1 coreceptor usage from CCR5 to CXCR4, which correlates with the subsequent accelerated decrease in $CD4^+$ T cell count and disease progression, occurs in the late stage of HIV-1 infection in about half of HIV-infected individuals.[10–14]

It has been reported that in HIV-1 subtype C-vertically infected children, R5 variants are predominantly transmitted and the virus may evolve to use CXCR4 as a coreceptor in older children,[15] and that X4 variants emerge later as disease develops in HIV-1 subtype B-infected children.[6–9] However, the late appearance of X4 variants with relation to disease progression is less clear in children than in adults.[16] Especially in HIV-1-infected infants disease progression was not necessarily associated with the switch in HIV-1 coreceptor usage.[17,18] In one study the coreceptor switch of HIV-1 was found in two of six rapid-progressor infants after vertical infection.[19] In another study, 14 of 15 infants with rapid disease progression harbored viruses that used CCR5 as a coreceptor, and only the remaining one had a virus that used both CCR5 and CXCR4.

In developed countries the clinical profile of HIV-1 infection in children shows a bimodal distribution, with approximately one-quarter developing severe symptoms and dying within the first 24–36 months of life (rapid progressors). Most children, however, develop AIDS more slowly, with some surviving beyond 5 years (slow progressors).[20] A similar bimodal disease pattern is seen among children living in

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developing countries,[21] although considerably less is known about the underlying virological and immunological factors, which may differ from those in developed countries.

Recently several longitudinal studies regarding the effect of highly active antiretroviral therapy (HAART) in the dynamics of evolution of HIV tropism in patients under HAART were carried out and reported a higher prevalence of X4 variants in those HIV-1-infected individuals exposed to HAART than in drug-naive individuals.[22–29] In addition, effective HAART was reported to enhance CCR5 to CXCR4 coreceptor switch.[30] However, the dynamics of viral tropism during the course of HIV-1 infection in persons exposed to antiretroviral therapy (ART) still remain unclear. Moreover, there are indeed limited reports regarding the effect of ART on HIV-1 coreceptor usage among children.

The aim of this study was to investigate the dynamics of coreceptor usage and whether ART enhanced the coreceptor switch among non-B subtype HIV-1-vertically infected children in Kenya.

Materials and Methods

Study population

As of August 2007, 95 HIV-1-infected children resided in a children's home in Nairobi. These children were born to HIV-1-infected mothers who either died of, or were too debilitated by HIV/AIDS and could not offer basic care to the children. All children were admitted into the home by their first birthday, where their HIV-1 status was confirmed serologically at 18 months of age. None of these children had a history of previous exposure to ARVs by the time of admission. Of the 95 children 81 were followed up at least three times during the period between 2000 and 2007, and blood samples were obtained from them every 6 months since the year 2000. Of the 81 children, 41 received ART consisting of two nucleoside reverse transcriptase inhibitors (NRTIs) and one nonnucleoside reverse transcriptase inhibitor (NNRTI). The mean duration of ART varied among those children was 7.6 years with range of 1 to 15 years. The remaining 40 did not receive ART during the follow-up period.

This study was approved by the Kenya Medical Research Institute's National Ethical Review Committee on behalf of the Kenyan Government and conducted according to the national and international regulations governing the use of human subjects in biomedical research. The study was conducted within the continuing antiretroviral, medical, and healthcare programs of the institution without additional demand for blood samples solely for research purposes.

$CD4^+$ T cell counts and plasma viral loads

$CD4^+$ T cell counts of peripheral blood were determined using the FACSCOUNT (Becton-Dickinson, Beiersdorf, Germany). Plasma HIV-1 RNA loads were determined by the Amplicor HIV-1 Monitor kit version 1.5 (Roche Diagnostics, Alameda, CA) using the standard procedure (with detection limit of 400 copies/ml) according to the manufacturer's instructions.

Extraction and amplification of plasma HIV-1 viral RNA

HIV-1 RNA was extracted from 100 μl of plasma using SMITEST EX-R & D (Medical & Biological Co. Ltd., Fukushima, Japan) according to the manufacturer's instructions. A part of the HIV-1 group M *env* gene covering the C2V3 region (corresponding to 6975–7520 nt in HIV-1 HXB2) was amplified by both one-step RT-PCR and nested polymerase chain reaction (PCR) with primers M5 (5′-CCAATTCCCATAC ATTATTGTGCCCCAGCTGG-3′ and M10 (5′-CCAATTGT CCCTCATATCTCCTCCTCCAGG-3′) in the first round and M3 (5′-GTCAGCACAGTACAATGCACACATGG-3′) and M8 (5′-TCCTTGGATGGGAGGGGCATACATTGC-3′) in the second round,[31] according to the manufacturer's instructions. Amplification was done with one cycle of 95°C for 10 min and 35 cycles of 95°C for 30s, 55°C for 30 s, and 72°C for 1 min with a final extension of 72°C for 10 min. PCR amplification was confirmed by visualization with ethidium bromide staining of the gel.

Sequencing, cloning, and subtyping of the env-C2V3 region

The amplified PCR products were cloned using the TOPO TA Cloning kit (Invitrogen, Carlsbad, CA) and sequenced as described previously,[31] to take into account both the majority and minority virus populations.

The sample nucleotide sequences were aligned with HIV-1 subtype/circulating recombinant form (CRF) reference sequences from the Los Alamos database using CLUSTAL W (version 1.83), with minor manual adjustments. A phylogenetic tree was constructed by the neighbor-joining method, and its reliability was estimated by 1000 bootstrap replications. The profile of the tree was visualized with TreeViewPPC1.6.5.[31] To improve the accuracy of HIV-1 subtyping, we used the National Center for Biotechnology Information (NCBI) genotyping tool (http://www.ncbi.nih.gov/projects/genotyping/formpage.cgi) and the REGA subtyping tool (http://dbpartners.stanford.edu/RegaSubtyping/), as needed.

Determination of the predicted coreceptor usage of HIV-1

The predicted coreceptor usage of HIV-1 was determined based on the *env*-V3 loop amino acid sequences. R5 and X4 variants were identified according to (i) the 11/25 amino acid rule [uncharged residues at position 11 of V3 (mostly serine/glycine), negatively charged residues at position 25 (mostly glutamic (E)/aspartic (D) acid), and a net charge of the V3 loop less than +5 have been reported to predict CCR5 chemokine receptor usage. Conversely, positively charged residues at position 11 or 25 (mostly arginine (R)/lysine (K)) and a net charge of the V3 loop equal and more than ±5 have been reported to predict CXCR4 chemokine receptor usage], and (ii) the net charge of the V3 region, which was calculated by subtracting the number of acidic amino acids (aspartate and glutamate) from the number of basic amino acids [lysine, histidine (H), and arginine]. A net charge of equal and less than +5 in the V3 region has been shown to predict CCR5 chemokine receptor usage (R5 variants), whereas a net charge of more than +5 has been shown to predict CXCR4-chemokine receptor usage (X4 variants).[32–36] In our study, a net charge of +5 was considered to predict an R5 phenotype, unless this was accompanied by the appearance of either an arginine or lysine amino acid residue at positions 11 or 25.

Determination of the rate of disease progression

The children were categorized into three groups, rapid progressors, slow progressors, and the others, based on the rate of disease progression. *Slow progressors* were the children over 10 years of age when they received ART, and were classified in stage N1 or A1 according to the Centers for Disease Control and Prevention (CDC) classification for children. Children over 10 years of age who did not need ART were also included in this category. *Rapid progressors* were the children who received ART within the first 5 years of their lives either because they had an onset of severe clinical manifestations (CDC category C) and/or profound immune suppression (CDC category 3).

