In Uganda, the HIV Network for Prevention Trials (HIVNET) 012 study recently demonstrated that single-dose nevirapine (Nvp) prophylaxis is effective for preventing mother-to-child transmission (MTCT) of human immunodeficiency virus type 1 (HIV-1). This exploratory study examines the relationship between HIV-1 subtype, MTCT, and the development of Nvp resistance (NvpR) in women enrolled in HIVNET 012. For 102 women (32 whose infants were HIV-1 infected by age 6–8 weeks and 70 whose infants were uninfected), HIV-1 subtypes included 50 (49%) subtype A, 35 (34%) subtype D, 4 (4%) subtype C, 12 (12%) recombinant subtype, and 1 unclassified. There was no apparent difference in the rate of MTCT among women with subtype A versus D (adjusted odds ratio [OR], 1.24; 95% confidence interval [CI], 0.45–3.43). NvpR mutations were detected more frequently at 6–8 weeks postpartum in women with subtype D than in women with subtype A (adjusted OR, 4.94; 95% CI, 1.21–20.22). Additional studies are needed to further define the relationship between HIV-1 subtype and NvpR among women receiving Nvp prophylaxis.

Little is known about the relationship between human immunodeficiency virus type 1 (HIV-1) subtype (clade) and HIV-1 transmission or pathogenesis. In Uganda, subtypes A and D account for most HIV-1 infections and are present at similar rates [1, 2]. We determined the HIV-1 subtypes of Ugandan women enrolled in a clinical trial of nevirapine (Nvp) prophylaxis to prevent mother-to-child transmission (MTCT) of HIV-1. In the HIV Network for Prevention Trials (HIVNET) 012 study, pregnant Ugandan women received a single dose of Nvp at the onset of labor, and infants received a single dose of Nvp within 72 h of birth. That regimen significantly reduced the rate of HIV-1 MTCT [3, 4]. The efficacy, simplicity, and low cost of the HIVNET 012 Nvp regimen make it attractive for use in resource-limited settings [5]. Here, we examine the relationships among HIV-1 subtype, MTCT, and the development of Nvp resistance (NvpR) in women who received Nvp prophylaxis during the HIVNET 012 study.

Methods

Study visits and results for HIVNET 012. Detailed methods and results of HIVNET 012 are reported elsewhere [3]. Women...
had not received prior antiretroviral therapy and did not receive antiretroviral therapy after the single dose of Nvp, which is consistent with the standard of care in Uganda. Among 311 women receiving Nvp, there were 320 live births (8 multiple births); nearly all women breast-fed [4]. HIV-1 infection was diagnosed in infants before age 18 months by HIV-1 RNA polymerase chain reaction (PCR) and was confirmed by an additional HIV-1 RNA PCR or HIV-1 culture. Of the 320 infants, 49 were infected with HIV-1, despite Nvp prophylaxis, 37 (including one set of twins) of them by age 6–8 weeks [4].

Subtype analysis study subjects. In HIVNET 012, plasma samples were collected from women at 7 days and 6–8 weeks after delivery. Samples collected 6–8 weeks after delivery were used for analysis for Nvp<sup>a</sup> in a previous study [6]. The same samples were used for HIV-1 subtyping in this report. The subtype analysis study subjects included women whose infants were infected with HIV-1 by age 6–8 weeks, despite Nvp prophylaxis. Samples obtained 6–8 weeks after delivery were available from 33 (92%) of 36 of those women. We also analyzed HIV-1 from a random sample of 72 women who received Nvp whose infants were uninfected and alive at age 6–8 weeks. Women with uninfected infants were excluded if their virus load was <2000 HIV-1 RNA copies/mL at baseline or at 6–8 weeks after delivery, to provide sufficient HIV-1 RNA for analysis.

Phylogenetic analysis of HIV-1 subtypes. HIV-1 sequences corresponding to protease amino acids 1–99 and reverse-transcriptase (RT) amino acids 1–324 (297 and 972 nt, respectively) were obtained from plasma HIV-1 in a previous study [6] (GenBank accession nos. AF388065–AF388166). For HIV-1 subtyping, we used the biase editor Seqapp v1.9a169 (available at http://iubio.bio.indiana.edu/soft/molbio/seqapp/) to create nucleotide alignments. Alignments included reference sequences recommended by the Los Alamos National Laboratory for HIV-1 subtype analysis (http://hiv-web.lanl.gov/). Alignments were performed manually and did not require gap striping. Distances between the sequences were calculated with DNADist, using the Kimura-2 parameter as an optimal substitution model with a transition-transversion ratio of 1.5. (PHYLIP, version 3.572; available at http://evolution.genetics.washington.edu/phylip.html). Neighbor-joining and consensus were used to create phylogenetic trees with 500 bootstrap replications (SeqBoot). Consensus trees were displayed with TreeView [7]. Bootstrap values >80 were considered to be acceptable for subtype assignment.

