Maternal-Fetal DNA Admixture Is Associated with Intrapartum Mother-to-Child Transmission of HIV-1 in Blantyre, Malawi

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Background. The mechanism of mother-to-child transmission (MTCT) of HIV-1 is not well described.

Methods. Of 328 HIV-infected mother-infant pairs, we identified 91 that had discordant angiotensin I–converting enzyme and glutathione S-transferase M1 alleles. Maternal alleles in cord blood were quantified with real-time polymerase chain reaction, as indicators of microtransfusions.

Results. HIV-1 infected infants had more maternal DNA in cord blood than their uninfected counterparts. Increased maternal DNA in cord blood was associated with preterm delivery, low birth weight, and maternal immunosuppression.

Conclusion. Intrapartum MTCT was associated with placental microtransfusions. The associations among placental microtransfusions, in-utero MTCT, maternal immunosuppression, and poor birth outcome should be further investigated.

Although the rate of mother-to-child transmission (MTCT) of HIV-1 has been significantly reduced in the developed world, in 2006 there were an estimated 530,000 children newly HIV-1 infected [1]. Among populations for whom replacement feeding is unfeasible, it has been estimated that 12% of MTCT occurs in utero before 36 weeks’ gestation, 29% occurs between 36 weeks’ gestation and delivery, 20% occurs during delivery, and the remaining 39% occurs during prolonged breast-feeding [2]. Remarkably, despite this wealth of knowledge about the timing of MTCT of HIV-1, the mechanism of transmission remains unclear.

For in-utero and intrapartum transmission, suggested routes of HIV-1 transmission include ascending infection, exposure to HIV-1 in the birth canal, and passage through the placental barrier. By measuring placental alkaline phosphatase in umbilical cord blood, our group has previously found an association between placental microtransfusions and intrapartum MTCT [3]. To better understand microtransfusions, we assessed them by quantifying the amount of maternal DNA in umbilical cord blood.

Materials and methods. Participants presenting to the Antenatal Ward at Queen Elizabeth Central Hospital, Blantyre, Malawi were enrolled into a prospective cohort study designed to assess the relationship between malaria and MTCT of HIV-1 [1–3]. Women and their newborn infants received single-dose therapy with nevirapine, in accordance with the HIV Network for Prevention Trials (HIVNET) 012 protocol [4]; no women received ongoing antiretroviral treatment. Mother-infant pairs were included in this substudy if the mother delivered a live, singleton child by vaginal delivery and if matched umbilical cord and maternal peripheral blood samples were available. Infant HIV status was determined by real-time polymerase chain reaction (PCR) against HIV-1 DNA, as described elsewhere [5], in accordance with the methods of Luo et al. [6]. Infants were considered to have been infected with HIV-1 in utero if they were HIV-1 DNA positive within 48 h of birth; they were considered to have been infected with HIV-1 intrapartum if they were both HIV-1 DNA negative at birth and HIV-1 DNA positive at 6 weeks; and they were considered HIV-1 negative if they were HIV-1 DNA negative at 6 weeks [7]. N values in this section of the text are provided when data were missing for >5 participants. This study was approved by the University of North Carolina–Chapel Hill institutional review board and the Malawi College of Medicine Research Ethics Committee; informed consent was obtained from all participants.

Samples were prepared as follows. Immediately after delivery, the umbilical cord was clamped and cut. The umbilical cord was cleaned with saline, the umbilical vein was located, and blood
was aspirated by use of a needle and syringe. Whole blood was put into tubes containing EDTA, centrifuged in a clinical centrifuge at 300 g for 5 min, and the plasma was removed. Whole blood pellets were stored at −80°C until processed. Genomic DNA was isolated from maternal peripheral blood samples and from venous umbilical cord blood pellets by use of the Qiagen DNA Mini Kit, in accordance with to the manufacturer’s instructions. Matched maternal and umbilical cord genomic DNA were amplified with PCR for the polymorphic glutathione S-transferase M1 gene (GSTM1) and angiotensin I converting enzyme intron 16 (ACE) gene, in accordance with the methods described by Lo et al. [10] using the primers listed in table 1. When the maternal sample had an ACE allele that was not present in the infant, this was defined as an informative ACE pair. Matched pairs that were uninformative for ACE were screened for a GSTM1 insertion, and samples were considered GSTM1-informative when the maternal sample was GSTM1 insertion—positive and the infant was GSTM1 insertion—null; only GSTM1 insertion—null infants whose umbilical cord blood also supported the amplification of an ACE allele were included.