Results

Predicted HIV-1 coreceptor usage

Of the 41 children on ART, 35 had HIV-1 that used CCR5 as a coreceptor at baseline (the first time point at which sample analysis was done). The mean age, viral load,and $CD4^+$ T cell count of these 35 children at baseline were 5.5 years old (range: 1–12), 5.2 $\log_{10}$ copies/ml (range: 3.9–6.1), and 537 cells/μl (range: 93–1760), respectively. In 7 (20%) of them the virus switched coreceptor usage from CCR5 to CXCR4, and in 28 the virus used CCR5 as a coreceptor during the follow-up period. The duration from the start of ART to the time of HIV-1 coreceptor switch varied considerably (mean: 2.6 years, range: 0.5–5.2 years) (Table 1). The remaining six children on ART had HIV-1 that used CXCR4 as a coreceptor from baseline to the end of the study.

Of the 40 children without ART, 32 had HIV-1 that used CCR5 as a coreceptor at baseline. The mean age, viral load, and $CD4^+$ T cell count of these 32 children at baseline were 8.0 years old (range: 3–19), 4.8 $\log_{10}$ copies/ml (range: 2.3–6.0), and 684 cells/μl (range: 70–1335), respectively. In three (9.4%) of them the virus switched the coreceptor usage, and in 29 the virus used CCR5 as a coreceptor during the follow-up period. The remaining eight children without ART had HIV-1 that used CXCR4 as a coreceptor from baseline to the end of the study. Although more of the treated children had HIV-1 that switched coreceptor usage from CCR5 to CXCR4, the difference in the rate of the coreceptor switch between treated (7/35, 20%) and untreated (3/32, 9.4%) children ($p = 0.38$) was not statistically significant (Table 1).

The seven children whose viruses switched their coreceptor usage started ART at younger ages than the 28 children whose viruses used CCR5 as a coreceptor from baseline to the end of the study (mean 5.3 and 7.6 years, respectively). The mean age of the children with HIV-1 coreceptor switch with and without ART was 7.3 and 9.7 years, respectively. The difference in the age of the coreceptor switch between treated and untreated children was not significant ($p = 0.31$).

Chronological changes of the env-V3 amino acid sequences from the serial study points for the 10 children whose HIV-1 showed the coreceptor switch with and without ART are shown in Fig. 1. No significant association was observed between the changes in coreceptor usage and plasma viral load in the children.

HIV-1 coreceptor switch with different rates of disease progression

Of the HIV-1-infected children who had the virus that used CCR5 as a coreceptor at recruitment, 10 started ART by the age of 5 years (rapid progressors) and 23 did not need ART by the age of 10 years (slow progressors). The rate of coreceptor usage was strongly higher in rapid progressors (4/10, 40%) than slow progressors (2/23, 8.7%), though the association was not statistically significant ($p = 0.053$) (Table 2).

HIV-1 subtypes

Phylogenetic analysis based on the *env*-C2V3 region revealed that all the 81 children were infected with non-B subtype HIV-1: subtypes A1 ($n = 65$), A2 ($n = 4$), D ($n = 9$), C ($n = 2$), and CRF_02AG ($n = 1$) (data not shown). No significant relationship between HIV-1 subtype/CRF and

TABLE 1. CHARACTERISTICS OF THE STUDY CHILDREN AT BASELINE, ART START, AND CORECEPTOR SWITCH

		Baseline mean (range)			*ART start mean (range)*			*Coreceptor switch mean (range)*			
	Coreceptor usage	*Viral load*[a]	*CD4*[+b]	*Age*[c]	*Viral load*[a]	*CD4*[+b]	*Age*[c]	*Viral load*[a]	*CD4*[+b]	*Age*[c]	*Duration of ART (years)*
ART	R5▶R5 ($n = 28$)	5.3 (3.9–6.1)	472 (6–1566)	5.7 (1–12)	5.3 (3.9–6.1)	447 (93–1340)	6.6 (1–12)				
	R5▶X4 ($n = 7$)[d]	4.8 (3.6–5.4)	479 (178–1760)	5.0 (1–8)	4.7 (3.6–5.4)	357 (147–1442)	4.7 (2.5–7)	4.8 (4.2–5.4)	677 (157–1439)	7.3 (3–12)	2.6 (0.5.–5.2)
	X4▶X4 ($n = 6$)[e]	3.5 (2.3–5.0)	590 (17–1620)	11.2 (7–18)							
No ART	R5▶R5 ($n = 29$)	4.8 (2.3–6.0)	697 (70–1637)	8.1 (2–19)							
	R5▶X4 ($n = 3$)	4.4 (3.3–5.2)	573 (338–700)	7.7 (5–11)				4.8 (4.6–5.1)	462 (411–550)	9.7 (7–12)	
	X4▶X4 ($n = 8$)	4.4 (2.9–5.3)	716 (345–1570)	8.6 (6–13)							

[a]Log (copies/ml).
[b]$CD4^+$T cell count (cells/μl).
[c]Years old; R5, CCR5; X4, CXCR4.
[d]One of the seven children had already received ART at baseline.
[e]All the six children in this group had received ART at baseline.

Child ID	Date of sample collection	V3 amino acid sequence 11 25	Viral load (log/ml)	net charge	11/25 amino acid	predicted phenotype
36m*	aug,02	CTRPGNNTRESVRIGPGQAFYATKDVIGDIRQAHC	4.9	+3	S/D	R5
	apr,03	.I..S.....I.....	5.8	+3	S/D	R5
	feb,04	.....R.I.....IG.....	5.6	+4	S/D	R5
	Oct,05	......K..R.I.....RV..T.NVIR.....	5.1	+7	S/V	X4
38m*	mar,03	....ST...K.....GEIT.....	4.6	+5	S/E	R5
	dec,03	....S....K.....GEIT.....	4.8	+5	S/E	R5
	feb,04	....SSP..TR.A..R.....SAIT.T..K.Y.	4.6	+6	R/A	X4
	sep,05	....S.P..RR.A.....SAIX.T..T.Y.	4.9	+6	R/A	X4
51m*	apr,03	....N....KG.H.....S.FT.GNI.....K.Y.	5.3	+5	G/N	R5
	nov,04	....N....KG.H.....S.FT.GNI.....K.Y.	5.2	+5	G/N	R5
	apr,05	....N....KG.H.....SLFT.GNI.....K.Y.	5.5	+5	G/N	R5
	oct,05	....N....KG.H.....SLFT.GNI..N..K.Y.	5.4	+6	G/N	X4
69m*	mar,03	.I..N....QGIH.....WV.N.....E....Y.	5.4	+4	G/D	R5
	may,04	.I..N....QGIH....R..WV..K.V.IK...Y.	5.1	+7	G/K	X4
85f*	feb,03	....N....K.I.....T....G.IT.....	4.5	+4	S/D	R5
	dec,03	....N....K.IH....RT....G.IT.....	3.5	+5	S/D	R5
	apr,04	....N....K.IH....RT....G.IT.....	4.9	+5	S/D	R5
	apr,05	....N....K.I.....T....G.IT.....	4.3	+5	S/D	R5
	sep,05	....N....K.IH....RT....G.I..N.....	4.5	+6	S/D	X4
89f*	feb,04	..S.T...SRGIHM...RS....D.I..N.....	5.2	+5	G/D	R5
	jul,04	..SNTSS.SRGIHM...RS....D.I..N.....	5.0	+5	G/D	R5
	mar,05	..S.T...SRGIHM...RS....D.I..N.....	5.5	+5	G/D	R5
	oct,05	..SRT...SRGIHM..LRS....DRI..N.....	4.8	+6	G/R	X4
91f*	mar,05	....N....K.IHF.....L.T.DNI..N....Y.	4.9	+4	S/N	R5
	mar,06	....N....R.IH.....L.T.NRI..N.....	4.2	+6	S/R	X4
	aug,06	....N....KGIHF.....L.T.NRI....KK.Y.	4.0	+6	G/R	X4
21f**	jul,03	....S....K.IHL...R.....G.I.....	4.2	+5	S/D	R5
	dec,04	....N....K.IHL...R.....GRI..N.....	4.7	+7	S/R	X4
	apr,06	....S....K.IHL.A.R.....GRI.....	4.2	+6	S/R	X4
49f**	aug,02	.S..S....K.....G.IV.....	4.2	+5	S/D	R5
	jun,03	.S.....K.....G.IV.....	4.5	+5	S/D	R5
	feb,04	.S.....K.....V...GAIV.....	4.1	+5	S/A	R5
	mar,05	.S.....K..H.....GAIV....R...	4.6	+6	S/A	X4
72f**	Jul,02	.I.VN....Q..L.....MG.I..N..D...	5.2	+3	S/D	R5
	Jun,03	.I..Y.....GTHM...K.YFT...I.....D...	5.1	+4	G/D	R5
	feb,04	.I..N....Q..N.....MG.I.....D...	NT	+2	S/D	R5
	sep,04	....N....K.IHF.....L.TNNII..N..D...	5.1	+6	S/I	X4

