Genomic human immunodeficiency virus type 1 RNA variation in mother and child following intra-uterine virus transmission

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In order to study the relationship between virus populations in a human immunodeficiency virus type 1 (HIV-1)-infected mother and her infant, we analysed a 276 bp fragment, including the V3 region, of genomic HIV-1 RNA purified from serum. Samples were collected from the mother 6, 4 and 2 months prior to delivery, during delivery and 10 months after childbirth (samples MA to ME, respectively) and from the infant at birth (cord blood) and the ages of 6 weeks and 9 months. A heterogeneous sequence population was observed in the maternal samples (mean nucleotide variation of 2.4 to 4.2%, range 0 to 8.3%). Until the age of 6 weeks the sequence population in the infant was highly homogeneous (mean nucleotide variation < 0.7%, range 0 to 2.5%). At 9 months of age, the infant’s virus population showed more heterogeneity (mean nucleotide variation of 1.8%, range 0.4 to 3.6%) and a drift in the consensus sequence was observed. The evolution of the V3 region in the mother was characterized by accumulation of amino acid substitutions diverging from the virus population observed in the infant. The mean nucleotide distance between the maternal sequence populations and the sequence population of the child at birth was 2.8, 2.6, 3.7, 5.2 and 5.3% for the samples MA, MB, MC, MD and ME, respectively. Nearly complete replacement at position 308, previously described as antigenically important, from a proline to a histidine was observed during pregnancy, whereas all clones of the child’s virus at birth and at 6 weeks contained a proline at that position. In conclusion, intra-uterine transmission is associated with a homogeneous sequence population in the child at birth, which is more closely related to the sequence population present in the mother during the first and second trimester of pregnancy than to the sequence population at delivery.

Introduction

Genetic analysis of human immunodeficiency virus type 1 (HIV-1) strains following sexual or parenteral transmission generally shows a genetic distance between the consensus sequence of the virus donor and virus recipient of less than 1% (Wolfs et al., 1992; Kleim et al., 1991; McNearney et al., 1990). The virus population of the recipients at seroconversion is extremely homogeneous, indicating that a limited number of variants or even one particular variant had initiated the infection in the new host. The high similarity between the consensus sequence of donor and recipient fits with transmission of randomly selected variants present in the donor.

Following vertical transmission, Wolinsky et al. (1992) observed that, in two out of three mother–infant pairs studied 9 to 14 weeks after childbirth, the variant transmitted to the infant represented a minor genotype in the maternal sequence set. They suggested that particular variants could be preferentially transmitted, such as variants that escape the maternal immune response or with tropism for certain cells. It has been demonstrated that the HIV-1 envelope shows genetic variation within a person over time (Wolfs et al., 1990, 1991; Holmes et al., 1992). In perinatally infected children, transmission may have occurred many months before birth (Courgnaud et al., 1991; Lewis et al., 1990; Mano & Chermermann, 1991). It cannot be discounted, therefore, that the virus population observed in the child at birth represents a major variant in the mother at the actual time of transmission earlier in pregnancy. In order to test this hypothesis, we have examined sequentially collected samples from mother and child. Viral RNA was purified from serum and cDNA was amplified by PCR. A region of 276 bp, including the V3 region, was analysed.

Methods

Sera. Sequential sera were collected from an HIV-1 seropositive woman and her infected infant, who were participating in the Dutch prospective study of HIV-seropositive women and their children. The

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mother was asymptomatic (CDC stage II), p24 antigen-negative (Abbott Laboratories) and CD4 counts were between 10^4 and 0.5 x 10^5/l during pregnancy and between 0.4 x 10^4 and 0.5 x 10^5/l up to 25 months after childbirth. Because of a dubious positive serology for syphilis, penicillin was administered during the second trimester of pregnancy. Apart from this, the pregnancy was uncomplicated. The birth weight was 2820 g and she showed no signs of congenital syphilis. She was classified as P1-B (asymptomatic, immunological disorders) up to the age of 25 months; p24 antigen was detectable from the age of 6 weeks.

The maternal samples examined were collected 6, 4 and 2 months prior to delivery (corresponding to 12, 21 and 21 weeks of gestation and called, respectively, samples MA, MB, MC), during delivery (sample MD) and 10 months after childbirth (sample ME). The child’s samples were collected at birth (cord blood, sample TA), and at the ages of 6 weeks (sample TB) and 9 months (sample TC).