Quantitative real-time PCR (qPCR) (PE Biosystems 7300) was performed on the umbilical cord genomic DNA against the informative maternal ACE or GSTM1 allele by use of the primer sets and probes listed in table 1. The thermal profile consisted of an initial incubation at 50°C for 2 min, then 95°C for 10 min, followed by 50 cycles of 95°C for 15 seconds and 56°C for 1 min, in a reaction volume of 25 μL (5 μL template DNA, 2 μmol/L of each primer, 0.8 μmol/L probe, and 12 μL of Taqman Universal PCR Master Mix [Applied Biosystems]). All reactions were run in duplicate along with no-template control reactions. Serial dilutions of GSTM1, ACE insertion—containing, or ACE deletion—containing maternal DNA demonstrated that the assay was linear over 3 logs (R² = 0.99). To control for the total amount of DNA in the umbilical cord blood, all qPCR reactions were normalized to RNase P concentration by use of the TaqMan RNase P Control Reagent Kit (Applied Biosystems).

The efficiency of each assay was calculated using the slope of each assay’s calibration curve, in accordance with the methods described by Dorak [8]. Because the efficiencies of the target and reference genes were all within 5% of each other (data not shown), the comparative threshold (Ct) method was used to quantify the amount of maternal-specific allele in the umbilical cord DNA [8] by use of the following equation:

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\text{maternal-fetal DNA admixture} = \frac{\text{fractional concentration}}{2^{\frac{\text{average target gene Ct} - \text{average RNaseP Ct}}}}
\]

To account for any differences between the GSTM1, ACE-insertion and ACE-deletion qPCR assays, we identified 3 maternal DNA samples positive for all 3 target alleles. Serial dilutions of these samples were run in duplicate, against all 3 assays, to determine interassay accuracy. The GSTM1 and ACE deletion reactions gave similar dilution profiles, whereas the ACE insertion profile consistently resulted in lower Ct values than the other 2 assays. This discrepancy was corrected by multiplying all ACE-insertion values by 0.895, which normalized the 3 informative-gene assays (figure 1).

Samples were excluded from data analysis if there was no detectable amplification in both the informative gene and the RNase P assay (n = 4). Samples that had detectable amplification in the RNase P assay but lacked detectable amplification in the informative gene assay (n = 42) were rerun using 4 times more genomic DNA. The limit of detection for the informative gene assays was estimated to be Ct = 40; samples below this limit were all assigned Ct values of 40 (n = 42).

Because of likely differences in the mechanism of transmission, in-utero and intrapartum transmission were analyzed as independent outcomes. DNA admixture data were presented as a fractional concentration, defined as the ratio of the normalized target gene (ACE or GSTM1) to RNase P, as described above. The relationship between the DNA admixture and dichotomous variables was tested with the Wilcoxon rank sum test. Binary coding for the following variables was created by dichotomization at the median: duration of labor (10 h) (n = 89), placental delivery time (5 min) (n = 90), high HIV-1 RNA concentration (>4.2 log10 copies/mL; n = 56), and low placental weight (<540 g) (n = 91). Women were tested for syphilis using the rapid plasma reagin test (RPR), and all RPR-reactive serum samples were tested with the Treponema pallidum hemagglutination assay (TPHA) (n = 91). Women with a RPR-reactive result followed by a TPHA-reactive result were considered syphilis seroreactive. Placental malaria parasitemia (91 participants tested) and chorioamnionitis were determined via placental histology, as described in Mwapasa et al. [9]; only 47 of the 91 participants were examined for chorioamnionitis. All other clinical characteristics were determined by the study nurse at the time of delivery. Associations with P < .2 are noted in Results. All analyses were run on Stata (version 10.0, Stata).

**Results.** Matched maternal and umbilical cord genomic DNA was available from 328 of the 565 mother-infant pairs who met the eligibility criteria. Of the 328 samples available, 91 (28%) were ACE or GSTM1 informative. The percentage of informative pairs is consistent with the findings of Lo et al., who detected
32% informative pairs in their population of 156 matched pairs [10]. If we dichotomize the fractional concentration of maternal DNA at 10^−2, where there is a natural inflection of the data, 39 (43%) of the 91 infants had an appreciable amount of maternal DNA in their umbilical cord blood; this result is similar to the 40% reported by Lo et al. [10].

Among the 91 informative mother-infant pairs, 69 infants were exposed but uninfected, 13 infants were infected in utero, and 9 infants were infected intrapartum. The median log_{10} fractional concentrations of maternal DNA in umbilical cord blood were −4.9, −2.6, and −1.3 for uninfected infants, infants infected in utero, and infants infected intrapartum, respectively (figure 2A). As expected, DNA admixture was significantly higher in the umbilical cord blood of intrapartum-infected infants than in that of uninfected infants (P = .02). The difference between infants infected in utero and uninfected infants was not as robust (P = .06).

