

# SECOND GENERATION MOTHER-TO-CHILD HIV TRANSMISSION IN SOUTH AFRICA IS CHARACTERISED BY POOR OUTCOMES

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## **ABSTRACT**

### **Background**

The worldwide incidence of pregnancy for perinatally HIV-infected women is increasing. Subsequently, there is growing risk of second-generation mother-to-child HIV transmission. The infant clinical outcomes for such a phenomenon have yet to be described.

### **Methods**

As part of a wider observational study in KwaZulu-Natal, South Africa, six *in utero* HIV-infected infants were identified as being born to perinatally HIV-infected mothers. Blood results and clinical data were collected in the first 3 years of life. In two cases, sample availability allowed confirmation by phylogenetic analysis of grandmother-to-mother-to-child HIV transmission.

### **Results**

Due to difficulties with acceptance, disclosure, poor health, and being themselves long-term non-progressors, all six mothers had difficulty administering twice daily combination antiretroviral therapy (cART) to their infants. None of the infants followed maintained suppression of viraemia on cART. One infant died, and another was lost to follow-up.

### **Conclusion**

As increasing numbers of second-generation mother-to-child HIV-infected infants arise, it is important to highlight that this represents an extremely vulnerable group. In order for them to survive and thrive, these infants' mothers require their specific needs to be addressed and given intensive support.

## INTRODUCTION

With improvements in HIV treatment and care, the number of perinatally HIV-infected children surviving to adulthood is increasing (1,2). In 2016 there were approximately half a million perinatally HIV-infected adolescents worldwide. Pregnancy outcomes for perinatally HIV-infected women have been reported from various regions worldwide, including the Americas (3,4,13–15,5–12), Europe (16–21) and India (22), but notably absent are publications from sub-Saharan Africa, where most perinatally HIV-infected young women reside (2). Findings from these reports include high rates of sexually transmitted infections, immunosuppression, and antiretroviral therapy (ART) resistance. Most studies have found similar, or lower, mother-to-child transmission (MTCT) rates compared to non-perinatally HIV-infected women. However, a study undertaken in the United States and Puerto Rico, the largest to date, found a two-fold increased risk (14). Despite these transmissions occurring, the clinical outcomes for this second-generation of perinatally HIV-infected children are unknown. This case series describes the clinical outcomes in six occurrences of second-generation mother-to-child HIV transmission.

## METHODS

**Study cohort:** Mother-child pairs with confirmed *in utero* (IU) HIV transmission were identified in the first weeks following delivery as part of an observational study exploring the feasibility of very early combination ART (cART) in KwaZulu-Natal, South Africa, as described elsewhere (23). All infants had a confirmed positive HIV total nucleic acid (TNA) PCR at birth. Based on the history reported by the mothers at enrolment, six of 191 (3.1%) transmitting mothers were identified as having themselves been perinatally HIV-infected.

**cART and routine HIV care and monitoring:** cART and routine HIV care for the mothers and infants were provided by local government-run health clinics, according to the South African National HIV Treatment Guidelines (24). The mothers were treated with an efavirenz (EFV)-based regimen as first-line therapy, and a ritonavir-boosted-lopinavir-based (LPVr) regimen as second-line therapy in the occurrence of drug resistance. The infants were prescribed a nevirapine (NVP)-based regimen until 4 weeks of age, when they were changed to a LPVr-based regimen, given as 3 syrups twice daily.

In parallel with the routine care of the patients, clinical research staff collected data, and took blood samples one to three monthly from the mothers and infants enrolled in the study. In two

of the cases of second-generation MTCT, the maternal grandmother was also enrolled, and blood drawn. Blood samples were used to measure CD4+T cell counts using flow cytometry (BD, Franklin Lakes, NJ, USA). Plasma viral load (VL) was measured by Nuclisens EasyQ v2.0 HIV-1 RNA PCR, (bioMérieux, Marcy l'Etoile, France), with a limit of detection of 20 HIV RNA copies/mL in the first instance, or <100 in the case of low sample volume. Viral suppression was defined as a VL <20 HIV RNA copies/mL. Drug resistance mutation (DRM) testing was performed on plasma at a local clinical diagnostics laboratory by Sanger sequencing using the HIV-1 Genotyping kit (Applied Biosystems, Waltham, MA, USA). Resistance phenotype was derived from the Online Stanford Database (24).