Molecular cloning of the V3 region. Genomic HIV-1 RNA was isolated from 50 µl of serum, according to Boom et al. (1990). Conversion of viral RNA to cDNA, amplification by nested PCR, cloning and extraction of plasmid DNA were performed as described (Wolfs et al., 1991) with some slight modifications in the reverse transcription (RT) reaction and amplification by PCR. One fourth of the isolated RNA was converted to cDNA in an RT mixture containing 10 pmol of the 3'V3 NOT primer (5' GATTTAGGTGACACCTATAGGGTCGCCCTTCCTGAGGA 3', 0.4 pmol) and the 5'KSI primer (15 pmol) under the same conditions as described above. Amplification was performed for 20 cycles. Each cycle consisted of 30 s at 96 °C, 1 min at 50 °C and 2 min at 72 °C.

Consensus sequence. A 276 bp fragment, including the whole V3 region, was analysed (HXB2 positions 7031 to 7312, Los Alamos 1990). A consensus sequence was made by assigning the nucleotide or deduced amino acid most frequently found in the individual clones to each position. Consensus sequences were based on 11 to 22 clones.

For the direct sequences, the deduced amino acid sequence at heterogeneous positions was based on the highest peaks confirmed in both the forward and reverse direction, or on the highest peak in one direction when comparable peaks were observed in the other direction. For positions with comparable peaks in both directions, or discordant peaks in forward and reverse direction, no consensus was indicated.

Calculations. All calculations were performed with nucleotide sequences. Pairwise comparisons were performed to establish nucleotide distances between sequences. Positions where an alignment gap had to be introduced were excluded from the calculations. Intrasample sequence variation is expressed as the mean nucleotide distance of all pairwise comparisons between sequences obtained from a sample. Intersample sequence variation is expressed as the mean nucleotide distance of all pairwise comparisons between the sequences obtained from the different samples. From the samples MD and ME, sequences were obtained from two separately performed RNA extractions and cloning experiments. All the sequences obtained from these samples were used for calculation of the intra- and intersample variation. When comparisons were made between consensus sequences, positions where no consensus could be indicated (because two nucleotides were equally common) were excluded.

Phylogenetic analyses. The phylogenetic analysis programs used, the maximum-likelihood (Felsenstein, 1982) and the neighbour-joining methods (Saitou & Nei, 1987), were taken from the PHYLIP package (Felsenstein, 1989). The maximum-likelihood method was used as implemented in the DNAML program that is part of PHYLIP. For the neighbour-joining method, pairwise distances were calculated using the two-parameter estimation method described by Kimura (1981). Bootstrap analysis was performed on the neighbour-joining tree, using the programs SEQBOOT, DNADIST and CONSENSE from PHYLIP. Phylogenetic analyses were performed with a subset of the sequences. From each sample, only sequences differing by more than one nucleotide from other sequences within that sample were used. For the neighbour-joining method it was necessary to exclude identical sequences derived from different samples. The trees were plotted using the DRAWTREE program from PHYLIP.

Results

Genomic HIV-1 RNA was isolated from maternal serum samples collected 6, 4 and 2 months prior to delivery, during delivery and 10 months thereafter (samples MA, MB, MC, MD, ME, respectively). From the child, the samples were collected at birth (cord blood) and at the ages of 6 weeks and 9 months (samples TA, TB, TC, respectively). Following amplification of cDNA by PCR, a 276 bp fragment, including the V3 region, was sequenced directly as well as after molecular cloning. From the samples MD and ME, sequences were obtained from two separately performed RNA extractions and
lysine (K) at position 24, and variants which had a glutamate (E) at position 21 and variants which had a K and glutamate (E), respectively, at these positions (Fig. 1). Considering the sensitivity of the first PCR and the intensity of the bands observed (data not shown), the amount of cDNA obtained is estimated at 10 to 100 copies for the maternal samples and the cord blood sample and more than 100 copies for the samples TB and TC. From each serum sample, 11 to 22 clones of cDNA were sequenced. Fig. 1 shows the deduced amino acid sequences aligned with the consensus sequence of the cord blood sample. Inactivating mutations were observed in four out of 126 sequences (a frameshift in MB-11 and TC-11, and a stop codon in ME-10' and TC-6) corresponding to a frequency of 10⁻⁴.