DNA admixture was also associated with poor birth outcomes. Among all 91 DNA isolates from umbilical cord blood, increased amounts of maternal-fetal DNA admixture were associated with low birth weight (median log_{10} fractional concentration in samples obtained from low birth weight infants, −1.3 vs. −4.8 copies/mL; P = .02) and preterm delivery (median log_{10} fractional concentration during preterm delivery, −1.3 vs. −4.8; P = .005).

Immunological status also appeared to influence microtransfusions. A higher fractional DNA concentration was found in the umbilical cord blood of infants born to mothers with low CD4 T cell counts (<200 cells/mL; P = .04), compared with infants born to mothers with high CD4 T cell counts. However, no difference in the fractional concentration of maternal DNA was found when women with high HIV-1 concentrations were compared to women with low HIV-1 concentrations (data not shown).

Obstetric factors such as delayed placental delivery, visible placental tears, sex of the newborn, vaginal and/or vulval tears, episiotomy, duration of membrane rupture >4 h, labor >10 h, primigravidity, and low placental weight were not associated with DNA admixture (P > .2). In addition, other infections such as chorioamnionitis (16 cases), syphilis (1 case), and placental malaria (5 cases) were also not associated with DNA admixture, although the sample sizes were small. The relationship

**Figure 2.** Box and whisker plots of factors associated with log_{10}transformed maternal-fetal DNA admixture. A, HIV-1 vertical transmission status; B, preterm delivery (<37 weeks gestation); C, low CD4 T cell counts (<200 cells/mL); D, low birth weight (<2500 g). Line, median value; box edges, 25th and 75th percentile; whisker edges, upper adjacent values; dots, outside values. P values were determined by the Wilcoxon rank sum test. NT, uninfected infant; IU, infant infected in utero; IP, infant infected intrapartum.
between these infections and MTCT of HIV-1 in the parent cohort is described elsewhere [5].

**Discussion.** In both breast-feeding and non-breast-feeding populations, a majority of MTCT of HIV-1 occurs at or around the time of delivery through unknown mechanisms [2]. The 2 most likely routes of intrapartum HIV-1 infection are birth canal exposure or transplacental passage, and in this study, we observed that maternal-fetal DNA admixture was associated with intrapartum MTCT. This observation is consistent with our previously reported findings that linked elevated umbilical cord placental alkaline phosphatase with intrapartum MTCT [3]. Like umbilical cord placental alkaline phosphatase activity, we interpret maternal-fetal DNA admixture as a measurement of placental microtransfusions, and therefore, we conclude that microtransfusions increase the risk of intrapartum MTCT of HIV-1.

In a previous study by Lo et al. [10], separation of theuffy coat from the plasma allowed the investigators to determine the amount of both cell-associated and cell-free maternal DNA in the umbilical cord. Because we extracted genomic DNA from pelleted whole blood, we were unable to determine whether the maternal DNA was cell-derived, which would have provided an important clue to the nature of the microtransfusions (i.e., whether the microtransfusions contained maternal cells). A second limitation of these data is that the associations detected between MTCT and DNA admixture are derived from transmission groups with small sample sizes, and therefore they should be interpreted with caution. Although this presents difficulty in the interpretation of the nonsignificant association between DNA admixture and in-utero MTCT, the significant association with intrapartum MTCT is consistent with our previously published findings [3]. Finally, also as a result of the small sample size, we were unable to do multivariate modeling, which precluded adjustment of the data for covariates known to be associated with MTCT of HIV-1.

In conclusion, when combined with our previous microtransfusion findings [3], this study supports placental microtransfusions as a mode of intrapartum MTCT of HIV-1. In an attempt to synthesize these data with the existing literature, we propose the following model of MTCT: low CD4 T cell counts, through an unknown mechanism, compromise the maternal-fetal placental barrier, which, in turn, leads to both poor birth outcomes and placental microtransfusions. The resulting placental microtransfusions allow the passage of HIV-1 directly into the infant bloodstream, leading to increased rates of MTCT of HIV-1. The nature of the placental insult remains ill defined, but it could arise either through the physical stress of labor and delivery or via specific infections, such as malaria, chorioamnionitis, or syphilis; alternatively, the nature of the insult could be less important than the inflammation itself, which may provoke a common inflammatory response, such as tumor necrosis factor-α production [11, 12]. The novel association between placental microtransfusions, in-utero MTCT, low CD4 T cell count, and poor birth outcome is intriguing and it, along with our model, should be evaluated in a larger study.

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**References**