FIG. 1. Changes in the HIV-1 V3 amino acid sequences during follow-up of the 10 children whose infected viruses switched from CCR5 to CXCR4 coreceptor usage. A net charge of less than and more than +5 in the V3 region was considered as CCR5-using (R5) and CXCR4-using (X4) variants, respectively, and a net charge of +5 was considered as R5 variants, unless this was accompanied by the appearance of either an arginine or lysine residue at position 11 or 25 of the V3 amino acid sequences. *, **Children whose HIV-1 showed a switch in coreceptor usage from CCR5 to CXCR4 with treatment (*) and without (**) treatment. NT, not tested.

TABLE 2. HIV-1 CORECEPTOR USAGE IN ASSOCIATION WITH THE RATE OF DISEASE PROGRESSION

	Change in coreceptor usage[a]	*Number of children (on ART)*	*Mean age (range) at ART start/recruitment*	*Mean age (range) at switch*	*Children with coreceptor switch*
Rapid progressor	R5→R5	6 (6)			
	R5→X4	4 (4)	3.0 (1–4)	4.8 (3–6)	40%[b]
Slow progressor	R5→R5	21 (6)			
	R5→X4	2 (0)	7.5 (6–9)	12.0 (10–14)	8.7%[b]

[a]R5, CCR5; X4; CXCR4.
[b]$p = 0.053$.

coreceptor usage was observed among the children (data not shown).

Discussion

In the current study we conducted a longitudinal study to investigate the evolution of the *env*-V3 region in terms of coreceptor usage among non-B subtype HIV-1-infected Kenyan children in relation to ART. These children were vertically infected with HIV-1 and have been virologically and immunologically followed up since the year 2000. Most studies on viral evolution and coreceptor usage from HIV-1-infected adults are often compromised by a lack of knowledge of the duration of infection. It makes this study particularly useful that the precise timing of HIV-1 infection is known in the children studied.

Recently it has been reported that the prevalence of X4 variants was higher in HIV-1-infected individuals exposed to ART than in drug-naive individuals,[22–25] and that effective HAART enhanced the coreceptor switch from CCR5 to CXCR4.[30] In our study, however, no significant difference in the rate of the coreceptor switch between the children with and without ART was observed ($p = 0.38$). In addition, the duration of time from the start of ART to the time of HIV-1 coreceptor switch varied considerably from 0.5 to 5.2 years (mean: 2.6 years) in our study, though it was expected to be synchronized if ART was directly associated with a switch in HIV-1 coreceptor usage. These results suggest that switching of coreceptor usage from CCR5 to CXCR4 among HIV-1-infected children is not directly influenced by ART.

We further analyzed the children who harbored HIV-1 who showed a switch in coreceptor usage according to the rate of disease progression. The rate of HIV-1 coreceptor switch was found to be strongly higher in the rapid progressors (40%, 4/10) than the slow progressors (8.7%, 2/23) ($p = 0.053$). The strong association between rapid disease progression and HIV-1 coreceptor switch in our study may suggest that those factors associated with rapid disease progression in children, such as high viral load at infection,[32] poor cell-mediated immune responses,[33] lack of neutralizing antibodies,[34] and the biological properties of the virus, would be more directly implicated. Studies using animal models also showed that reduced HIV-specific immunity may result in HIV-1 coreceptor switch.[35]

In the current study, the predicted coreceptor usages based on the env-V3 amino acid sequence according to previous reports[37,38] were not always correlated with those based on the amino acids residues at positions 11 and 25[39–41] (Fig. 1). Therefore, we mainly used the net charge of the env-V3 amino acid sequence to predict HIV-1 coreceptor usage, except when the net charge of the env-V3 amino acid sequence was +5, in which cases we used amino acid residues at positions 11 and 25 to predict the coreceptor usage.[39–41] Phenotypic assay for HIV-1 coreceptor usage might be needed to confirm our prediction of the coreceptor usage.

In conclusion, our data suggest that ART does not enhance the HIV-1 coreceptor switch from CCR5 to CXCR4. This switch in HIV-1 coreceptor usage was associated with rapid disease progression among non-B subtype HIV-1-infected children. We are currently carrying out more detailed analyses on the genetic host factors associated with delayed or rapid disease progression among HIV-1-infected children, hoping to focus more light on the possible factors that influence the HIV-1 coreceptor switch.

Sequence Data

GenBank accession numbers of the sequences reported in this study are EU602350 to EU603148 for env-C2V3.

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Disclosure Statement

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Sequence Note

HIV Type 1 Genetic Diversity in Moyale, Mandera, and Turkana Based on *env-C2-V3* Sequences

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Abstract

The genetic diversity of HIV-1 subtypes circulating in three districts of northern Kenya, i.e., Turkana, Mandera, and Moyale, was studied. DNA sequences encoding a portion of the *env-C2-V3* region of the virus were amplified by PCR and sequenced directly. One hundred and fifty-nine samples were successfully sequenced in the *env-C2-V3* region and analyzed. From the analysis, 57% were subtype A1, 27% were subtype C, 9% were subtype D, and the remaining 7% were unclassified. This study showed that HIV-1 subtype A1 was the dominant subtype in circulation in this region, though there was a significant percentage of HIV-1 subtype C in circulation there.

THE GREATEST DIVERSITY OF HIV-1 STRAINS is demonstrated in sub-Saharan Africa where all the HIV-1 subtypes have been isolated.[1] An outstanding property of HIV-1 is its unusually high genetic variability. Sequences accumulated in the past two decades reveal three distinct lineages: M (main), O (outlier), and N (new or non-M, non-O). Group M viruses dominate the pandemic and include nine major clades (A–D, F–H, J, and K) and 43 recombinant circulating forms (CRFs).[2]

In some subtypes, further phylogenetic structures can be identified leading to the classification of subsubtypes.[3] Phylogenetic analysis of circulating subtypes has proved useful in tracking the spread of HIV in different populations and geographic regions.[4] The development over the past two decades of molecular methods for manipulation of RNA and DNA has afforded molecular virologists the ability to study viral genomes in detail that had previously not been possible.[5] The major methods for detection of viruses in the clinical laboratory today include (1) identification of cytopathic effects (CPE) in cell cultures, (2) use of fluorescent antibodies directly on specimen material, (3) enzyme immunoassays for antigen detection, and (4) amplification techniques with viral genomes as targets.[5]

The experimental determination of the linear arrangement of bases in a viral genome is the ultimate form of subtyping. Sequencing of the genome has the potential to distinguish even between parent and progeny if a single mutation has occurred in the replicative process.[5] Small portions or specific variable regions of a viral genome can be sequenced for the purpose of subtyping. Here only the portions of the genome that confer the subtype can be sequenced. In most cases the comparison will include only the regions that are subjected to immunological pressure by the host (i.e., the major antigenic epitopes of the surface proteins). However, in other cases the untranslated regions of the genomes are unique to a subtype because they contain random mutations that may persist indefinitely in the complete absence of immunologic pressure. The use of polymerase chain reaction (PCR) amplification in conjunction with sequencing and recent developments in automated sequencing have made it possible to obtain a fully analyzed sequence of a small (e.g., 1-kb) portion of a viral genome within about 3 days after obtaining a clinical isolate or, in some cases, after receiving a clinical specimen.[5] PCR has made it possible to amplify minute amounts of DNA or RNA and then sequence directly or clone the products for sequencing. The most frequently sequenced portion of the HIV-1 genome is the *V3* and flanking regions of the gp120 in the *env* gene.[6] This is due to the biological importance of this area.[7] Sequence analysis of this gene region has been shown to provide accurate phylogenetic relationships.[8,9]