**Viral sequencing and phylogenetic analysis:** In two cases (2 and 5), there was sample available for deep sequence analysis of the transmitted founder virus. HIV from mother and infant plasma within 28 days of delivery was reverse transcribed and amplified. Libraries were prepared using Nextera XT and deep-sequencing was then performed using Illumina MiSeq (26). Raw reads were blasted against Los Alamos Sequence Database (<http://www.hiv.lanl.gov/>) HIV-1 “Web Alignments 2017” to obtain the HXB2 alignment positions for each read. From the aligned sequences, nucleotide diversity  $\pi$  was calculated (27) and compared to 22 other transmitting mother-child pairs from the wider cohort for whom NGS data were available.

In another two cases where the grandmother, mother and infant blood samples were available (Cases 4 and 6), phylogenetic analysis of HIV *gag* was undertaken. Viral RNA was isolated from plasma using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany). The HIV Gag-Protease region was amplified by reverse transcription using Superscript III One-Step Reverse Transcriptase kit (Invitrogen, Waltham, MA, USA). Second round PCR was used to amplify *gag* using BioTaq Polymerase (Bioline, London, UK). PCR products were purified using a QIAquick PCR purification kit (Qiagen, Hilden, Germany). Population sequences were generated using Sanger sequencing with the Big Dye ready reaction terminator mix V3 (Applied Biosystems, Waltham, MA, USA). Sequence data were analysed using Sequencher v.5.4.6 (Gene Codes Corporation, Ann Arbor MI USA). Nucleotides were aligned and a Maximum Likelihood Tree was constructed using the full-length *gag* sequences from the two case families and 24 other mother-child pairs from the same cohort, rooted on subtype C consensus sequence, in Geneious v10.0.9 (Biomatters Ltd, Auckland, NZ). In the same two

families, DNA was extracted from whole blood using the DNeasy Blood and Tissue Kit (Qiagen, Hilden, Germany). The DNA was used to determine HLA-A/B/C type by amplifying and sequencing Exons 2 and 3 for each locus and comparing results to the IMGT-HLA database ([www.ebi.ac.uk/ipd/imgt/hla/](http://www.ebi.ac.uk/ipd/imgt/hla/)).

**Consent and ethical approval:** Written informed consent for the use of clinical data and blood samples, was obtained from the mothers and grandmothers enrolled. The study was approved by the KwaZulu-Natal Bioethics Research Ethics Committee and the Oxfordshire Research Ethics committees.

## RESULTS AND DISCUSSION

We identified six IU HIV-infected infants born to mothers, who were themselves perinatally infected with HIV. The clinical and virological characteristics for these six cases are summarised in Table 1 and Figure 1A-D. Of note, periods of high plasma viraemia in both the mothers and children were frequent (Fig 1A&C). In two cases, access to maternal grandmother blood samples, as well as from the second-generation mother-child pair, enabled phylogenetic analysis of HIV *gag* and HLA typing to confirm HIV transmission across the three generations (Fig 2A-B). In the four cases where lack of sample availability prevented phylogenetic analysis of viral sequences, grandmother-to-mother transmission was determined by history alone.

These mothers had several features in common (Table 2). First, the fact that they had survived to 18-23 years to give birth themselves means they were relative slow progressors. ART only became available through the South African Department of Health in 2004 (28) and, until September 2016, was only initiated according to clinical or immunological criteria (29). The perinatally HIV-infected women included in this report were among the minority (25%) of perinatally HIV-infected children who can survive beyond 10 years without cART (30). Most strikingly, in Case 4, the mother had a CD4<sup>+</sup> T cell count above 600 cells/ $\mu$ L after 18 years without cART: a long-term non-progressor phenotype. Their own survival without cART may have made it difficult for these young women to realise the life-saving importance of cART for their infants. Case 4 was an example of this, where the mother refused to continue cART for her child after 6 months, citing the fact that she herself had not needed cART as a child.

However, despite it being more difficult to navigate adherence counselling in these cases, these mothers may have other advantages. Although HLA type does not predict paediatric slow progression (31), there may be other inherited immunological attributes that improve their children's survival (32). Going forward, with better infant diagnosis, earlier treatment initiation and improved cART coverage, this survival effect phenomenon will diminish.