**Intrasample diversity and evolution of V3 coding sequences in the mother**

The sequence population of the mother was heterogeneous with mean intrasample variations of 2-4, 2-7, 3-9, 4-2 and 3-6% (range 0 to 8-3%) for the samples MA, MB, MC, MD and ME, respectively. In each sample, subgroups of variants were observed, for example variants which had a glutamate (E) at position 21 and lysine (K) at position 24, and variants which had a K and E, respectively, at these positions (Fig. 1).

During pregnancy, a substitution at position 39 was observed. A proline predominated, both in the clones as well as in the direct sequences, until 2 months before delivery. At the time of delivery, a histidine had become predominant and remained until 10 months after delivery.

Substitutions within the V3 domain were also observed at other positions (R³⁶, V³⁸, N⁵⁹ and Q⁸⁸; Fig. 1). In the samples collected during pregnancy and delivery, only variants with a combination of some of these substitutions, i.e. R³⁶, V³⁸ and H⁵⁹ or R³⁶, H³⁸, N⁵⁹ and Q⁸⁸, were observed (i.e. MC-3, MD-5 and MD-9; Fig. 1). The majority of the sequence population (16/20 sequences) of the sample collected 10 months after childbirth was characterized by variants containing all of these substitutions (Fig. 1, sample ME). In the sample collected at the time of delivery, two major groups of sequences were observed. One group was characterized by sequences identical or closely related (nucleotide distance < 1%) to the sequence of clone MD-5 (representing 45% of the sequence population) and the other by sequences identical or closely related to MD-20 (representing 23% of the sequence population). The nucleotide distance between the sequences MD-5 and MD-20 was 6-9%. Ten months later, variants closely related to the sequences MD-5 or MD-20 were no longer observed (sample ME; Fig. 1). Based on the 5' regions of the sequence population of sample ME, two groups of sequences could be distinguished. They differed from each other at positions 9 (silent mutation), 21, 23, 24 and frequently at positions 1 and 28. Closely related 3' regions were observed in both groups of sequences (i.e. ME-5 and ME-1'). Variants with a 5' region (up to position 54) comparable to these two groups of sequences had already been observed in earlier samples (i.e. compare MC-3 with ME-1, MD-19 with ME-8' and MD-20,8' with ME-1'). However, when comparing the 3' regions of these sequences with the corresponding variants of the ME sample, considerable differences were noticed. They differed at least at five (MC-3 and ME-6,10) but mostly at eight to nine positions (i.e. MC-3 and ME-1 or MD-20,8' and ME-1'; Fig. 1). Variants with identical 5' regions and very different 3' regions were also observed in the sequence set of samples MC and MD (i.e. MC-7 and MC-5, nine substitutions). It is noteworthy that no variants intermediate between the different sequences were observed.

Interestingly, we observed sequences with a 5' region (nearly) identical to some variants, whereas the 3' region was identical to other variants: variants ME-6 and ME-10 (combination of a 5' region of variants like MC-3 with a 3' region, starting from positions 61 to 63, of variants such as MD-9), and MD-5' (combination of a 5' region of variants like MD-7 and a 3' region, starting from positions 61 to 65, of variants such as MD-6).

In addition to sequences of molecular clones, direct sequences were also obtained. The method used was found to be unsatisfactory for determining the frequency of each nucleotide at heterogeneous positions. The height of the peaks was found to be dependent on the kind of nucleotide and the position in the sequence. For example, at position 55 it was observed in the clonal sequences that the G peak of the codon GAT (encoding D) was much higher than the first A peak of the codon AAA (encoding K). The same was true for position 24 (codon GAA, encoding E or codon AAA, encoding K). This could explain the consistently observed difference between the consensus sequences and direct sequences at these positions (Fig. 1). However, when judged at particular positions, the direct sequences confirmed the observation in the clonal sequence population. For example, the clonal sequence sets of samples MC and MD were typically heterogeneous at position 20 (N or K) and 22 (A or T). Heterogeneity at these positions was also only observed in the direct sequences of these samples and not in the direct sequences of the other samples (Fig. 1). The same was found for position 38 (V or I) for the sequence sets of samples MD and ME.
Intrasample diversity and evolution of the V3 coding sequences in the child