Kenya has predominantly HIV-1 subtype A1 in both nonrecombinants (55%) and recombinants. This is based on near

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full-length sequences that have been generated.[10] Subtypes C and D occur as nonrecombinants but to a much lesser extent than subtype A1, and they are also found in recombinants.[10] A full-length subtype G has been found previously from Kenya, with recombinants between A1, A2, and D; A2 and D; A1 and D; A1 and G; A1 and C; A1, C, and D; and C and D also reported.[10]

There have been several molecular epidemiological studies of HIV-1 subtypes in Kenya. Most of these studies have centered on the analysis of partial sequences within the gp120 coding region of *env*. This involved the analysis of the *C2-V3* region.[11] These studies determined that the majority of the sequences analyzed were HIV-1 subtype A1 (71–87%), with significant components of HIV-1 subtype D (7–29%) and HIV-1 subtype C (7–17%). In a study carried out in 2002[10] where 41 near full-length sequences were analyzed, a high proportion of recombinants was seen (40%). These full-length sequences also showed that there was a near absence of pure subtype C and D strains, only 2.4% for each. Almost all the nonrecombinants were HIV-1 subtype A1, which comprised 56% of all strains.

The purpose of this study was to carry out a rapid analysis of samples collected from Northern Kenya using *env*-C2-*V3* primer-based amplification and direct sequencing to determine the genetic diversity of HIV-1 strains circulating in this region.

The subjects who were enrolled in this study were individuals who resided in areas of northern Kenya that bordered Ethiopia, Somalia, and Sudan. Three main sites, Mandera, Turkana, and Moyale, were used as contact points for recruitment of patients. The target subject population consisted of antenatal clinic (ANC) attendees, cases of sexually transmitted diseases (STDs), blood donors, tuberculosis patients, and children born to HI-positive mothers. Patients were also drawn from the outpatient departments of the District hospitals (in Moyale and Mandera) or inpatients already tested positive for HIV-1 and counseled within the health facility framework. In the Turkana District, subjects were selected from known HIV-positive patients attending the AMREF supported health facility in Lokichogio. Subjects were also drawn from the existing refugee sites, especially from those health facilities supported by the UNHCR with the assistance of medical personnel in charge. The subjects were enrolled from May 2003 to March 2006. The specific areas from which the HIV-positive samples were collected were Lokichogio and Kakuma (Turkana District), Sololo and Moyale District Hospital (Moyale District), and Mandera District Hospital. All the experiments in this study were conducted after obtaining approval from the KEMRI/National Ethical Review Committee.

Five-milliliter blood samples were collected with informed consent, as well as with anonymous epidemiological data, including age, sex, and citizenship, from clinics and district hospitals in northern Kenya using an approved protocol. The samples were confirmed to be positive for HIV-1 antibodies using a rapid detection kit (Determine HIV1/2; Abbot, Japan).

Peripheral blood mononuclear cells were prepared from whole blood using 10% ammonium chloride lysis of red cells. Proviral DNA was extracted from peripheral blood mononuclear cells using DNAzol (Molecular Research Center Inc., Cincinnati, OH). A part of the HIV-1 group M *env* gene covering the *C2-V3* region (corresponding to 6975–7520 nt in HIV-1HXB2) was amplified by nested PCR using primers M5 (5′-CCAATTCCCATACATTATTGTGCCCCAGCTGG-3′) and M10 (5′-CCAATTGTCCCTCATATCTCCTCCTCCAGG-3′) in the first round and M3 (5′-GTCAGCACAGTACAATG-CACACATGG-3′) and M8 (5′-CCTTGGATGGGAGGG-GCATACATTGC-3′) in the second round. The cycling conditions for this PCR were similar, i.e., a hot start at 95°C followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 1 min, and a final extension of 72°C for 10 min.[11] PCR amplification was confirmed by visualization with ethidium bromide staining of agarose gels. The amplified products were sequenced di-

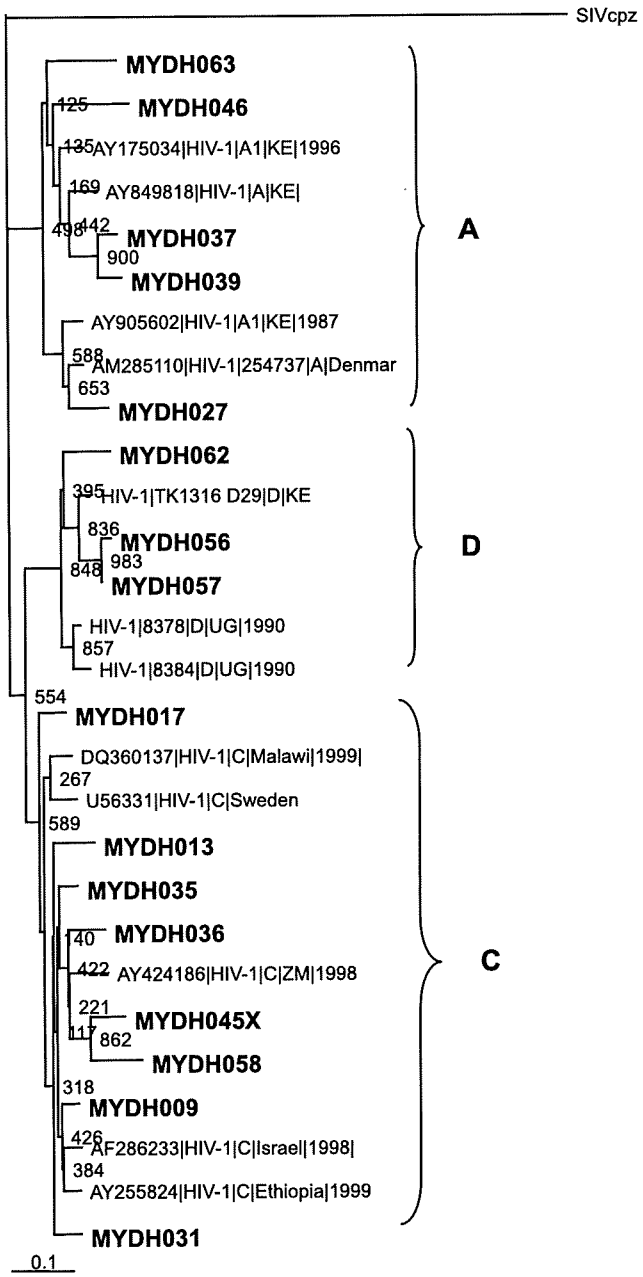

FIG. 1. A representative tree of sequences generated from samples collected in Moyale.

rectly using the second PCR primers of the *C2-V3* region that were used to generate the amplicons. The BigDye Terminator DNA sequencing kit from Applied Biosystems was used to sequence the DNA amplicons on the ABI Prism 310 genetic analyzer (Applied Biosystems, Foster City, CA).

