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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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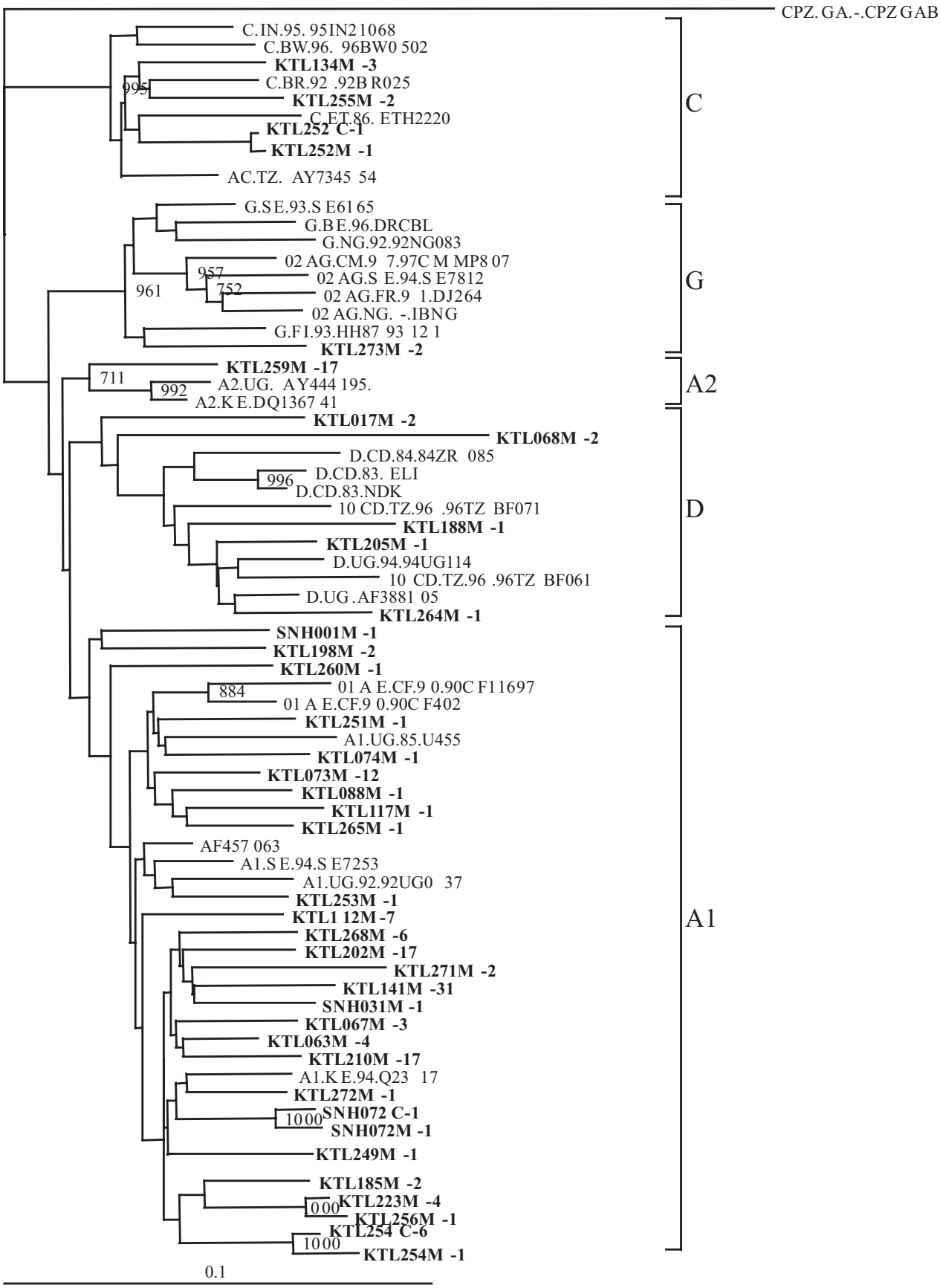

FIG. 1. Phylogenetic tree based on a part of the pol-RT gene (697 bp) of the 39 samples analyzed. The study sequences are in boldface.

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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