A second potential risk factor for ART-experienced mothers is the high risk of HIV drug resistance (33). Even with the relatively limited exposure to cART in these cases, four out of six of the mothers had Non-Nucleoside Reverse Transcriptase Inhibitor (NNRTI) drug resistance mutations (DRMs), and two had the Nucleoside Reverse Transcriptase Inhibitor (NRTI) DRM M184V postnatally (Table 1). Although, for Cases 1 and 2 who had both NNRTI and NRTI DRMs, switching to a regimen they were susceptible to did not alter their viral load at all, suggesting ongoing non-adherence. Of note, mothers in Cases 1, 2, 3 and 6, despite being pregnant and having a history of failing NNRTI therapy, were continued on an NNRTI. This highlights the need for greater awareness of the almost ubiquitous NNRTI DRMs after viraemia and previous NNRTI use (34), and the urgent requirement to suppress pregnant women's viral load to prevent MTCT. With the increased use of cART in children in South Africa, resistance will only become more of a problem (35). A recent study in Canada found DRMs in all perinatally HIV-infected pregnant women tested, and nine out of eleven had resistance to three neonatal ART drugs (10). Resistance usually develops from periods of sub-optimal adherence, and this commonly occurs during adolescence (36).

A third factor these mothers have in common is in adaptation of the virus to shared HLA class I types through the passage of HIV. It has been very clearly shown both in mother-to-child and in adult-to-adult that transmission of viruses pre-adapted to the HLA class I molecules expressed by the recipient dramatically accelerates disease progression (37,38). In the examples described here, selection pressure on the virus has been imposed by two individuals sharing the identical HLA haplotype before being transmitted to a third sharing that same haplotype (illustrated by both cases 4 and 6, Fig 2B).

A fourth factor these mothers share is the fact that they are, or were only recently, themselves adolescents. As mentioned above, this is a time of high-frequency cART non-adherence. In Cases 1, 2, 3 and 6, the mothers had initiated cART in late childhood, but stopped taking their treatment during adolescence. The developmental transition from childhood to adulthood is a

vulnerable life-stage (39), particularly when partnered with chronic illness (40). Specific features that may add complexity to their HIV-infection, include stigma from having what is commonly known as a sexually transmitted infection, the burden of daily oral medication, delayed puberty, and subtle cognitive impairment (41). Although it is difficult to disentangle how much is due to the disease itself rather than the socioeconomic circumstances linked to HIV risk, perinatally HIV-infected adolescents have also been shown to have high rates of mental disorders, substance abuse, risky behaviours, and reduced cognitive functioning (42). These issues likely contribute to the high rates of virological failure in adolescents compared to adults, seen across many settings (43), including South Africa (36).

A fifth factor these mothers have in common is the issues around disclosure. In all cases but one disclosure of diagnosis was made during adolescence. The mother in Case 3 was informed of her HIV status at age 15, and her subsequent depressive symptoms and non-adherence is an example of the potentially detrimental psychological effects of delayed disclosure (44). The local KwaZulu-Natal guidelines recommend full disclosure to have occurred by 10 years of age (45). Cases 3, 5 and 6 did not disclose their HIV status to their partner, putting their partners at risk for transmission HIV infection. Case 2 and 3 did not disclose to their family or the household who regularly care for the child, in fear of losing their support. This non-disclosure limited the opportunities for the children to receive ART doses, and is a commonly cited reason for sub-optimal adherence (46). A study in the United States found that children and adolescents who have been aware of their diagnosis for longer, have higher rates of disclosure (47), suggesting that acceptance of HIV diagnosis may have been a key missing factor in these cases. Nevertheless, the pervasive HIV stigma in South Africa, intermixed with poverty and gender power imbalances (48,49), can make disclosure more difficult and dangerous than in other settings, and certainly is not unique to perinatally infected mothers.