The generated sequences were aligned manually based on the alignment of Los Alamos database reference sequences for subtyping. Sequences were aligned using CLUSTAL W version 1.81[12] with subsequent inspection and manual modification. The frequency of nucleotide substitution in each base of the sequences was estimated by the Kimura two-parameter method.[13] Phylogenetic trees were constructed by the neighbor-joining method, and their reliability estimated by 1000 bootstrap replications. Alignments were gap stripped for the generation of trees. Resulting trees were visualized using Treeview.[14]

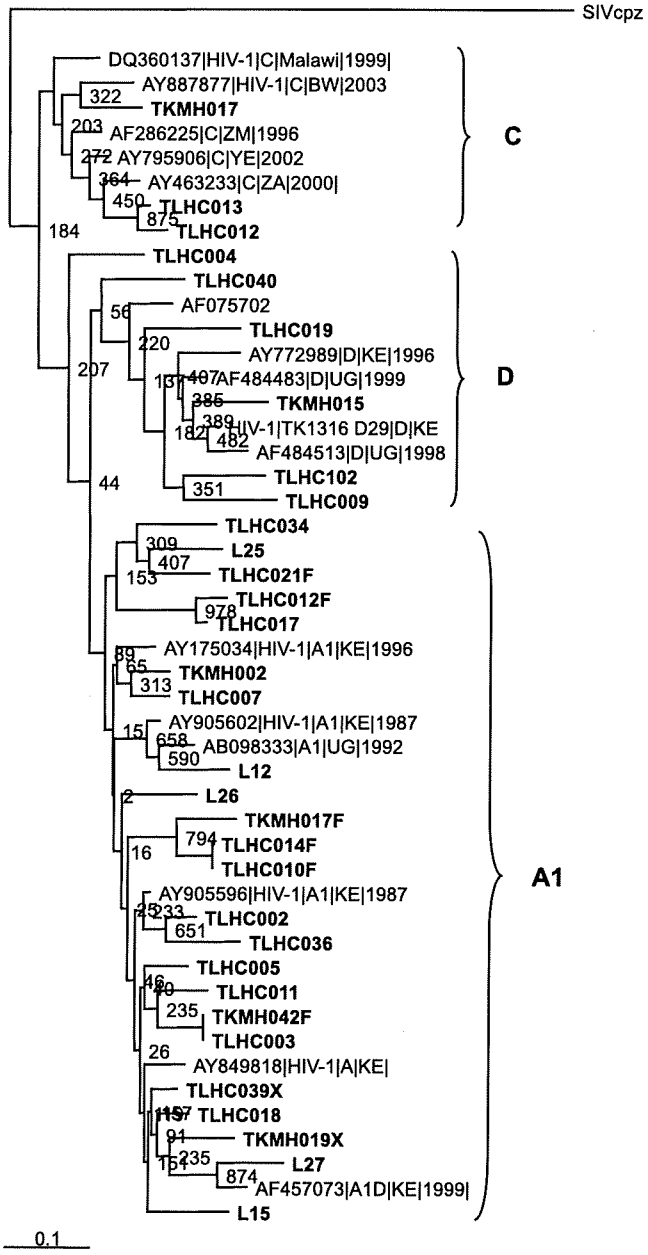

FIG. 2. A representative tree of sequences of samples collected from Turkana.

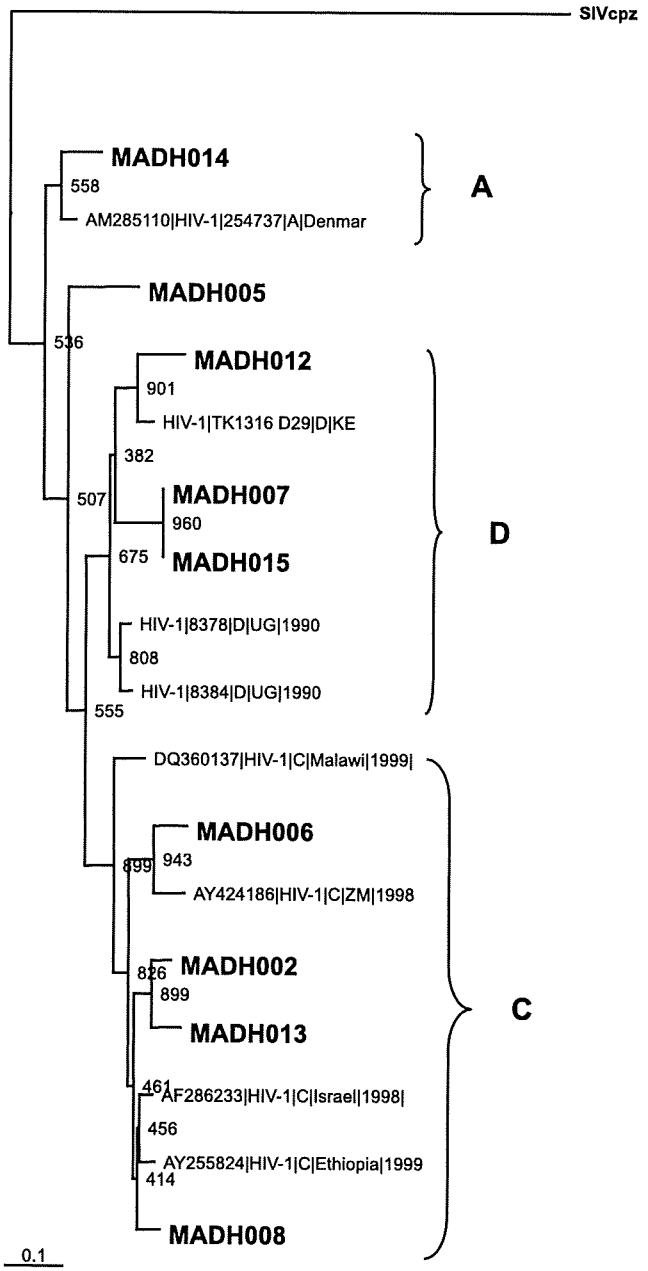

FIG. 3. A representative tree of sequences generated from samples collected from Mandera.

One hundred and fifty-nine samples were successfully analyzed and grouped into different subtypes. Fifteen of these were from the town of Mandera, 76 from Turkana, and 68 from Moyale. Representative trees of each of the three towns of northern Kenya are indicated in Figs. 1–3.

From the analysis of samples collected from Moyale and amplified with *env-C-V3* primers, 69% were HIV-1 subtype A1, 27% were HIV-1 subtype C, and 4% were HIV-1 subtype D as shown in the representative phylogenetic tree (Fig. 1). The HIV-1 subtype A1 samples clustered with reference sequences mainly from Kenya showing that the viruses were of Kenyan origin. The samples that were HIV-1 subtype C

clustered with reference sequences from Malawi, Zambia, and Ethiopia. The HIV-1 subtype D sequences clustered with reference sequences from Kenya and Uganda.

Analysis of HIV-1-positive samples from Turkana with *env-C2-V3* primers showed that 75% of the sequences were HIV-1 subtype A1, 15% were HIV-1 subtype C, and 10% were HIV-1 subtype D (Fig. 2). The HIV-1 subtype A1 sequences clustered with reference sequences from Kenya and Uganda indicating that these viruses were of East African origin. The HIV-1 subtype D viruses clustered with reference sequences from Kenya and Uganda too. Those that were HIV-1 subtype C clustered with reference sequences from Zambia, Yemen, Botswana, and South Africa. Analysis of *env-C2-V3* sequences from Mandera indicated that 40% were HIV-1 subtype A1, 47% were HIV-1 subtype C, and 13% were HIV-1 subtype D (Fig. 3).

A similar analysis was carried out in 2005 but it involved analysis of 72 HIV-1-positive samples in the *env-gp41* region.[15] Here we have reported the analysis of 159 samples in the *env-C2-V3* region. This analysis, like many others done in Kenya in the past, indicates that HIV-1 subtype A-1 is by far the most dominant circulating subtype in Kenya, though in this study it was seen that a significant percentage of the sequences were HIV-1 subtype C, i.e., higher than has been reported in other parts of Kenya.

All the sequences generated in this study were deposited in GenBank with the following accession numbers: AY952815, AY952818–19, AY952824–29, AY952835–38, AY952840–AY952857, and DQ155149–DQ155282.

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Disclosure Statement

No competing financial interests exist.