Statistics. We evaluated the association of subtype with Nvp<sup>a</sup> and MTCT by logistic regression analysis, adjusting for baseline covariates such as virus load and CD4 cell counts. All statistical analyses were done with SAS software (version 8.1; SAS Institute).

Results

HIV-1 subtypes of women in HIVNET 012. Amplification and sequencing of HIV-1 protease and RT were successful for 102 of 105 available samples, including those from 32 of 33 women whose infants were infected at age 6–8 weeks and from 70 of 72 women whose infants were uninfected at that age (table 1). Subtypes were clearly assigned for 29 of 32 women with HIV-1–infected infants and for 60 of 70 women with uninfected infants: subtype A, 50; subtype D, 35; and subtype C, 4. The pol sequences from 12 of the remaining 13 women were identified as recombinant by the Recombinant Identification Program (available at http://hiv-web.lanl.gov/). Individual segments within those pol regions (varying in length and position among the women studied) were confirmed as either subtype A or D by further phylogenetic analysis. Detailed analysis of the recombinant sequences will be presented elsewhere. The subtype of HIV-1 from one sample could not be determined.

Relationship between HIV-1 subtype and MTCT. The HIV-1 subtyping analysis described above included 32 women whose infants were HIV-1 infected by age 6–8 weeks and 70 women whose infants were not HIV-1 infected (table 1). In this selective subsampling of women in the HIVNET 012 trial, the relative proportion of women with HIV-1–infected infants was similar for those with subtype A (15 of 50) versus subtype D (11 of 35) (odds ratio [OR], 1.24; 95% confidence interval [CI], 0.45–3.43). Three of 4 women with subtype C had HIV-1–infected infants, but the numbers were too small for meaningful statistical analysis.

Relationship between HIV-1 subtype and Nvp<sup>a</sup>. Sequences

<table>
<thead>
<tr>
<th>Transmission status</th>
<th>A</th>
<th>D</th>
<th>C</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>K103N</td>
<td>2</td>
<td>2</td>
<td>3</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>K103N+Y181C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>K103N+Y181C+V106A</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Y181C</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>V106I</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>No. with mutations</td>
<td>3</td>
<td>3</td>
<td>7</td>
<td>1</td>
<td>7</td>
</tr>
<tr>
<td>No. without mutations</td>
<td>12</td>
<td>32</td>
<td>17</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>15</td>
<td>35</td>
<td>11</td>
<td>24</td>
<td>32</td>
</tr>
</tbody>
</table>

NOTE. Data are no. of women infected with A, C, D, or other (recombinant or unclassified) HIV-1 subtype with each mutation or combination of mutations. Nvp<sup>a</sup> mutations were detected in plasma samples collected from women 6–8 weeks after delivery (6–8 weeks after Nvp administration). These women had infants who were infected (+) or who were not infected (−) with HIV-1 by age 6–8 weeks.

Table 1. Nevirapine resistance (Nvp<sup>a</sup>) mutations detected in women in the HIV Network for Prevention Trials 012 Study, by transmission status and human immunodeficiency virus type 1 (HIV-1) subtype.

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obtained from the analysis described were analyzed previously for the presence of Nvp* mutations (A98G, L100I, K103N, V106A, V108I, Y181C, Y188C, and G190A) [6]. Nvp* mutations were detected in 18 (18%) of 102 women at 6–8 weeks postpartum (table 1) [6]. Of note, the analysis was done with population sequencing, which often fails to detect minor variants (e.g., those that represent <25% of the virus population). In this study, we compared the rate of Nvp* mutations in women with subtype A versus subtype D. A higher proportion of women with subtype D developed Nvp* than did those with subtype A (10/35 vs. 6/50, respectively; OR, 2.93; 95% CI, 0.95–9.03). Most of the women who developed Nvp* had the K103N mutation, either alone or in combination with other Nvp* mutations [6]. The pattern of Nvp* mutations detected in women with subtypes A and D was similar.

The higher rate of Nvp* in women with subtype D did not appear to reflect more advanced disease status. Baseline (pre-Nvp) virus loads and baseline (pre-Nvp) CD4 cell counts were similar among women with subtypes A and D (median virus loads, 4.5 log_{10} HIV RNA copies/mL; interquartile range [IQR], 4.1–5.3 log_{10} HIV RNA copies/mL, vs. 4.6 log_{10} HIV RNA copies/mL; IQR, 4.0–4.8 log_{10} HIV RNA copies/mL, respectively; median CD4 cell counts, 425 cells/µL; IQR, 215–608 cells/µL, vs. 377 cells/µL; IQR, 213–530 cells/µL, respectively). However, after controlling for baseline virus load and baseline CD4 cell count, logistic regression analysis showed a substantial enhancement in the OR for the development of Nvp* in women with subtype D versus subtype A (adjusted OR, 4.94; 95% CI, 1.21–20.22). We previously demonstrated an association between virus load and the development of Nvp* in this cohort [6]. Further statistical analysis of data in this report revealed that women with subtype A had a higher proportion of higher virus loads than did women with subtype D. Therefore, adjusting for virus load enhanced the OR.