A sixth factor shared by these mothers is poor maternal health. Cumulative years of virological failure, whether due to non-adherence or resistance, or in Case 5, a late diagnosis, means that perinatally HIV-infected mothers may likely have substantially impaired immunological function (10,17). Poor maternal health can affect the child in multiple ways: limiting the mother's ability to care for the child, increasing exposure to co-infections, and impairing infant immune development (50). Case 6 is an example of the former. The mother developed chronic diarrhoea, eventually diagnosed as *Cystoisospora belli* infection. In the delay to diagnosis, the mother had profound weight loss, and became so unwell she could no longer administer cART

to her child. Her child had been aviraemic since 2 months of age but rebounded to 14 000 HIV RNA copies/mL at this time.

For Case 5, the mother was severely immunocompromised (CD4+ T cell count 50 cells/ $\mu$ L) at delivery, which likely led to fetal immune dysfunction, manifesting as poor growth, a low CD4+ T cell count, and pancytopenia with suspected sepsis at birth. At 3 months of age the child died from sepsis and malnutrition, in keeping with the increased mortality risk for children born to mothers with low CD4+ T cell counts (51,52). In deep sequence analysis of plasma viral RNA, this pair's HIV showed high viral diversity (Fig 3), in the mother likely due to the many years of high maternal viraemia. The unusually high viral diversity observed in this child's virus at birth suggests that more than one viral strain had been transmitted to the child, consistent with the rapid disease progression in this instance.

Although our conclusions are limited by the small sample size, from a population of children with a background high rate of virological failure (51,53,54), this case series demonstrates universally poor adherence in the second generation of mother-to-child transmission of HIV. Our study was not designed to incorporate a formal maternal psychological assessment, so could not analyse the mothers' experiences in-depth, but we have obtained enough information to prime further qualitative studies. Furthermore, the true magnitude of the problem of second-generation MTCT remains unknown.

In South Africa and worldwide, the number of perinatally HIV-infected girls surviving to childbearing age and having children of their own is increasing. Optimising their care, and preventing transmission of HIV to their infants, requires careful consideration and specific insights. The first action must be preventing unplanned pregnancies, and improving the support for adolescents living with HIV, including early disclosure. If they become pregnant, it must be ensured that these young women get adequately treated to reduce transmission risk and optimise maternal physical and mental health. There should be a low threshold for suspecting ART resistance, and tailored adherence counselling to deal with issues of stigma, adolescence, orphanhood, and onward disclosure. In the occurrence of second-generation mother-to-child transmission, recognising the profound vulnerability of these children is essential to ensure they can survive and thrive.



### **Table 1 legend**

**Characteristics of six mother/child pairs with 2nd generation mother-to-child HIV transmission in KwaZulu-Natal, South Africa.**

### **Figure 1 legend**

**Clinical and virological characteristics of six mother-child pairs with HIV infection from KwaZulu-Natal, South Africa.**

Timelines span from delivery until last seen by the study. Case 5 was censored early due to death, and Case 4 due to loss to follow-up.

(A) Plasma viral load (VL) of the children and (C) of the mothers in  $\log_{10}$  HIV RNA copies/mL. (B) CD4+ T cell percentage of the children and (D) absolute CD4+ T cell count of the mothers, in cells/ $\mu$ L. Normal CD4+ T cell percentage range (10<sup>th</sup> to 90<sup>th</sup> centile for HIV-uninfected infants) shown in grey shading in panel B.

### **Figure 2 legend**

**Second generation mother-to-child HIV transmission confirmed by showed genetic relatedness of both the HIV virus and participants.**

(A) Phylogenetic clustering of HIV *gag* using a Maximum Likelihood phylogenetic tree of 24 mother-infant pairs from the study rooted on subtype C consensus sequence. The grandmother-mother-infant trios from Case 4 and 6 are indicated in colour. The legend indicates phylogenetic distance between mother-infant pairs and is expressed as nucleotide substitutions per site.

(B) HLA Class I typing from the grandmother-mother-infant trios from Case 4 and 6. Shared alleles indicated in bold and indicate likely genetic relatedness within each trio.

### **Figure 3 legend**

#### **High HIV diversity**

Nucleotide diversity  $\pi$  of deep-sequenced HIV virus from 23 IU transmitting pairs, within 28 days of delivery. The high nucleotide diversity of virus from the infant in Case 5 is indicated in pink, and suggests more than one viral strain was transmitted, likely a consequence of the mother's longstanding HIV infection.

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