The objective of this study was to determine whether the maternal infecting human immunodeficiency virus (HIV) type 1 clade affects mother-to-child transmission frequency. Mothers in the mother-to-child HIV-1 transmission study in Nairobi, Kenya, were grouped by HIV-1 status of their first enrolled child: uninfected, perinatally infected, or postnatally infected. Restriction fragment length polymorphism (RFLP) analysis was used to determine HIV-1 viral clades of nested polymerase chain reaction products from HIV-1 protease or p24 genes. When inconclusive, sequencing determined the clade. Clade distributions within the groups were compared. The 3 groups displayed a uniform clade distribution. The predominant clades were A (59%) and D (20%). Clades B, C, F, mixed, and recombinant infections comprised the remainder (21%). No significant association was seen between clades A and D and either frequency or mode of vertical transmission. RFLP analysis revealed 2 clade B infections, 9 mixed, and 5 p24/protease recombinant infections in the study population.

As the magnitude of the AIDS pandemic continues to grow, so does the number of children infected with human immunodeficiency virus (HIV) type 1 due to the ever-increasing numbers of HIV-1-infected women of childbearing age. For this reason, researchers have sought to determine which factors influence HIV-1 transmission from mother to child. Factors known to be associated with mother-to-child HIV-1 transmission are high maternal plasma virus load [1], low CD4 cell counts [1], advanced maternal disease [1], low maternal plasma vitamin A (associated with AIDS progression) [1], duration of breast-feeding [2], and close maternal-child HLA matching [3]. On the positive side, administration of antiretrovirals to the breast-feeding [2], and close maternal-child HLA matching [3]. One of the remaining questions is whether there is a relationship between the infecting clade of HIV-1 and mother-to-child transmission (either perinatally or postnatally). Studies from Thailand suggest that HIV-1 clade E may be transmitted more efficiently via heterosexual transmission than clade B virus [5], although this has been disputed [5]. Based on this idea and other controversial findings that suggest clade C virus has spread more rapidly than clade B virus via heterosexual transmission [5], we hypothesized that HIV-1 of different clades might have variable mother-to-child transmission frequencies, due either to clade-specific differences in viral pathogenicity or cellular tropism for cells of the placenta or reproductive tract.

Materials and Methods

Study population. In the mother-to-child transmission study cohort in Nairobi, Kenya, women admitted to the labor and delivery ward of a major maternity hospital were invited to participate in the study. With their permission, cord blood serum samples were screened by ELISA for HIV-1 antibody. We enrolled women who were HIV-1 seropositive and an equal number of unselected HIV-1-seronegative women and their infants. Within 24 h of delivery, blood samples were taken for analysis from mothers agreeing to participate in the study. The HIV-1–positive mothers and their infants were then seen at 2, 6, 10, 14, 18, and 24 weeks and at 3-month intervals thereafter [2, 6]. As per World Health Organization recommendations and the Government of Kenya policy at the time during which women in this study were recruited, HIV-1–infected mothers were not discouraged from breast-feeding unless they had safe alternative methods of nutrition for their infants [7].

The children of HIV-1–positive mothers were monitored for HIV-1 infection by either Western blot or EIA. For the purpose of follow-up on the HIV-1 status of their child, blood samples were obtained from children at ages 2, 6, 14, and 24 weeks, and at 3-
to 6-month intervals thereafter. Polymerase chain reaction (PCR) was done by use of \textit{vif}, \textit{nef}, and \textit{env} primers [8] developed from sequences of African HIV-1 strains.

The HIV-1 infection status of infants born to HIV-1–seropositive mothers was classified as follows. Infants who were classified as HIV-1 uninfected were children who lost their maternal antibodies and remained seronegative and seropositive children who died or were lost to follow-up at age <1 year and who were PCR negative on ≥2 occasions after delivery beyond 2 weeks of life.