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Sequence Note

Prevalence of Nevirapine-Associated Resistance Mutations after Single Dose Prophylactic Treatment among Antenatal Clinic Attendees in North Rift Kenya

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Abstract

The use of single dose nevirapine to prevent mother-to-child transmission of HIV has been reported to induce drug-resistant mutations and reduce options for antiretroviral treatment for HIV-infected mothers and their children. To explore the status of nevirapine-resistant HIV genotypes in rural hospitals in the North Rift Valley Province of Kenya, samples collected 3 months after single dose nevirapine from 36 mothers and their children were analyzed. Resistance mutations were genotypically evaluated through proviral DNA amplification, cloning, and sequencing. Ten mothers (27.8%) had antiretroviral-associated resistance mutations of whom four (11.1%) had specific nevirapine (NNRTI) resistance-associated mutations. Three mothers (8.3%) transmitted the infection to their infants. This presence of nevirapine mutations in rural antenatal clinic attendees confirms the importance of integrating antiretroviral resistance monitoring as a key component in programs geared to prevention of HIV mother-to-child transmission.

The majority of children with HIV in Africa acquire the infection through mother-to-child transmission (MTCT), which occurs *in utero*, during labor, delivery, and while breastfeeding. The advent of antiretroviral treatment has dramatically reduced perinatal transmission. In 1998, a study in Côte d' Ivoire showed that a simpler drug regimen consisting of a 1-month course of zidovudine late in pregnancy could half the rate of transmission as long as the women avoided breastfeeding.[1] This was followed by a study in Uganda in 1999 that showed that one dose of nevirapine (sd-NVP) to the mother at the onset of labor and a dose given to the infant within 72 h of delivery were highly effective in reducing MTCT.[2] Unlike zidovudine, the sd-NVP regimen is simple to administer, affordable, and has moderate efficacy for the prevention of peripartum HIV-1 transmission. These advantages have led to a wide use of single-dose NVP to reduce vertical transmission of HIV in developing countries.

Despite the reduction of MTCT using sd-NVP, the development of drug resistance has been reported. In a study carried out in Uganda, 20% of the women had NVP-associated drug resistance 6 weeks after delivery[3] In Abidjan (Côte d'Ivoire), NVP resistance mutations were observed in 20.7% of women 1 month after sd-NVP.[4] A worrying consequence of the resistance has been the rapid selection of viral variants, which may cause antiretroviral failure and reduce treatment options for HIV-infected populations.

In Kenya, data on nevirapine resistance among antenatal clinic attendees at the programmatic level are scarce. The aim of this study was to determine the prevalence of HIV nevirapine-resistant genotypes and their effect on mother-to-child transmission among antenatal clinic attendees receiving treatment in three district hospitals in the North Rift Valley Province of Kenya.

From April 2005 to July 2006, 309 HIV-positive women attending antenatal clinics in Nandi Hills, Kapsabet, and Ki-

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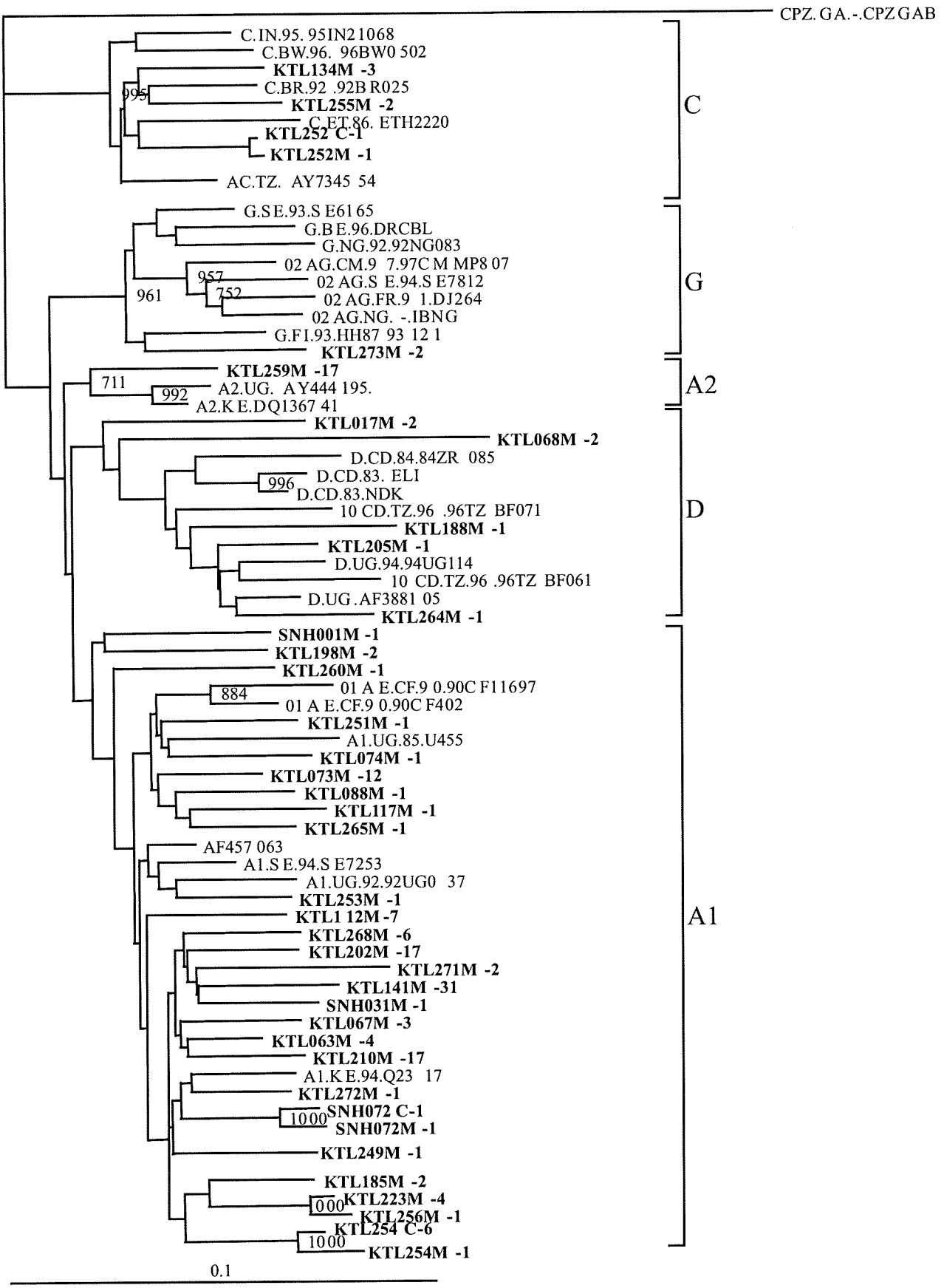

FIG. 1. Phylogenetic tree based on a part of the pol-RT gene (697 bp) of the 39 samples analyzed. The study sequences are in boldface.

tale hospitals were, with informed consent, requested to participate in the study. The study sites were among the countrywide centers receiving treatment through the President Emergency Plan For AIDS Relief (PEPFAR) initiated by U.S. President George Bush in 2003.[5] This study was approved by the Kenya Medical Research Institute Scientific Steering Committee and Ethical Review Board (Ref. KEMRI SSC No. 822). As part of the prevention of mother-to-child transmission of HIV campaign under the PEPFAR program, mothers were receiving one tablet of 200 mg of nevirapine to take it at the onset of labor and 0.6 ml (6 mg) nevirapine suspension in a luer lock syringe to give to the baby within 72 h of delivery. The HIV-positive women were counseled on feeding choices according to Kenya National AIDS Control Program guidelines[6] involving either exclusive breastfeeding with early weaning or formula feeding. Three months after delivery, a postnatal follow-up was done including taking a blood sample from both mother and child and gathering clinical data including previous history of use of antiretroviral treatment.