Discussion

Of 102 women analyzed in this study, 50 (49%) had subtype A, 35 (34%) had subtype D, 4 (4%) had subtype C, and 12 (12%) had recombinant HIV-1, which is similar to the distribution of subtypes found in Uganda in recent epidemiologic studies [1, 2]. Because we analyzed the subtypes of the HIV-1 pol region only, it is possible that the subtypes of other HIV-1 genes may differ, reflecting the high rate of HIV-1 intersubtype recombination in Uganda [8]. The relatively high rate of recombination we observed in the pol region is not surprising, since subtypes A and D cocirculate in Uganda. Recombination between different HIV-1 subtypes likely occurs in the setting of dual HIV-1 infection. We have documented dual infection with subtypes A and D in Uganda in 2 pregnant women and an infant [9, 10].

Recent reports compared the rate of disease progression in persons infected with subtype A versus subtype D. One study of women in Senegal with non-A subtypes (C, D, or G) found that the women were more likely to develop AIDS within 5 years of infection than were women with subtype A [11]. Two other studies [12, 13], including one from Uganda [13], found no significant difference in disease progression in persons with subtype A or D. In our cohort, virus loads and CD4 cell counts before Nvp administration were similar among women with subtypes A and D. Because clinical outcome of women in HIVNET 012 was monitored for only 6–8 weeks, it was not possible to evaluate the relationship of HIV-1 subtype with clinical outcome.

We found no difference in the rate of MTCT in women with HIV-1 subtype A or D, which is consistent with a recent study from Kenya, which found no significant difference in the frequency or mode of MTCT in women with HIV-1 subtypes A or D [14]. More extensive studies are needed to define further the role of HIV-1 subtype on MTCT in different clinical settings.

We observed a higher rate of Nvp* in women with subtype D than in those with subtype A after single-dose Nvp prophylaxis. However, this analysis was based on relatively few women. Confirmation in larger studies is needed to evaluate further the influence of subtype on selection of Nvp* after single-dose Nvp prophylaxis. A higher rate of Nvp* in women with subtype D HIV-1 could reflect a higher replication rate of subtype D HIV-1. HIV-1 subtypes also differ in the frequency of amino acid polymorphisms at positions associated with antiretroviral drug resistance. Such differences may influence the fitness of HIV-1 with antiretroviral drug resistance mutations, such as K103N. Fitness differences could favor the selection of resistant variants or could help to maintain those variants in plasma after an antiretroviral drug is discontinued. Our finding of a higher rate of Nvp* in women with subtype D 6–8 weeks after single-dose Nvp prophylaxis could reflect more frequent selection of Nvp-resistant variants or more sustained circulation of those variants in women after delivery. Further studies are needed to evaluate the relative fitness of subtype A versus subtype D with Nvp* mutations and to compare the kinetics of emerging and fading of those variants in women receiving Nvp prophylaxis. Studies are underway to evaluate the selection of Nvp* and clinical outcome of infants in HIVNET 012 infected with subtypes A or D HIV-1.

The HIVNET 012 Nvp prophylaxis regimen is being implemented worldwide. If findings from this exploratory study are confirmed, they would suggest that the rate of Nvp* may vary among women receiving the regimen, depending on the subtypes prevalent in a particular geographic region. The impact of Nvp* on women receiving single-dose Nvp prophylaxis to prevent HIV-1 MTCT is not clear. Our previous analysis of women who received Nvp prophylaxis in HIVNET 012 revealed that Nvp* faded from detection over time and was not associated with a higher risk of MTCT with the first use of Nvp prophylaxis [6]. We also found little evidence for transmission of Nvp-resistant HIV-1 from women to their infants [6]. Currently, no evidence exists that Nvp* influences clinical progression of HIV-1 infection. In countries where the HIVNET
012 regimen is most likely to be implemented, treatment options for HIV-1 infection are extremely limited. If treatment options were expanded in the future in those countries, women with NvpR could be offered treatment with other antiretroviral drugs. The potential selection of NvpR in women receiving the HIVNET 012 regimen must be balanced against the simplicity, efficacy, and cost-effectiveness of the regimen. Implementation of this regimen could prevent HIV-1 infection in millions of HIV-1 exposed infants over the next decade.

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