Children who were considered infected with HIV-1 were further classified as being either perinatally or postnatally infected. Perinatally infected infants were considered to have acquired HIV-1 infection in utero, during labor, or shortly after delivery. This group included seropositive children who were persistently PCR positive more than once or who had only their first PCR obtained before age 6 months with a negative result and children for whom no PCR testing was done and who were always seropositive when tested. Postnatally infected children included (1) children ultimately found to be infected, who were persistently PCR negative before age 6 months (on ≥2 occasions); (2) children born to mothers who seroconverted after delivery, who were seronegative at birth and subsequently seroconverted; and (3) children, for whom no PCR results were available, who lost maternal antibodies and then subsequently seroconverted. Children who were aged <12 months when last seen alive who were antibody positive and for whom there was insufficient PCR data to determine their status were classified as indeterminate and were not included in this study. For the purpose of this study, only the status of the first child (index child) of each mother was included in the analysis. The frequency and timing of mother-to-child transmission for different viral clades was then compared.

\textit{Preparation and initial analysis of samples.} Peripheral blood mononuclear cells (PBMC) were isolated from whole blood of HIV-1–infected women by ficoll-hypaque density gradient centrifugation. The PBMC were then drawn off and stored in liquid nitrogen until use. DNA was extracted by means of the Puregene-Gentra (Minneapolis, MN) protocol for DNA purification from body fluids and was stored at −20°C. A single, 30-cycle PCR was carried out on the DNA samples for HLA by use of DQ\textsubscript{a} primers [9], to determine that DNA had been isolated in quantities sufficient for amplification of product visible on an agarose gel. PCR was also carried out for the HIV-1 genes \textit{vif}, \textit{nef}, and in some cases \textit{env} by using the primers previously described. Only samples with sufficient DNA for positive amplification of \textit{vif}, \textit{nef}, or \textit{env} by single-round PCR were used for viral clade determination.

\textit{Restriction fragment polymorphism analysis (RFLP).} RFLP analysis was done by use of the protocol developed by Janini et al. [10]. In brief, HIV-1–specific primers for the protease gene were used on template DNA derived directly from PBMC lysates in a nested PCR reaction that amplified a 297-bp fragment including the entire protease gene. PCR amplification was repeated on the samples that did not amplify initially. For confirmation of the results obtained, HIV-1 primers specific for the p24 region of the \textit{gag} gene of the virus were used in a nested PCR reaction to amplify a 311-bp fragment that included part of the p24 gene. Analysis of the p24 gene was also useful for further differentiation between B and D clades that could not effectively be subtyped by analysis of the protease gene alone.

\textit{Sequencing of indistinct samples.} Samples for which a clade could not be assigned by the RFLP methodology were subtyped by sequencing of the sample’s protease or p24 genes by the dideoxy chain termination method. Sequences obtained were compared on DNASTAR (DNASTAR, Inc., Madison, Wisconsin) by use of the Megalign program with published consensus sequences representative of each clade to determine subtype with the greatest level of homology.

\section*{Results}

One sample from each of 154 mothers was amplified with primers specific for the protease and p24 genes. Of these samples, 102 were from the group in which the index child was HIV-1 negative, 28 were from the group in which the first child was perinatally infected, and 24 were from the group in which first children were infected postnatally. We were able to definitively assign infecting clades for 130 mothers of the cohort (table 1). Twenty-four samples were indeterminate by RFLP analysis and subsequent sequencing.

\textit{Distribution of HIV-1 characteristics in the Nairobi mother-to-child transmission cohort.} As seen in table 1, the distribution of HIV-1 clades was about the same for each group of mothers. There was no difference in clade distribution between

\begin{table}[h]
\centering
\caption{Distribution of the human immunodeficiency virus type 1 clades among different transmitting groups of mothers in Nairobi, Kenya.}
\begin{tabular}{|c|c|c|c|c|}
\hline
Subtypes & Total & None & Perinatal & Postnatal & Any mother-to-child \\
\hline
A & 76 (58) & 52 (61) & 14 (58) & 10 (42) & 24 (53) \\
B & 2 (2) & 1 (1) & 0 (0) & 1 (4) & 1 (2) \\
C & 3 (2) & 2 (2) & 1 (4) & 1 (4) & 2 (4) \\
D & 26 (20) & 14 (16) & 6 (25) & 6 (25) & 12 (27) \\
F & 8 (6) & 6 (7) & 1 (4) & 1 (4) & 2 (4) \\
Mixed & 9 (7) & 6 (7) & 1 (4) & 2 (8) & 3 (7) \\
Recombinant & 5 (4) & 4 (5) & 1 (4) & 0 & 1 (2) \\
Total determined & 130 (100) & 85 (100) & 24 (100) & 21 (100) & 45 (100) \\
Not determined & 24/154 (16) & 17/102 (17) & 4/28 (17) & 3/24 (13) & 7/52 (13) \\
\hline
\end{tabular}
\end{table}