Up until the age of 6 weeks a very homogeneous sequence population was observed in the infant, with a mean intrasample variation of 0.7% (range 0 to 2.5%) and 0.6% (range 0 to 1.5%) for the samples TA and TB, respectively. One particular sequence (identical to TA-6 and TB-1) predominated in both samples (Fig. 1). Differences between this major variant and the minor variants were mainly caused by randomly occurring substitutions. Only a minority of the substitutions were observed in more than one sequence of the infant's sequence sets (D₂₀, G¹⁷, N₅⁵ and R⁷₀; Fig. 1). The results of the direct sequences were in agreement with a very homogeneous sequence population: positions with double peaks confirmed in both the forward and reverse direction were not observed in sample TA, and only at one position in sample TB (position 70: K or R). This homogeneous population did not appear to be due to amplification of a limited number of cDNAs and, subsequently, sequencing of identical copies. The first PCR of both samples yielded a visible product on an ethidium bromide-stained agarose gel, corresponding to
Fig. 2. Distribution of nucleotide sequence similarities within the maternal (a) and infant's (b) sequence sets. For each sample, the fraction of the total number of pairwise comparisons with a given percentage similarity are shown. For example, if 91 pairwise comparisons were obtained from a sample and 10 comparisons showed a nucleotide variation of 1%, the fraction with a similarity of 99% is 11% (10/91). For reasons of clarity, only the results for the maternal samples MA (the most homogeneous sample) and MD (the most heterogeneous sample) are shown. The total numbers of pairwise comparisons were 91, 231, 105, 55 and 136 for, respectively, the samples MA, MD, TA, TB and TC. (a) ---, 6 months before delivery; --, at delivery. (b) ---, cord blood; ---, 6 weeks; ..., 9 months after birth.

The virus circulating at 9 months of age was more heterogeneous than at earlier time points, with a mean intrasample distance of 1.8% (range 0.4 to 3.6%). Fig. 2 shows, in a similarity histogram, the intrasample genetic diversity of the maternal samples MA and MD, representing, respectively, the most homogeneous and heterogeneous maternal sequence set as well as the samples from the child. From this it is clear that the distribution of differences between sequences within a sample is much broader in the maternal samples than in the samples from the child.

The predominant variant present in the samples TA and TB was no longer observed in the sample collected 9 months after birth (sample TC, Fig. 1). Compared to the predominant variant of the samples TA and TB, the sequence population of sample TC frequently contained amino acid substitutions at six positions, namely V to I, K to E, D to N, T to R, K to R and A to V (Fig. 1). The substitutions at positions 55 and 70 had already been observed in the sequence population of sample TB (TB-4 and TB-12; Fig. 1).

Fig. 3. Nucleotide distance between the predominant sequence present in the cord blood, presumed to represent the sequence of the virus that had initiated the infection in the infant, and the maternal clonal sequences. Each dot represents the result of a pairwise comparison between a maternal clonal sequence and the sequence from the child. The results are given separately for each maternal sample. The nucleotide distance to the maternal consensus sequence of a sample is shown by a box.

Comparison of sequence populations of mother and infant

The predominant sequence observed in the cord blood and in the sample collected at the age of 6 weeks appears to represent the maternal variant that had initiated the infection in the child. In Fig. 3, the nucleotide distance of the maternal clonal and consensus sequences to this
predominant variant is shown for each maternal sample. All maternal samples except for sample ME included a minority of variants, representing 5 to 15% of the maternal sequence population, closely related (nucleotide distance < 1%) or identical (MD-10) to the predominant variant in the child at birth. The population of sequences of the maternal samples collected during pregnancy, especially during the first and second trimester (samples MA and MB), were more closely related to the variant transmitted to the child than of the samples collected during delivery and 10 months after childbirth. The mean nucleotide distance between the maternal sequence populations and the sequence population of the child at birth was 2.8, 2.6, 3.7, 5.2 and 5.3%, respectively, for the samples MA, MB, MC, MD and ME.