Our study design involved analyzing viral genotypes of mother–child pairs that visited the clinic 3 months after delivery. Peripheral blood mononuclear cells were extracted from whole blood by density gradient centrifugation using Ficoll-Paque Plus (Pharmacia) and DNA extracted using DNAzol (Invitrogen) and ethanol precipitation. The extracted proviral DNA was used for polymerase chain reaction (PCR) amplification. A region of the HIV-1 *pol* gene including the reverse transcriptase sequence (Pol-RT; corresponding to nt 2513–3209 in HIV-1_{HXB2}) was amplified by nested PCR with primers RT 18 (5′-GGAAACCAAAAATGATAGGGGGAATTGGAGG-3′) and KS 104 (5′-TGACTTGCCCAATTTGTTTTCCCACTAA-3′) in the first round and KS101(5′-GTAGGACCTACACCTGTTCAACATAATTGGAAG-3′) and KS 102 (5′-CCCATCCAAAGAAATGGAGGAGGTTCTTTCTGATG-3′) in the second round. Amplification was carried out with 1 cycle of 95°C for 10 min and 35 cycles of 95°C for 30 s, 55°C for 30 s, and 72°C for 1 min, and final extension of 72°C for 10 min. The PCR amplification was confirmed by ethidium bromide staining of samples electrophoresed on a 1.5% agarose gel.

The PCR products were cloned using the TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and sequenced as previously described.[7] At least five clones per sample were analyzed to obtain a consensus sequence. Phylogenetic relationships of newly derived viral sequences were estimated from comparisons with those of previously reported HIV-1 group M from the Los Alamos sequences database using the CLUSTAL W profile alignment option. Genetic distances were calculated by the two-parameter method of Kimura and the phylogenetic tree constructed by the neighbor-joining method with its reliability being estimated by 1000 bootstraps.[8] The tree profile was visualized with Tree View PPC version 1.6.5.

The RT nucleotide sequences (697 bp) were translated into the corresponding 232 amino acids using Genetic Information Processing software (Genetyx-Win) version 4.0 (Gene-

TABLE 1. DRUG ASSOCIATED MUTATIONS DEDUCED AND LEVEL OF RESISTANCE[a]

Sample ID	*Subtype*	*NRTI mutations (Clones)*	*NNRTI mutations (Clones)*	*Drug associated and level of resistance*
KTL067M	A1	K219Q (1/11)		AZT and d4T, Low
KTL088M	A1	V118I (1/5)		3TC, low
KTL188M	D		Y181C (2/14)	NVP and DLV, High; EFV, Low; ETR, Intermediate
KTL210M	A1		Y181C (2/5)	NVP and DLV, High; EFV, Low; ETR, Intermediate
			G190A (1/5)	EFV, Intermediate; ETR, Low; NVP, High
KTL223M	A1	V118I (1/13)		3TC, low
KTL255M	C	K65R (1/6)		ddI, ABC, 3TC, FTC, and TDF, Intermediate; D4T, Low
KTL254C	A1		V106A (1/9)	NVP, High; DLV, Intermediate; EFV and ETR, Low
KTL252M	C		K103N (20/22)	NVP, DLV, and EFV, High; ETR, Low
			Y188C (1/22)	DLV, EFV and ETR, Low; NVP, High
KTL252C	C	Y115F (1/22)	Y188C (9/21)	ABC, Intermediate; TDF, Low DLV, EFV, and ETR, Low; NVP, High
KTL264M	D	K219E (1/8) L74I (1/8)		AZT and d4T, Low DDI, Intermediate; ABC, Low
KTL259M	A1		G190A (2/5)	NVP, High; EFV, intermediate; ETR, Low
KTL273M	G	K65R (1/8)		ddI, ABC, 3TC, FTC, and TDF, Intermediate; D4T, Low

[a]M-mother, C-child, D4T-stavudine, ABC-abacavir, 3TC-lamivudine, DDI-didanosine, FTC-emtricitabine, TDF-tenofovir, DLV-delavirdine, EFV-efavirenz, NVP-nevirapine, ETR-etravirine, AZT-zidovudine

tyx, Tokyo, Japan). After a successful translation, the possible mutation points associated with drug resistance were determined using the HIVdb Program from the Stanford University HIV Drug Resistance Database (http://hivdb.stanford.edu/pages/algs/HIVdb.html).

In this study, 3 out of the 36 mothers (8.3%) transmitted the infection to their newborns in the presence of nevirapine prophylaxis. Of the total of 39 (36 mothers and 3 infants) samples successfully amplified and sequenced, 28 were subtype A1 (71.8 %), 5 subtype D (12.8%), 4 subtype C (10.3%), 1 subtype A2 (2.6%), and one subtype G (2.6%) (Fig. 1).

Analysis of the sequences revealed that samples from 10 mothers and 2 children had viral genotypic evidence of drug-associated resistant mutations (Table 1). Specifically, the mutations associated with nevirapine Y181C, K103N, G190A, Y188C, and V106A were detected in six of the resistant cases. The K103N mutation, which causes high resistance to NVP when present alone,[4] was detected in one mother (KTL252M) while the Y181C mutation, which has been reported to have an impact only when present with other nonnucleoside reverse transcriptase inhibitor (NNRTI) mutations, was detected in two mothers (KTL188M and KTL210M). The G190A mutation, which causes high mutation to NVP and intermediate mutation to EFV, was detected in two mothers as minor populations. In one case, the mother (KTL254M) had no drug-associated mutation but the child (KTL254C) had an NVP-associated mutation (V106A) detected as a minor population (Table 1). This suggests that the child may have acquired the mutation in response to the NVP syrup administered postnatally.

Although the women self-reported that they had not previously been exposed to NRTIs in their counseling and questionnaire responses, drug resistance mutations associated with NRTIs were detected in seven cases (19.4%). The detected mutations included D67N, K219Q/E, V118I, K65R, Y115F, and L74I. All the mutations were found as minor populations (one clone in each case). The consistency of these data with findings from other co-workers[9] who found similar minor clone populations among drug-naive populations led us to rule out laboratory error in our sampling procedures.

Our analysis shows that like other parts of the country the predominant circulating subtype in North Rift is A1. Based on the pol RT region their prevalence is similar to our recent findings from the Nairobi STI clinic[10] denoting a near uniform epidemic in the country except for Northern border regions where subtype C predominates.[11]

This study was designed to evaluate the prevalence of RTI resistance-associated mutations after a single-dose nevirapine regimen through analysis of proviral DNA. Though direct sequencing using plasma is the gold standard, it has been shown that peripheral blood mononuclear cells can be reliably used for drug resistance genotyping.[12] We cloned the samples in our attempt to detect minor populations that may not have otherwise been detected if we were to directly sequence only. We have shown in another study that minor populations later proliferate to dominant strains causing treatment failure.[13]

Four of the 36 (11.1%) mothers had detectable resistance to NVP 3 months after delivery. Because we did not sample before NVP use, we could not determine if the mothers had the nevirapine mutations before prophylaxis. Among the mothers, K103N, Y181C, Y188C, and G190A mutations were detected. This is one of the first reports of RTI resistance-associated mutations among women and infants on a single-dose nevirapine regimen to reduce MTCT of HIV-1 in Kenya. The findings are similar to studies in neighboring Uganda in which 20% of HIV-infected women treated with single-dose NVP to prevent perinatal transmission were found to have developed resistance to NVP during follow-up.[3]

Due to a small sample size and short follow-up period, it was not possible for us to determine the mutation that was frequently selected in this population. Previous studies have, however, shown that the K103N mutation was selected more frequently than Y181C in women following single-dose NVP.[14] In most of our subjects, the mutations were detected as minor populations. The majority of the clones (90.9% and 42.9%, respectively) had mutations in only one mother–child pair (KTL252M/C). The large number of subjects with NRTI mutations despite the absence of evidence of exposure to the regimen is consistent with other reports in which drug resistance has been detected in drug-naive individuals.[15] The efficacy of the treatment regimen containing NRTI could be compromised in NRTI-naive patients already harboring resistant viruses.

Our findings suggest the need to incorporate antiretroviral drug resistance testing as an important secondary endpoint in PMTCT assessment. However, as this may not be feasible in sub-Saharan Africa, periodic monitoring among nevirapine-exposed women and children should be considered instead. The high incidence of resistance mutations as minor populations in our study calls for the use of clonal sequencing or similar methods in current national antiretroviral resistance monitoring surveys to detect evolving variants that may have a future negative impact on antiretroviral treatment programs.