\textit{NOTE.} Data are no. (%) of each of clades A–D, F, mixed infections, and recombinant infections as seen in each of transmission groups (n = 130), and no. of samples amplified to which a definitive clade could not be assigned (not determined).
perinatal, postnatal, or any mother-to-child transmission, compared with the distribution among pairs without transmission. The predominant subtypes in this cohort were clade A (42%-61% of samples) and D (17%-25% of samples). In addition, RFLP analysis yielded a small number of clade B, C, and F in this sampling, and a handful that appeared to be either mixed or recombinant. Table 1 includes only the 130 mothers for whom a definitive clade has been assigned to date.

**Identification of recombinant, mixed, and clade B isolates in Nairobi.** RFLP analysis identified 2 HIV-1 clade B viruses in the study group. These samples were sequenced and compared on DNASTAR by use of Megalign with consensus sequences from GenBank to confirm viral clade. Sequencing of these isolates was carried out to elucidate that they were indeed clade B, since this clade was not identified in Kenya before this study. In addition, RFLP analysis suggested a relatively high degree of protease/p24 recombination (5) and mixed infections (9) endemic in these women, despite their belonging to a relatively low-risk group. Furthermore, there were 3 cases in which HIV-1-positive mothers with a mixed HIV-1 infection transmitted HIV-1 to their child. In all 3 cases, the children had a mixed infection identical to that of their mothers.

**Discussion**

The similarity in the distribution of clades A and D among the different transmission groups in this study (table 1) suggests there is no difference in the relative rates of mother-to-child transmission of these 2 clades. There was not a great enough representation of clades B, C, and F in this sampling, however, to enable us to make a statement concerning the relative transmission rates of these clades in our cohort. Although clades B, C, and F were not represented well, perinatal mother-to-child transmission rates in Kenya, at ~25% [2], are comparable with rates in other parts of the world. More specifically, in the absence of any interventions, the perinatal transmission rates of typically North American and European B clade virus is ~19%-25% [11]. In South Africa, where clade C virus is most prevalent, the perinatal transmission rate is 24% [12]; in Thailand, where clade E is predominant, the perinatal transmission rate is 24.2% [13]. That perinatal transmission rates from around the world are so similar despite the prevalence of different clades suggests that HIV-1 viral clade does not have an effect on the rate of transmission of the virus from mother to child. The apparently high prevalence of recombinant and mixed infections in the population is a consequence of the presence of several HIV-1 clades in Kenya [14]. Thus, it is not surprising that individuals infected with >1 subtype of the virus have been described [10]. The small number of recombinant infections seen using this technique (5) as opposed to mixed infections (9) is probably a consequence of studying only recombination of the p24 and protease genes (and of only using a screening technique to detect them). The relative closeness of these 2 genes on the HIV-1 genome (<1 kbp) would indicate that crossover events occurring between the genes would not occur as frequently as between the more distantly situated protease and env genes. This method probably only identifies a small portion of the recombination events actually taking place, thus making our estimation of the number of recombinant infections artificially low. In addition, because RFLP is a screening technique for clade identification, sequencing of mixed and recombinant infections is required for confirmation.

The presence of a small number of clade B infections (2) in our population indicates that clade B is present, albeit in small numbers, in Kenya’s general population. This finding is significant because this is the first time clade B HIV-1 has been identified in the Nairobi cohort and is a further indication of the effect of travel on the spread of HIV-1 [6].

There were 24 cases in which the clade could not be assigned when RFLP analysis was indeterminate, despite sequencing of the protease gene. Clades could not be assigned as the sequences did not cluster specifically with a single clade. The inability to determine the clade of these samples may be due to the availability of an inadequate number of comparative protease sequences. Recombination within the protease gene may also be responsible for producing chimeras to which clades could not be assigned. Further, the presence of multiple clades in some areas, coupled with the high spontaneous mutation rate seen in HIV-1 replication, ultimately results in a myriad of viral quasispecies that may or may not cluster with a defined HIV-1 clade.

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**References**