Phylogenetic analyses were performed by means of the maximum-likelihood and the neighbour-joining method with a subset of the sequences (see Methods). The results from both methods are comparable, but by no means identical (Fig. 4). Both dendrograms demonstrate the formation of two tight clusters of the child's sequences together with a subset of the maternal sequences. The sequences of sample TA and TB cluster with the maternal sequences MA-7, MA-11, MB-4 (MB-6, MB-3, not represented in the trees), MC-9, MC-13 (MC-11, not represented in the trees) and MD-10 (cluster 1). The sequences of sample TC cluster with MA-8, MB-7 (MB-11, not represented in the trees) and MD-4' (cluster 2). Both methods also agree in the sequences present within clusters 3 to 6, although the branching within these clusters was not always identical for the different methods, especially in cluster 6. In the neighbour-joining tree, this cluster contained two major branches, representing the two subgroups of sequences observed in sample ME. This was not observed in the maximum-likelihood tree. The main differences were noted in the choice of the branching points of the out-lier sequences (sequences outside the numbered clusters). Bootstrap analysis values were generally low (below 50%) in the neighbour-joining analysis. This is to be expected, because in a data set with relatively low variability the classification depends on a small number of positions. Since in bootstrapping the same analysis is repeated with subsets of the data where a number of positions have
been omitted, this will result in large variation in tree topology. In large clusters, relocation of one sequence is very likely to happen, and this lowers the bootstrap values rapidly.

**Discussion**

In order to examine the genetic relationship between the virus population in an HIV-1-infected mother and her infant, we analysed a 276 bp fragment, including the V3 region, of genomic HIV-1 RNA purified from serum samples sequentially collected from mother and child. In the mother, a continuous change in sequence populations was observed. It is unlikely that these changes represent sampling artefacts. The results of the direct sequences were in agreement with the clonal sequence sets, which excludes major sampling errors at the level of cloning. Although in one of the two tested samples a difference was observed between the sequence populations obtained from two different RNA extractions (sequence set ME, Fig. 1), it seems unlikely that sampling artefacts at the level of extraction could explain the observed differences. The continuity in the accumulation of amino acid substitutions within the V3 region strongly suggests that the change in sequence populations represents adaptive evolution. During pregnancy, a nearly complete replacement was observed at position 39 (corresponding to position 308 of the V3 loop) from a proline to a histidine (Fig. 1). A complete substitution at this position has previously been observed in two other patients (Wolfs et al., 1991). Replacements of amino acids at position 308 were found to influence the antigenic properties of peptides mimicking the central region of the V3 loop (Wolfs et al., 1991; Zwart et al., 1992). In the child, only a proline was observed in the first weeks of life and remained preserved until the age of 9 months. This may result in an antigenic distinction between the virus population in the mother and child at time of delivery. In the maternal samples, we observed variants that could have resulted from recombination of two other variants observed within the maternal sequence sets (representing 3-6% of the total number of maternal sequences). It has been observed that recombination of HIV genomes occurs efficiently in vitro under certain selection pressures (Clavel et al., 1989; Vartanian et al., 1991). Recent evidence for recombination in vivo was given by Groenink et al. (1992) who found that three out of eight sequences of biological clones seem to have been the result of recombination. It could be that the observed sequences in our samples were introduced by recombination events in vitro. Recombination during amplification by PCR has been described, but found to occur infrequently (<1%) (Vartanian et al., 1991). Considering an efficiency of 5 to 10% of the RT reaction (M. Cornelissen, personal communications; Zhang et al., 1991), the number of RNA molecules present in each RT reaction of the maternal samples will have been less than $10^4$. Because of the low amount of RNA molecules present in the reaction, combined with the conditions used in our experiments and the fact that template switching is a forced mechanism (i.e. introduced by breakages in the RNA strand), the chance of template switching during the RT reactions seems very low, probably less than 1% (Luo & Taylor, 1990; Ouhammouch & Brody, 1992). Recombination events in vivo could explain the observation of sequences with identical 5' regions and very different 3' regions (i.e. MC-7, MC-5; Fig. 1) and vice versa (the two subgroups of variants observed in sample ME; Fig. 1). When this is true, intermediate variants will not be observed because they do not exist. Another explanation for not observing intermediate variants could be that most of the substitutions result in variants with lower fitness. Therefore, there is little chance of detection of these intermediates when a limited number of clones are sequenced. However, the combination of particular substitutions could result in variants with a higher relative fitness and these variants will multiply and are subsequently detected.