The sequences have been deposited in the GenBank database, with accession numbers EU386189–EU386340.

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Disclosure Statement

No competing financial interests exist

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Human Papillomavirus Infection and Cervical Abnormalities in Nairobi, Kenya, an Area With a High Prevalence of Human Immunodeficiency Virus Infection

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Human papillomavirus (HPV) infection and cervical abnormalities, and their association with human immunodeficiency virus (HIV) infection were studied in 488 women who visited a health center in Nairobi. PCR-based HPV and cervical cytology tests were carried out on all participants, and peripheral CD4+ T cells and plasma HIV RNA were quantitated in HIV positive women. HIV were positive in 32% (155/488) of the women; 77% of these were untreated, and the others had been treated with anti-retroviral drugs within 6 months. Cervical HPV infection was detected in 17% of HIV negative and 49% of HIV positive women. Low-grade squamous intraepithelial lesions were observed in 6.9% of HIV negative and 21% of HIV positive women, while high-grade squamous intraepithelial lesions and cancer were seen in 0.6% and 5.8%, respectively. Multivariate analysis revealed that HIV and HPV infections were associated with each other. Cervical lesions were significantly associated with high-risk HPVs and with HIV infection, depending on HPV infection. HPV infection increased in accordance with lower CD4+ T cell counts and higher HIV RNA levels, and high-grade lesions were strongly associated with high-risk HPV infection and low CD4+ T cell counts. Immunosuppression as a result of HIV infection appears to be important for malignant progression in the cervix. Nationwide prevention of HIV infection and cervical cancer screening are necessary for the health of women in this area. High-risk HPV infection and low CD4+ T cell counts are the risk factors for cervical cancer. ***J. Med. Virol. 80:847–855, 2008.*** © 2008 Wiley-Liss, Inc.

KEY WORDS: HIV; HPV; cervical abnormality; HIV RNA; CD4$^+$ T cell count

INTRODUCTION

Uterine cervical cancer is the second most common cancer and the fifth most common cause of cancer mortality worldwide. Around 468,000 new cases of cervical cancer occur annually, and more than 233,000 women die from the disease worldwide. About 80% of such cases are in resource-poor developing countries [Parkin et al., 2001]. The highest incidence is observed in Latin America, the Caribbean, sub-Saharan Africa, and South and Southeast Asia [Parkin et al., 2001]. Cervical cancer remains a pervasive public health problem in developing countries. Implementation of nationwide cervical cancer screening has been successful in reducing the incidence and mortality of cervical cancer in many developed countries [Peto et al., 2004]. However, the high mortality rate from cervical cancer in women of child-bearing age may be one of the most important social problems in developing countries.

In sub-Saharan Africa, the annual incidence of cervical cancer is more than 93.9 per 100,000 women

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[Parkin et al., 2001]. Although the national incidence of invasive cervical cancer is unknown in Kenya, it has been estimated at 45 per 100,000 women [Gichangi et al., 2002]. In Kenya, 55% of cervical cancer cases are reported to be clinical stage 3 or more advanced, whereas this figure is only 25% in developed countries [Claeys et al., 2003]. Only 6% of women who present with invasive cervical cancer in Kenya have a history of screening [Gichangi et al., 2002]. The lack of a systematic cervical cancer screening program in Kenya appears to be a factor in the high incidence of progressive disease.

Human papillomavirus (HPV) has been identified as an important causative agent of cervical cancer. HPV DNA testing, rather than cytological testing, is recommended for cervical cancer screening in some resource-poor areas [Kuhn et al., 2000] and in those regions with high prevalence of the human immunodeficiency virus [HIV; Womack et al., 2000]. Cervical cancer is a major problem in areas of Africa with a high prevalence of HIV infection. Women with HIV infection are more likely to have a concurrent HPV infection [Temmerman et al., 1999; Womack et al., 2000], and HPV infection is associated with a greater risk for high-grade squamous intraepithelial lesions in HIV positive women than in HIV negative women. HIV-associated attenuation of HPV-specific immune responses may allow for persistent high-grade intraepithelial neoplasia, thus providing sufficient time for the accumulation of genetic changes important in progression to cancer [Palefsky, 2006]. Furthermore, compared to their non-infected counterparts, women infected with HIV and with invasive cervical cancer are more likely to present with advanced clinical disease [Maiman et al., 1997].

In Kenya, 11% of adult women were infected with HIV in 1996, and the incidence decreased to 8.7% in 2003. However, the incidence was higher in women living in large towns such as Nairobi, where it was 12% in 2003 [Ministry of Health, Kenya, 2005]. The high prevalence of HIV infection in Kenya may increase the incidence of cervical cancer and its precursor lesions, although Gichangi et al. [2002] have demonstrated that a two- to threefold increase in the prevalence of HIV in Kenya did not have a proportionate effect on the incidence of cervical cancer. Unlike cervical cancer, the incidence of Kaposi's sarcoma seems to mirror the incidence/prevalence of HIV, being increased significantly in HIV-infected individuals [Goedert et al., 1998]. It has been hypothesized that HIV-infected women die from HIV-related opportunistic infections before they develop invasive cervical cancer [Gichangi et al., 2002]. At the time of this report, the mean survival time for women with HIV infection in Kenya is 5 years, while typically more than 10 years elapse before the development of cervical cancer after HPV infection. Another possibility is that the diagnosis of subclinical cervical cancer may be missed in many women who die from opportunistic infections in AIDS, as many cases of cervical cancer are asymptomatic.

In the present study, a nested cross-sectional study was undertaken within an ongoing prospective study of HPV/HIV infection and cervical abnormality in Nairobi, Kenya, to establish their prevalence and any association between these conditions.

SUBJECTS AND METHODS

Study Design

This cross-sectional study was part of an ongoing cohort study conducted in Nairobi, Kenya, from November 2004 to August 2009. The subjects were sexually active women, aged 16–61 years old, who attended the Riruta Health Center in Nairobi from November 2004 to June 2005 for pregnancy, family planning, and gynecological concerns. The health center is located near a large slum town. After providing written informed consent, all of the women were invited to participate in this study. The recruitment criteria included women who were willing to undergo voluntary counseling and testing for HIV infection, a cervical Pap test, and an HPV DNA test. Ethics committee in Kenya Medical Research Institute (KEMRI) approved this study. More than 650 women volunteered, and 488 eligible women, including 83 pregnant women who were within 30 weeks of the gestation period, were evaluated in the present study. The remaining subjects were not eligible mainly because some information and data necessary for analysis were not available.

An educational talk about cervical cancer and its risk factors, screening methods, and management was presented to all of the women who visited the health center. The women who agreed to participate in this study were invited for a detailed explanation of the procedures involved. After an informed consent was signed, a structured questionnaire was administered, and a pelvic examination, Pap test, and HPV DNA test were performed in all participants. The questionnaire sought employment status, education, past and current sexually transmitted infection, and current pregnancy status. The first of two cervical scraped-cell samples was placed on a glass slide and fixed immediately in 95% ethanol for the Pap test, and the second was placed into a tube containing DNA extraction solution (10 mM Tris, pH 8.0, 1 mM EDTA, 2% SDS) for the HPV DNA test. After cytological screening, some abnormal and borderline cases ($n = 45$) were recalled and subjected to further examination by colposcopy and pathological diagnosis using punch biopsy specimens. HPV test samples were stored at $-20°C$. Blood samples were collected for screening of HIV. The samples were separated into serum and blood using a particle agglutination kit and were stored at $-20°C$.

HPV DNA Detection and Typing

DNA was extracted from cervical cell samples using a DNA extraction kit (SMI test). HPV L1 genes were amplified by PCR using modified GP5+ and GP6+ [de Roda Husman et al., 1995] multiprimers, designed to avoid mismatches between primer sequences and complement target HPV L1 genes. The modified GP