Up until the age of 6 weeks, the sequence population in the child was highly homogeneous (Fig. 2). It was characterized by one particular sequence which appears to represent the variant that had initiated the infection in the child. The sequence population in the cord blood represented a minor variant in the maternal sample collected during delivery (Fig. 1). This excludes detection of viral RNA in the cord blood due to contamination with maternal blood during delivery and confirms intraterine acquired infection. The variation observed in the samples TA and TB was mainly caused by randomly occurring substitutions. It has to be considered that such substitutions were introduced by Taq polymerase mis-incorporations during the PCR amplification. If this were true, the maximum misincorporation rate will have been about 0.2% (15 of 7176 nucleotides analysed for the samples TA and TB). This is comparable to the rate of 0.2% observed by Overbaugh et al. (1992), but much higher than observed by others (<0.03%) (Meyerhans et al., 1989; Oram et al., 1991). A recent study of the V1 region during the acute phase of infection suggests that the occurrence of random substitutions is characteristic of the early stage of the infection (Pang et al., 1992). Several studies have shown that, irrespective of the route of transmission, a homogeneous virus population is observed in the recipient (Wolfs et al., 1991, 1992; Wolinsky et al., 1992; Holmes et al., 1992; Pang et al., 1992; Cichutek et al., 1992). It is not clear if this means that, in general, the inoculum consists of a small amount...
of viable viruses from which only one virus particle succeeds in establishing an on-going infection (i.e. by infecting an activated T cell) or whether this is the result of selective outgrowth of the virus with (initially) the highest relative fitness. It is possible that the child was infected with more than one virus particle. The sequence population observed in the child 9 months after birth (sample TC) clusters with maternal sequences other than the sequence population present at birth (sample TA) and at the age of 6 weeks (sample TB) (Fig. 4, clusters 1 and 2). This could represent the outgrowth of another virus particle (i.e. transmitted during delivery). However, the observation of intermediate variants (TB-4 and TB-12; Fig. 1) between the sequence population present at birth and the sequence population of sample TC, support the idea that the change in sequence population resulted from evolution in the child. Because the virus populations in the mother and child represent closely related viruses replicating in closely related genetic backgrounds, it is possible that identical substitutions may have occurred in the two virus populations. This would explain the clustering of the TC sequences with particular maternal sequences. The change in sequence population in the child is more rapid than observed in three adults 11 to 13 months after infection (Wolfs et al., 1991; Cichutek et al., 1992) and may be a characteristic of perinatal acquired infection (M. M. Goodenow, personal communication).

The observed limited variation within the first 6 weeks could mean that the infant had become infected shortly before birth. However, limited variation could also be due to a lower level of replication of HIV-1 in utero compared to the period after birth (Borkowsky et al., 1992) or to a lower immune pressure in the unborn child and neonate. Because variants closely related to the one transmitted to the child were observed in all of the maternal samples collected up until delivery, a reliable timing of the transmission during the pregnancy could not be established. However, it is noteworthy that the sequence populations of the maternal samples collected during the first and second trimesters of pregnancy showed the highest similarity to the sequence population of the child at birth (Fig. 3), since in utero transmission has been described in first and second trimester fetuses (Courgnaud et al., 1991; Lewis et al., 1990; Mano & Chermann, 1991). Sequences identical or closely related (< 1% nucleotide difference) to the variant transmitted to the child represented a minority (< 15%) in all of the maternal samples. However, it must be noted that in none of the maternal samples did one particular variant predominate: only subgroups of related sequences could be observed. For this reason it is difficult to distinguish between the possibilities that infection in the infant is established by the transmission of a few randomly selected variants or that particular variants are selected for transmission or replication in the infant. The transfer of maternal antibodies to the fetus (Regelman et al., 1987) could favour the transmission and replication of variants escaping the maternal humoral immune response. Wolinsky et al. observed that a particular N-linked glycosylation site (at position 20, Fig. 1) was strikingly absent from the infant's sequence sets but present in the majority of the maternal sequence populations (Wolinsky et al., 1992; Wike et al., 1992). However, in our mother–child pair no differences were observed in potential N-linked glycosylation sites between the majority of the maternal sequences and the variant transmitted to the child (Fig. 1).

In conclusion, our research shows that intra-uterine acquired infection results in a homogeneous sequence population present in the child at birth and during the first weeks of life. This population is more closely related to the virus population present in the mother during the first two trimesters of pregnancy than at delivery. The observation of a continuous evolution of the V3 sequence population in the mother diverging from the virus transmitted to the child indicates that the time of collection of samples studied may be important for a proper understanding of the factors involved in transmission. Further study on mother–child pairs is needed in order to determine whether certain viral characteristics are associated with perinatal transmission.

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